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Nanoparticles loaded with antifungal drug for the treatment of skin infection

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***Abstract*---**Recent studies have concentrated on different formulations of polymeric nanoparticles. The oral bioavailability of water-insoluble medications has also increased because to advancements made possible by nanotechnology. Evaporation of the solvent and interfacial polymerization are two more methods that can be used in addition to these. Because of its nature as a macrolide pharmaceutical substance, it is especially prone to deterioration when exposed to organic solvents and heat; hence, these processes might not be optimal. On the other hand, coacervation is a sort of encapsulation that is less severe and more straightforward. There are two distinct types of nanoparticulate compositions. Although these formulations are more costly, researchers claim that they cause less damage to the kidneys. Many different resources have created delivery strategies that are both less dangerous to the kidneys and less costly. The current work utilized polyelectrolyte complexation technology in order to generate nanoparticle dosage forms with the intention of maximizing the oral bioavailability of amphotericin B (AmB). Oral bioavailability can be increased thanks to these polymers, which are also biocompatible and biodegradable.

***Keywords*---**amphotericin B, bioavailability, nanotechnology, skin disease.

Introduction

After new chemical entities have been characterised in vitro and in vivo, the next step is to make an attempt to synthesise already active medications in a suitable dose form. This is done with the goal of improving the inherent efficacy of the medications while simultaneously lowering the inherent toxicity. In the past several years, the discipline of formulation science has witnessed an enormous amount of expansion within the pharmaceutical industry. Formulation scientists have been working tirelessly over the course of the past few decades to find novel approaches to the delivery of pharmaceuticals. As other branches of research continue to make strides forward, the field of formulation science is also investigating novel concepts that might lead to improved methods of administering pharmaceuticals. The most recent example of adding nanotechnology into the formulation is as follows: Because of the drug's low water solubility, particles with a diameter of less than 1000 nanometers (nm) have a considerable influence on the material qualities of their surrounding environment. This antifungal medication is used intravenously for the treatment of systemic fungal infections. Amphotericin B is an antifungal antibiotic that is made by Streptomyces sp., and it is capable of destroying fungi. When one examines its atomic structure in greater detail, one can see that it is quite intricate. This type of medication is classified as an amphoteric due to the presence of two carboxyl groups in the ring that contains glucosamine and one main amino group in the ring that includes the prominent ring.

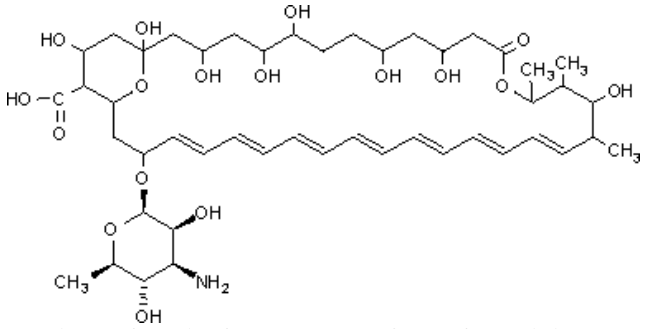

Fig.1 chemical structure of amphotericin B

Material & Methods

Characterization of nanoparticle dosage form containing amphotericin B
Particle size distribution and Polydispersity Index

By employing dynamic light scattering (DLS) with a Zetasizer Nano ZS, the researchers were able to measure the mean particle size as well as the distribution pattern of lyophilized nanoparticles (Malvern Instruments Ltd., UK). After being lyophilized, the nanoparticles were resuspended in DI water using an aliquot. After that, measurements were taken at an angle of 90 degrees to the light that was incident onto them. The value of each item is the average of the previous five measurements. The diameter was determined by using the autocorrelation function to compute the amount of light that was scattered from the particles. The Polydispersity Index, often known as PDI, is a measure of how

homogeneous a dispersion is and can vary from 0 to 1. Table 4 has an explanation of the findings.

Zeta Potential

The Zetasizer Nano ZS was utilized in order to carry out Laser Doppler Electrophoresis in order to obtain the zeta potential readings (Malvern Instruments Ltd., UK). At a temperature of 25 degrees Celsius and using ultrapure water as diluents to achieve the appropriate concentration, measurements were carried out in a folding capillary electrophoresis cell manufactured by Malvern Instruments Ltd. in the United Kingdom. The results of the zeta potential data analysis are reported as the mean of three replicate runs for each sample. The frequency shift or phase shift of an incoming laser beam that is induced by these moving particles is quantified as the particle mobility, and the application of the Smoluchowski or Huckel theories converts this mobility to zeta potential. Table 4 outlines the findings with their respective descriptions.

