Research Article

Design and Evaluation of Solid Lipid Nanoparticles of an Antibiotic-mannitol as Cryoprotectant

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ABSTRACT

Mannitol was used as a cryoprotactant to freeze-dry Doxycycline nanoparticles for colloidal drug administration in this work. in this research, the benefits include Medicines like vaccines and other injectables, which have a short shelf life and are commonly freeze-dried to increase their shelf life. A liquid may be treated more easily with aseptic handling because of this. The stability of a dry powder has been increased. In addition, water may be removed from the device without causing it to overheat. Reconstituted product dissolves quickly and readily. All of these concerns are avoided so that the product may be transported or stored at room temperature without suffering deterioration or degradation due to aggregation. Preparation of doxycycline SLNs included homogenization and sonication. Antimicrobial activity and in vitro anticancer activity are among the techniques employed in this investigation. Freeze drying was also a component of the process, as was particle size, drug loading, and drug release. The lipid solid's particle size distribution At various magnifications of the investigation, nanoparticles are determined to be 3.66, 6.58, 9.96, 4.97, 17.36, and 25.48mm in diameter. A freeze-dryer was used to freeze-dry the particles at -20°C and 0.4 pressure. Mannitol at various concentrations (5, 10, and 15 percent) was utilised as a cryoprotectant. Drug loading was determined to be 78 percent, while drug release was found to be 98 percent after 24 hr. Maximum inhibition of cell growth (69.9422) was seen at an IC_{50} concentration of 100g/mL of leaf crude extract (48.69g/mL). Doxycycline SLNs might be inferred to be more stable, less aggregate, and have fewer adverse effects when freeze-dried.

Keywords: Doxycycline, Solid lipid nanoparticles, Freeze-drying, Stability, Activity.

INTRODUCTION[1-7]

Solid lipid nanoparticles (SLNs) are intriguing colloidal drug delivery devices because they combine the properties of fat emulsions with polymeric nanoparticles. They can be used in a variety of ways, including intravenous, oral, topical, and pulmonary delivery. Polymeric nanoparticles and emulsions can be replaced with SLNs as a colloidal carrier system. Some of the advantages of SLNs include the ability to incorporate lipophilic and hydrophilic drugs, the ability to control drug release and targeting, increased drug stability, high drug payload, lack of carrier biotoxicity, no problems with large-scale production, sterilisation capability, and the good tolerability. The improved antibacterial efficacy of antibiotics when SLNs are utilised as a carrier is one of the benefits of these colloidal systems. Freeze-drying has been suggested as a method for improving colloidal nanoparticles' long-term stability. Other investigations have found that nanoparticle stability in an aqueous medium is a key impediment to their clinical usage. Also, before turning nanoparticles into dried particles, freeze-drying may cause various pressures that destabilise colloidal suspensions of

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nanoparticles. Cryoprotectants were employed to reduce SLN aggregations caused by stress during the freeze-drying process. Cryoprotectants such as mannitol were used. Nanoparticles' tiny and delicate structures may not be able to endure the freeze-drying process's stress. This research looked at the factors that might impact nanoparticle stability after lyophilization. The generated doxycycline SLNs could not be lyophilized directly, therefore cryoprotectants were required to prevent particle aggregation following reconstitution. The kind and proportion of cryoprotectants used, the duration of the freezing stage, the freezing temperature, the lyophilizer device's pressure, and other parameters can all affect the particle properties the following freeze-drying. This study looked at the effects of mannitol as a cryoprotectant in the freeze-drying process on particle size, shape, drug release profile, and antibacterial activity of doxycycline SLNs. Our objective was to create freeze-dried doxycycline SLNs while also optimising the lyophilization procedure to ensure that the physicochemical and antibacterial properties were intact. The doxycycline SLNs *in vitro* release was evaluated in pH-simulated media, while cytotoxicity was checked in a cancer cell line.

MATERIALS AND METHODS

Cholesterol (Vj-biotech lab, Coimbatore) was used as the lipid material of SLNs. Tween 80 (Vj-biotech lab) was used as a surfactant. Doxycycline hyclate (harish lab, vellore) was used as the active pharmaceutical ingredient. Ethanol and acetone (Vj-biotech lab) were organic solvents. Glycerol laurate (Vj-biotech lab) was used as an oily phase. Mannitol (Vj-biotech) was used as cryoprotactant.

PREPARATION OF ANTIBIOTIC DRUG SLNS[8-9]

Add 160mg of doxycycline hylate drug was homogenised at 11,000 rpm in deionized water with 1% w/w Tween80. Then, by heating to 70°C and stirring, 308mg of cholesterol as the lipid phase was dissolved in 24mL of a 3:1 (v/v) ethanol/acetone combination (equivalent to 18 mL ethanol and 6 mL acetone). Then, at 25°C, 1ml of glycerol laurate hot oily phase was added to the aqueous phase and homogenised for 6 min at 11,000 rpm. To obtain nanoparticles, the produced emulsion was sonicated in a bath sonicator and cooled to room temperature. SLNs were made at the optimal level and type of surfactant, homogenization rate and time.