Drug entrapment efficiency

After weighing and dissolving them in 0.5 milliliters of DMSO, ten milligrams of lyophilized nanoparticles were used. After centrifuging the solution for twenty minutes at a speed of 15,000 g, a volume of ten milliliters was obtained from thirty microliters of the supernatant by adding methanol. After that, spectrophotometric analysis at 405 nm was used to determine the quantity of AmB. After that, the proportion of drug association was computed based on the following equation:

Amount of AmB in nanoparticle × total particle mass × 100% Drug association (%) =
Particle mass tested × initial amount of AmB

***In Vitro* drug release study**

The dialysis membrane approach was used to accomplish the in vitro release of amyloid beta from nanoparticles (modified method of Italia et al., 2009). AmB nanoparticles with a molecular weight cut-off of 12,000 Da were dispersed in 0.5 ml of release medium, which was composed of 10 mM HEPES buffer containing 0.25 percent w/v sodium lauryl sulphate (SLS) in dialysis bags (Sigma). Each AmB nanoparticle contained an amount of entrapped drug that was equivalent to 1 mg. After being placed in screw-capped tubes that held 10 milliliters of release medium, the dialysis bags were placed in an orbital shaking water bath and incubated at 37 degrees Celsius (80 rpm). At predetermined time intervals of 5, 15, 30, 60, and 120 minutes, the entire release medium was replaced with a new buffer, and the quantity of AmB that was released into the medium was calculated using an HPLC technique that was devised. With the use of the following equation, we were able to determine the total quantity of AmB that was released from the nanoparticles.

Result & Discussion

Mean absorbance of the drug solutions of known concentrations for the preparation of the standard curve

Sample	Con^{c} of amphotericin B	Absorbance*****
1.	00.10µg/ml	0.008 ± 0.001
2.	00.25µg/ml	0.025 ± 0.003
3.	00.50µg/ml	0.065 ± 0.011
4.	1.000µg/ml	0.136 ± 0.023
5.	2.000µg/ml	0.266 ± 0.039
6.	3.000µg/ml	0.409 ± 0.054
7.	4.000µg/ml	0.543 ± 0.072
8.	5.000µg/ml	0.663 ± 0.085

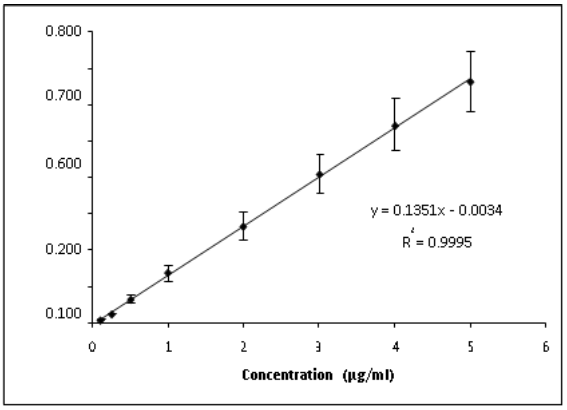

Fig. 2. Standard curve of pure amphotericin B by spectrophotometer

Detector response from hplc against corresponding concentrations of amphotericin B for preparation of thestandard curve

Calibration Sample	Concentration of amphotericin B	Area of amphotericin B *
Sample T1	0.10µg/ml	6.419 ± 0.768
Sample T2	0.25µg/ml	16.245 ± 1.893
Sample T3	0.50µg/ml	27.124 ± 3.140
Sample T4	1.00µg/ml	40.997 ± 3.154
Sample T5	2.00µg/ml	91.246 ± 5.127
Sample T6	3.00µg/ml	150.794 ± 7.182
Sample T7	4.00µg/ml	197.117 ± 11.431
Sample T8	5.00µg/ml	244.488 ± 9.467