Determination of Antibiotic Drug (FT-IR)

In this inquiry, a series of investigations were carried out to examine if there was any possible interaction between doxycycline and the lipid molecules discovered in DOX-SLN. FTIR analysis was utilised to investigate such a possibility in terms of stability and efficacy.

In order to characterise the probable interactions between the drug and the Lipid carrier in the solid-state, and FTIR spectrometer was used. The samples have been prepared, and the spectra have been recorded in the 4000–400 cm^{-1} region. The result is shown in the Figure 1,2.

Experimental Design[10]

To investigate the impact of various factors on the physicochemical characteristics of nanoparticles that have been created. The polymer concentration and varied speeds were chosen as independent variables. A total of six experiments were used in the design. The dependent variables were chosen to be the colloid system particle sizes and the drug encapsulation efficiency. The formulation of SLNs is shown in the Table 1,2.

Optimization[11]

The definition of optimizing is to make anything as excellent, functional, and effective as possible. During the creation of a new project, one usually experiments by following a series of methodical stages, carefully regulating each variable and modifying it one at a time until a good outcome is achieved. In the present

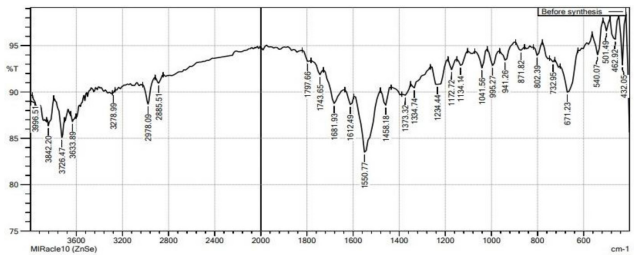

Figure 1: FT-IR Spectrum of Doxycycline Hyclate Pure Drug API.

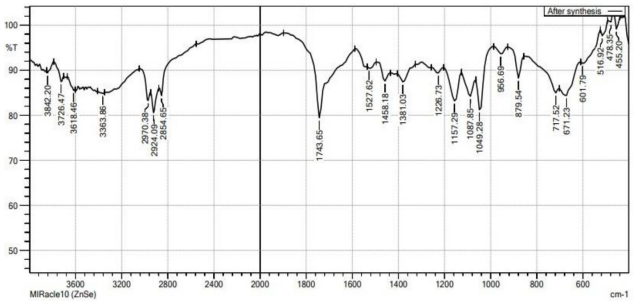

Figure 2: FT-IR Spectrum of Doxycycline SLNs.

Table 1: Ingredients of Doxycyline Composition SLNs.

Ingredients	Formulations					
	F1	F2	F3	F4	F5	F6
Doxycyline API	120mg	130mg	140mg	150mg	160mg	170mg
Cholesterol	300mg	302mg	304mg	306mg	308mg	310mg
Tween 80(W/W)	1%	1%	1%	1%	1%	1%
Ethanol/Acetone 3:1 volume ratio	18:6	18:6	18:6	18:6	18:6	18:6
Oilyphase(Glycerol Laurate)	1ml	1ml	1ml	1ml	1ml	1ml

Table 2: Formulations of Doxycyline SLNs.

Formulation	Drug/Polymer Concentration (ratio)	Sonication Time	Homogenization (rpm) rate
F-1	1:1	30 min	7000 rpm
F-2	1:2	45 min	8000 rpm
F-3	1:3	1 hr	9000 rpm
F-4	1:4	1.30 hr	10000 rpm
F- 5	1:5	2 hr	11000 rpm
F- 6	1:6	3 hr	12000 rpm

Table 3: Freeze Drying of Doxycyline Composition SLNs.

Sl. No	Cryoprotectant	Freezing Temperature	Cryoprotectant type
1	1%	-20	Mannitol
2	5%	-20	
3	10%	-20	
4	15%	-20	

study, the formulation F5 was chosen for freeze-drying process optimization research based on the findings of pre-optimization experiments.

Freeze Drying

Freeze drying is the removal of water or other solvents from a totally frozen sample under a vacuum, allowing the ice to transition straight from a solid to a vapour without going through a liquid phase. The particles were freeze-dried at -20°C under 0.4 bar in a freeze-dryer. As a cryoprotectant, different quantities of mannitol (5, 10, and 15%) were used. Freeze Drying of Doxycyline Composition SLNs are shown in the Table 3.