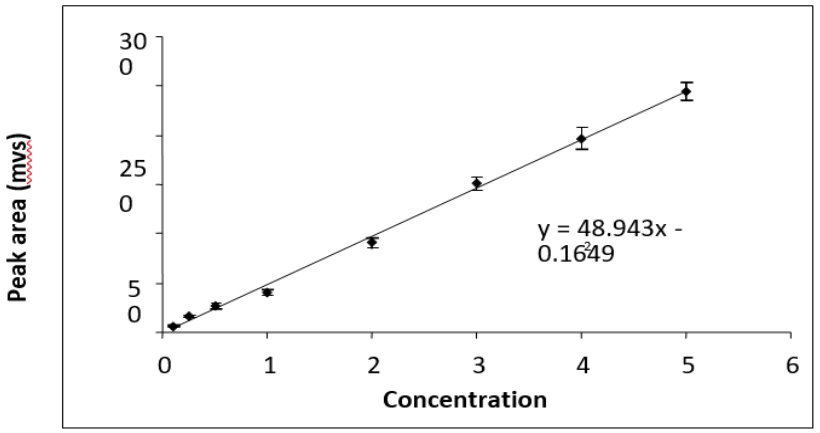

Fig. 5.3. Standard curve of pure amphotericin B by HPLC

Preparation of nanoparticle dosage form containing amphotericin B

The method of producing nanoparticles is determined entirely by the physicochemical characteristics of the drug that is going to be enclosed. In order to make amphotericin B nanoparticles suitable for oral administration, the polyelectrolyte complexation method was utilized at room temperature (a modified version of Tiyaboonchai et al., 2007). In order to create the formulation, we utilized zinc sulphate as a means of stabilizing the polyelectrolytes, as well as chitosan and dextran sulphate, all of which have water solubility polymers that are charged in the opposite direction from one another. In order to get a deeper comprehension of the characteristics of nanoparticles, the processing factors, such as physicochemical qualities and preparation circumstances, were investigated in this work. The effectiveness of drug attachment and the mechanism of drug release were both investigated, in addition to the size and appearance of the nanoparticles. In addition to this, it was demonstrated that the nephrotoxic potential of AmB in association with nanoparticles was in some way connected to the molecule's structural make-up. For the purpose of perfecting the formulation of amphotericin B nanoparticles, a Box-Behnken experiment was carried out to study the relationship between dextran sulphate, the pH of chitosan solution, and zinc sulphate as it pertains to the response variables.

Table 3 Composition and observed responses in box–behnken DESIGN

Independent variables			Dependent variables	
Batch No.				
Conc. of (DS) solution (mg/ml)				
pH of chitosan solution (X_2)				
Conc. of zinc sulfate (μM)				
Mean particle diameter (nm)				
(X_1)	(X_3)		(Y_1)	(Y_3)

F1	1.00	4.00	12.50	487.5	0.417	65.78
F2	1.00	3.00	43.75	582.3	0.311	49.49
F3	5.50	5.00	12.50	947.6	0.564	38.8
F4	5.50	4.00	43.75	321.2	0.253	80.24
F5	5.50	3.00	75.00	1923.4	0.614	22.78
F6	10.00	3.00	43.75	1433.7	0.473	37.23
F7	1.00	5.00	43.75	712.3	0.331	70.44
F8	5.50	3.00	12.50	514.8	0.248	62.38
F9	10.00	4.00	75.00	1147	0.545	41.71
F10	5.50	5.00	75.00	896.4	0.486	51.98
F10	5.50	4.00	43.75	337.1	0.238	83.17
F12	1.00	4.00	75.00	628.5	0.349	31.06
F13	10.00	5.00	43.75	1547.3	0.403	29.11
F14	5.50	4.00	43.75	310.4	0.247	89.43
F15	10.00	4.00	12.50	958.1	0.364	41.07

Polydispersityindex(Y_2)

Drug entrapment efficiency (%) Characterization of nanoparticle dosage form containing amphotericin B

Particle size distribution and Polydispersity Index
The average particle size and distribution pattern of lyophilized nanoparticles were determined by the use of a technique called dynamic light scattering (DLS). The chapter before this one has an in-depth discussion of the process that needs to be followed (section 4.6). The patterns of particle size distribution for the experimental batches. When it comes to transporting and entering tissues in a biological system, nanoparticles with a smaller particle size and a tighter distribution are far more effective than bigger particles with a wider dispersion. When compared to the other trial formulations, the F4 formulation has a particle size that is 321.2 nm smaller and a dispersion pattern that is narrower. Although F6 and F8 formulations have bigger particle sizes, their dispersion patterns are far more constrained. In Formulation F3, although having a larger particle size (947.6 nm), the distribution pattern is not affected in any way. Formulations F1, F11, and F14, on the other hand, have particles that are both smaller and more uniformly dispersed throughout the formulation. The Polydispersity Index, abbreviated PDI, is a metric that runs from 0 to 1 and is used to quantify the uniformity of dispersion. The findings of the testing batches are presented in Table 5.4 below. In the many different test formulations, the polydispersity index was anywhere from 0.238 to 0.614.

Zeta Potential

The zeta potential of a nanoparticle formulation is an important critical parameter that must be controlled to ensure the stability and uniformity of dispersed nanoparticles. In the course of this experiment, even little adjustments to the

parameters of the process did not have a discernible impact on the zeta potentials of the trial batches. The results are presented in Table 5.4 below. The zeta potential of the formulations that were put through the trials ranged anywhere from -27.1 to 30.0. If the particle suspensions that include the nanoparticles have a pH of 3, then the nanoparticles will have less protonated sulphate groups on their surface before dialysis.

Drug entrapment efficiency

Another important consideration in the process of developing nanoparticle formulations is the effectiveness of drug encapsulation. For the different formulations that were evaluated, the entrapment effectiveness ranged anywhere from 22.78 percent to 89.43 percent. The findings are shown in Table 4.

TABLE 4
Physical parameters of the trial batches of amphotericin B nanoparticles

Physical parameters Batch No.	Mean particle diameter* (nm) (Y_1)	Polydispersity index* (Y_2)	Drug entrapment efficiency* (%) (Y_3)	Zeta potential* (mV)
F1	487.5 ± 12.3	0.417 ± 0.022	65.78 ± 4.21	-30.2 ± 0.03
F2	582.3 ± 9.7	0.311 ± 0.017	49.49 ± 2.19	-33.1 ± 0.01
F3	947.6 ± 13.4	0.564 ± 0.034	38.80 ± 3.33	-29.1 ± 0.03
F4	321.2 ± 10.9	0.253 ± 0.016	80.24 ± 5.14	-31.7 ± 0.02
F5	1923.4 ± 27.2	0.614 ± 0.041	22.78 ± 2.61	-30.4 ± 0.02
F6	1433.7 ± 22.8	0.473 ± 0.019	37.23 ± 2.15	-28.6 ± 0.01
F7	712.3 ± 19.3	0.331 ± 0.015	70.44 ± 6.42	-30.8 ± 0.04
F8	514.8 ± 14.1	0.248 ± 0.019	62.38 ± 4.22	-32.2 ± 0.02
F9	1147 ± 25.4	0.545 ± 0.036	41.71 ± 3.91	-28.4 ± 0.02
F10	896.4 ± 16.4	0.486 ± 0.027	51.98 ± 6.82	-30.7 ± 0.03
F10	337.1 ± 7.5	0.238 ± 0.022	83.17 ± 7.34	-35.6 ± 0.04
F12	628.5 ± 17.6	0.349 ± 0.015	31.06 ± 2.66	-32.4 ± 0.01
F13	1547.3 ± 31.8	0.403 ± 0.021	29.11 ± 2.34	-27.1 ± 0.02
F14	310.4 ± 21.3	0.247 ± 0.014	89.43 ± 8.78	-36.0 ± 0.01
F15	958.1 ± 27.1	0.364 ± 0.026	41.07 ± 5.97	-30.2 ± 0.03

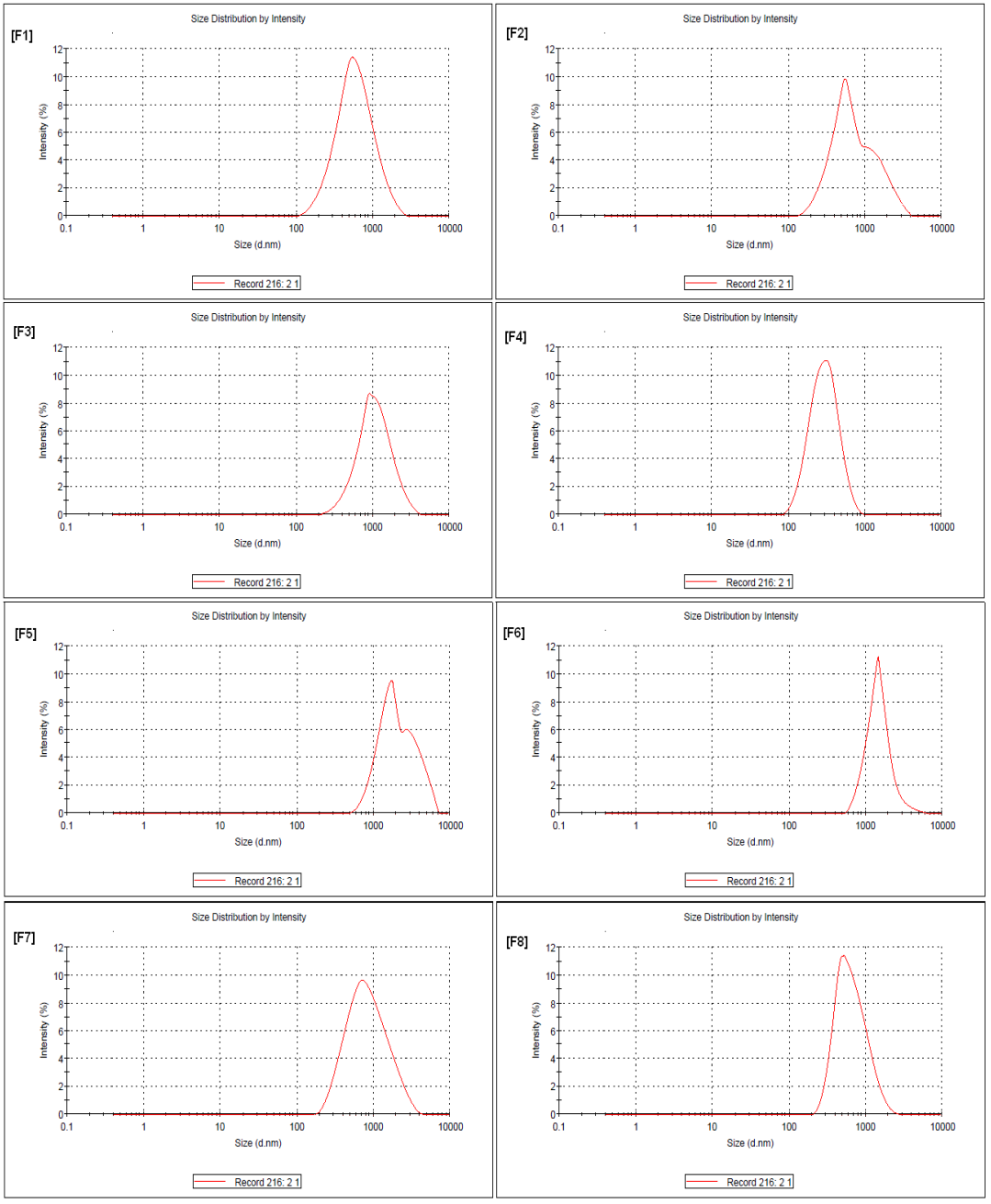

Fig. 5.4.A Particle size distribution pattern of different trial formulations (F1 to F8)

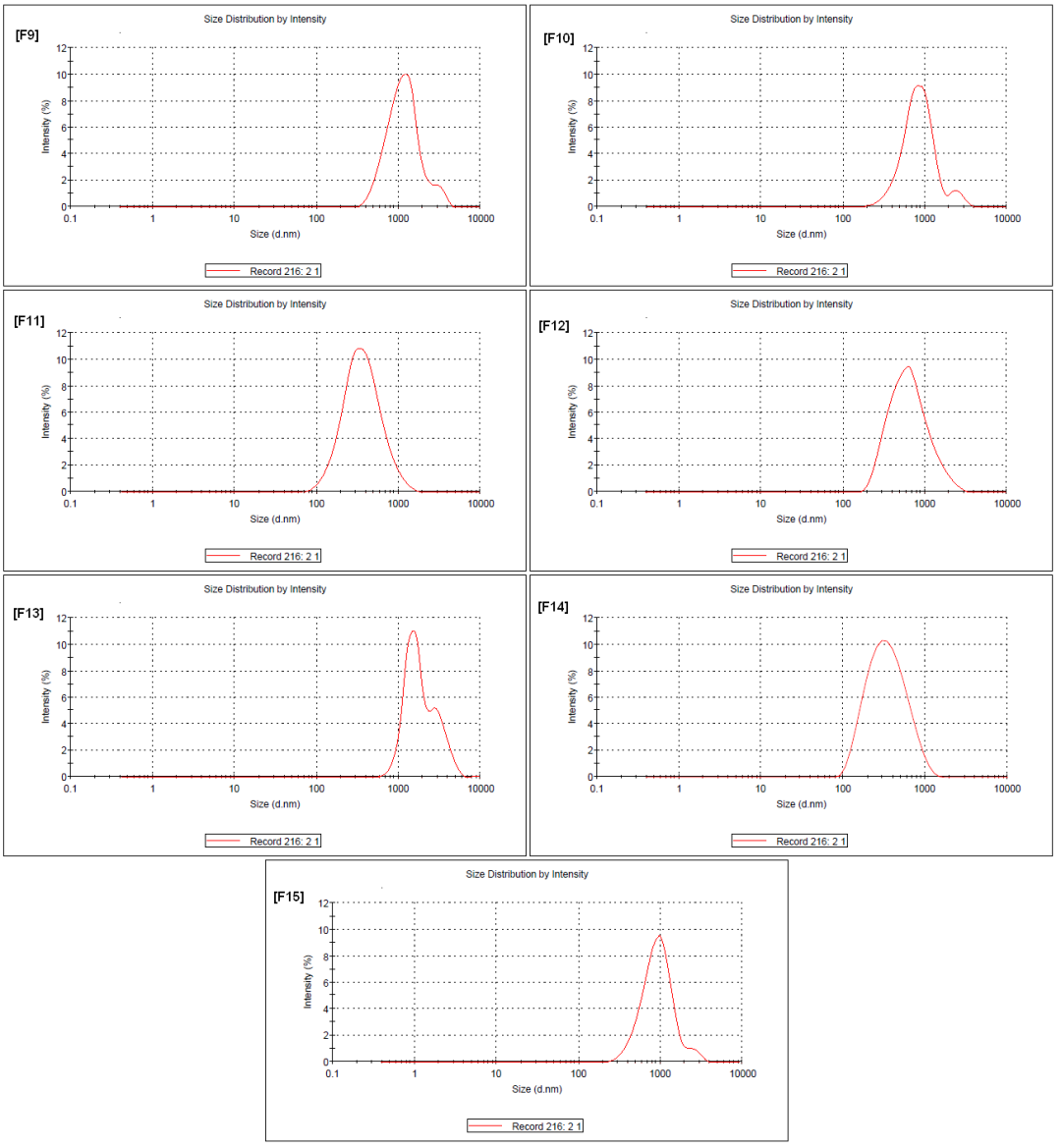

Fig. 5.4.B Particle size distribution pattern of different trial formulations (F9 to F15)

In Vitro drug release study

When conducting a release study, maintaining an absolute sink state might be challenging for medications that have a low solubility in water and are therefore difficult to disseminate. Because AmB has a limited solubility in water, it was required to include 0.25 percent SLS in the release medium. Research on the drug's release was the topic of conversation in the chapter before this one (section 4.6.4). The results of the tests on the trial formulae are presented in Table 5.5. The two batches of experimental items are so similar that there is no statistically significant difference between them. The results from each formulation

demonstrated that AmB could be readily released (more than 75 percent within 5 minutes). Based on these findings, it would indicate that the majority of AmB molecules are not really integrated into the side particles, but rather are adsorbing onto the surface of the particles instead. On the other hand, because AmB is not very soluble in water, it is expected that the rate of release in vivo will be lower than the rate that was tested in vitro. It is reasonable to anticipate a slow but steady rise in plasma concentration as a means of mitigating the toxicity. The patterns of release that were seen during the experimental formulations are shown in Fig.5.5.

TABLE 5
In-vitro release of amphotericin B from nanoparticles

Formulation No.	Cumulative % AmB released with time*				
	5 min	15 min	30 min	60 min	120 min
F1	76.32 ± 2.10	89.25 ± 2.81	95.14 ± 0.93	98.74 ± 0.37	99.67 ± 1.38
F2	75.44 ± 1.43	88.37 ± 2.32	94.26 ± 1.31	97.86 ± 1.12	98.79 ± 0.73
F3	78.40 ± 1.30	86.89 ± 1.65	95.77 ± 1.02	100.82 ± 0.64	101.75 ± 1.04
F4	77.52 ± 1.72	90.45 ± 1.47	96.34 ± 2.08	99.94 ± 0.88	100.87 ± 0.55
F5	76.64 ± 3.10	89.57 ± 3.14	95.46 ± 1.36	99.06 ± 1.12	99.99 ± 2.04
F6	79.60 ± 2.45	92.53 ± 2.66	98.42 ± 0.44	99.17 ± 0.46	100.78 ± 0.89
F7	78.72 ± 1.79	91.65 ± 1.14	97.54 ± 1.54	101.14 ± 0.72	99.17 ± 2.07
F8	77.84 ± 2.41	90.77 ± 3.01	96.66 ± 1.92	100.26 ± 1.19	101.19 ± 1.52
F9	80.80 ± 1.44	93.73 ± 1.42	99.62 ± 0.83	100.39 ± 2.04	99.88 ± 2.53
F10	79.92 ± 1.34	92.85 ± 0.87	98.74 ± 1.77	99.06 ± 0.94	102.14 ± 2.07
F11	79.04 ± 2.33	91.97 ± 2.11	97.86 ± 3.02	101.46 ± 1.02	102.39 ± 0.46
F12	82.00 ± 3.19	94.93 ± 0.97	96.09 ± 0.67	99.74 ± 3.11	101.03 ± 1.21
F13	81.12 ± 2.21	94.05 ± 1.43	99.94 ± 1.06	99.47 ± 1.20	99.26 ± 2.03
F14	80.24 ± 1.06	93.17 ± 2.08	99.06 ± 1.11	102.66 ± 0.78	103.59 ± 0.76
F15	83.20 ± 2.19	96.13 ± 1.74	98.61 ± 1.03	99.37 ± 1.77	101.79 ± 1.32

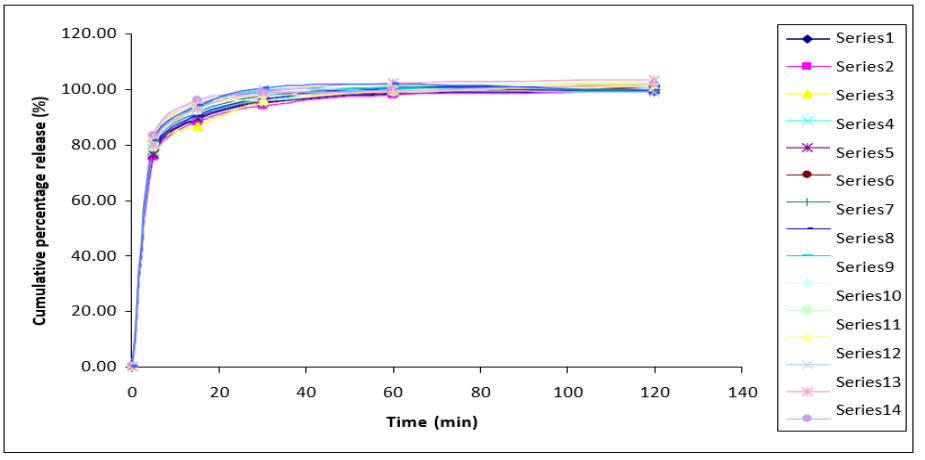

Fig..5.5 Mean cumulative % drug release profiles of AmB nanoparticle formulations prepared as per the experimental design

Conclusion & Future Prospective

Data from an inquiry on the use of nanoparticles to increase oral bioavailability of the water-insoluble medicine amphotericin B (AmB) are included in this master's thesis (AmB). Antifungal medication containing amphotericin B (AmB) is a polyene macrolide that is used to treat systemic fungal infections as well as visceral leishmaniasis. This drug has a poor capacity to dissolve in water, which contributes to its poor oral bioavailability. Therefore, the only form of AmB treatment that may be administered is by parenteral injection. There are now two different formulations of AmB. The first one is a micellar solution of AmB that uses deoxycholate as a surfactant. This solution has a high nephrotoxic potential. There are two distinct types of nanoparticulate compositions. Although these formulations are more costly, researchers claim that they cause less damage to the kidneys. Many different resources have created delivery strategies that are both less harmful to the kidneys and less costly. The current work utilized polyelectrolyte complexation technology in order to generate nanoparticle dosage forms with the intention of maximising the oral bioavailability of amphotericin B (AmB). Oral bioavailability can be increased thanks to these polymers, which are also biocompatible and biodegradable. Chitosan and dextran sulphate, both of which are water soluble polymers with opposing charges, are used in the formulation, while zinc sulphate is used as a stabiliser. A three-factor, three-level Box-Behnken experimental design was utilized in order to investigate the impact that dextran sulphate, the pH of the chitosan solution, and zinc sulphate had on response variables. The crystalline structure of the drug that was contained within the nanoparticles, the pattern of drug release seen in vitro, and the interaction between the polymer and the drug were all investigated. Bioavailability, nephrotoxicity, accelerated stability, and antileishmanial activity were some of the aspects of the novel formulation that were evaluated using rat models.

References

1. Allugunti V.R (2022). A machine learning model for skin disease classification using
2. convolution neural network. International Journal of Computing, Programming and Database
3. Gondal JA, Swartz RP, Rahman A. Therapeutic evaluation of free and liposome-encapsulated amphotericin B in the treatment of systemic candidiasis in mice. Antimicrob Agents Chemother. 1989 Sep;33(9):1544–1548.
4. Graybill JR, Craven PC, Taylor RL, Williams DM, Magee WE. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J Infect Dis. 1982 May;145(5):748–752.
5. Graybill JR, Palou E, Ahrens J. Treatment of murine histoplasmosis with UK 49,858 (fluconazole). Am Rev Respir Dis. 1986 Oct;134(4):768–770
6. Holmberg K, Meyer RD. Fungal infections in patients with AIDS and AIDS-related complex. Scand J Infect Dis. 1986;18(3):179–192.
7. Hospenthal DR, Rogers AL, Beneke ES. Effect of attachment of anticandidal antibody to the surfaces of liposomes encapsulating amphotericin B in the

treatment of murine candidiasis. Antimicrob Agents Chemother. 1989 Jan;33(1):16–18.
8. hrens J, Graybill JR, Craven PC, Taylor RL. Treatment of experimental murine candidiasis with liposome-associated amphotericin B. Sabouraudia. 1984;22(2):163–166.
9. Janoff AS, Boni LT, Popescu MC, Minchey SR, Cullis PR, Madden TD, Taraschi T, Gruner SM, Shyamsunder E, Tate MW, et al. Unusual lipid structures selectively reduce the toxicity of amphotericin B. Proc Natl Acad Sci U S A. 1988 Aug;85(16):6122–6126.
10. Katz NM, Pierce PF, Anzeck RA, Visner MS, Canter HG, Foegh ML, Pearle DL, Tracy C, Rahman A. Liposomal amphotericin B for treatment of pulmonary aspergillosis in a heart transplant patient. J Heart Transplant. 1990 Jan-Feb;9(1):14–17.
11. Kirsh R, Goldstein R, Tarloff J, Parris D, Hook J, Hanna N, Bugelski P, Poste G. An emulsion formulation of amphotericin B improves the therapeutic index when treating systemic murine candidiasis. J Infect Dis. 1988 Nov;158(5):1065–1070.
12. Kobayashi GS, Travis SJ, Medoff G. Comparison of fluconazole and amphotericin B in treating histoplasmosis in immunosuppressed mice. Antimicrob Agents Chemother. 1987 Dec;31(12):2005–2006.
13. Kumar, S. (2022). A quest for sustainium (sustainability Premium): review of sustainable bonds. Academy of Accounting and Financial Studies Journal, Vol. 26, no.2, pp. 1-18
14. Kusumawati, A. H., Wulan, I. R., & Ridwanuloh, D. (2020). Formulation and physical evaluation sheet mask from red rice (Oryza Nivara) and virgin coconut oil (Cocos Nucifera L). *International Journal of Health & Medical Sciences*, *3*(1), 60-64. https://doi.org/10.31295/ijhms.v3n1.148
15. Lopez-Berestein G, Bodey GP, Fainstein V, Keating M, Frankel LS, Zeluff B, Gentry L, Mehta K. Treatment of systemic fungal infections with liposomal amphotericin B. Arch Intern Med. 1989 Nov;149(11):2533–2536.
16. Lopez-Berestein G, Bodey GP, Frankel LS, Mehta K. Treatment of hepatosplenic candidiasis with liposomal-amphotericin B. J Clin Oncol. 1987 Feb;5(2):310–317.
17. Lopez-Berestein G, Fainstein V, Hopfer R, Mehta K, Sullivan MP, Keating M, Rosenblum MG, Mehta R, Luna M, Hersh EM, et al. Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. J Infect Dis. 1985 Apr;151(4):704–710.
18. Lopez-Berestein G, Hopfer RL, Mehta R, Mehta K, Hersh EM, Juliano RL. Liposome-encapsulated amphotericin B for treatment of disseminated candidiasis in neutropenic mice. J Infect Dis. 1984 Aug;150(2):278–283.
19. Lopez-Berestein G, Mehta R, Hopfer RL, Mills K, Kasi L, Mehta K, Fainstein V, Luna M, Hersh EM, Juliano R. Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposome-encapsulated amphotericin B. J Infect Dis. 1983 May;147(5):939–945.
20. Management 3(1), 141-147
21. Mardon DN, Gunn JL, Robinette E., Jr Variation in the lethal response in mice to yeast-like and pseudohyphal forms of Candida albicans. Can J Microbiol. 1975 Nov;21(11):1681–1687.

22. Patterson TF, Miniter P, Dijkstra J, Szoka FC, Jr, Ryan JL, Andriole VT. Treatment of experimental invasive aspergillosis with novel amphotericin B/cholesterol-sulfate complexes. J Infect Dis. 1989 Apr;159(4):717–724.
23. Sculier JP, Coune A, Meunier F, Brassinne C, Laduron C, Hollaert C, Collette N, Heymans C, Klastersky J. Pilot study of amphotericin B entrapped in sonicated liposomes in cancer patients with fungal infections. Eur J Cancer Clin Oncol. 1988 Mar;24(3):527–538.
24. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
25. Szoka FC, Jr, Milholland D, Barza M. Effect of lipid composition and liposome size on toxicity and in vitro fungicidal activity of liposome-intercalated amphotericin B. Antimicrob Agents Chemother. 1987 Mar;31(3):421–429.
26. Taylor RL, Williams DM, Craven PC, Graybill JR, Drutz DJ, Magee WE. Amphotericin B in liposomes: novel therapy for histoplasmosis. Am Rev Respir Dis. 1982 May;125(5):610