Particle Size[12]

Laboratory procedures are used to determine the particle size range and/or average or mean size of particles in a powder or liquid sample. The particle size measurement and analysis information, and the grinding circuit are to be optimized, thereby improving the quality of the beneficiation products, increasing the recovery rate and reducing energy consumption. The particle size distribution met the P80 criteria from 25 microns to 295 microns. The particle size of Freeze-dried Doxycycline is shown in the Table 4 and Figure 3.

Table 4: Particle size of Freeze dried Doxycycline Nanoparticles.

Sl. no	View field(µm)	Size(mm)
1	2 µm	3.66 mm
2	5 µm	6.58 mm
3	20 µm	9.96 mm
4	25 µm	14.97 mm
5	50 µm	17.36 mm
6	100 µm	25.48 mm

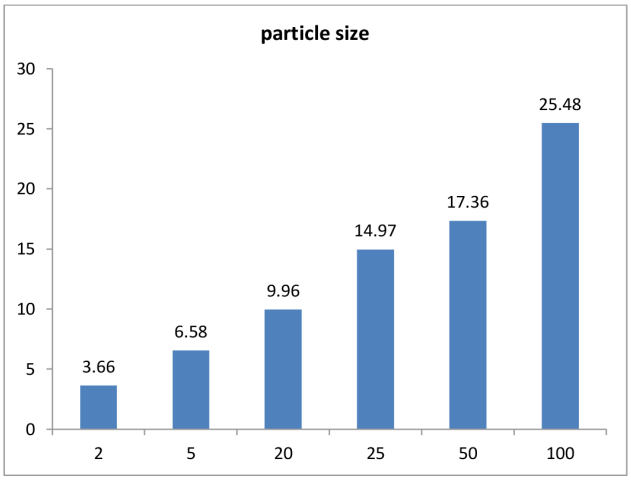

Figure 3: Particle size of Freeze-dried Doxycycline SLNs.

Drug Loading Efficiency[13]

Drug loading efficiency is influenced by both carrier and drug factors, such as molecular weight, drug solubility in the carrier, carrier volumetric size, and chemical interactions between the drug and the carrier. The materials were spun at 26,000 rpm for 45 min at 4°C using a Sigma Laboratories centrifuge to determine drug loading efficiency.

The drug concentration in the supernatant was measured with a spectrophotometer set to 208 nm, and the drug

Table 5: Time and Percentage of Drug Release.

Time (hr)	Percentage of drug release (%)	
	Before freeze-drying	After freeze-drying
4	18.30	35.28
8	24.15	54.16
12	32.16	62.28
16	54.57	75.61
20	67.59	82.52
24	78.24	98.11

loading efficiency was estimated using the equation below. The findings are presented as the average of three analyses with standard deviation.

$$\text{Drug} - \text{loading efficiency (LE\%)} = \frac{\text{Drug}_{\text{total}} - \text{Drug}_{\text{supernatant}}}{\text{Drug}_{\text{total}}} \times 100$$

Drug Release Studies[14]

Release study was done using the dialysis sack method by DO405 Dialysis tubing 23×15 mm. Five mL of the formulation was deposited in a dialysis membrane (10-12 KD) submerged in 50 mL phosphate buffer solution (pH 7.4) at room temperature (25±2°C) before and after freeze-drying.

Before being placed in the dialysis membrane, the freeze-dried particles were diluted with 5mL of deionized water. The free medication that was not placed on SLNs was removed using a Sigma Laboratories centrifuge at 26,000 rpm for 45 min at 4°C. The drug concentration was evaluated using UV spectrophotometry after one ml samples were taken at predefined intervals. The result is shown in the Table 5, Figure 4.

Morphology

The morphology of the nanoparticles was investigated. SEM images of doxycycline solid lipid nanoparticles freeze-dried using mannitol as a cryoprotectant before and after freezing. Before being analysed with an SEM, the nanoparticles were deposited on aluminium stubs and sputter-coated with a thin coating of Au/Pd. The result is shown in the Figure 5.

X-Ray Diffraction (XRD)[15]

The X-ray diffraction (XRD) analytical technique is based on the diffraction of X-rays by matter, particularly crystalline materials. X-ray diffraction is an elastic scattering (without loss of photon energy) that leads to increased interference when a more ordered material is investigated. For noncrystalline materials, the term "diffusion" is more appropriate.

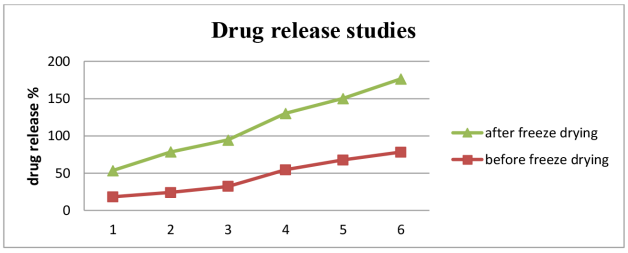

Figure 4: Release profile of Doxycycline solid lipid nanoparticles before and after Freeze-drying.

Sem Study

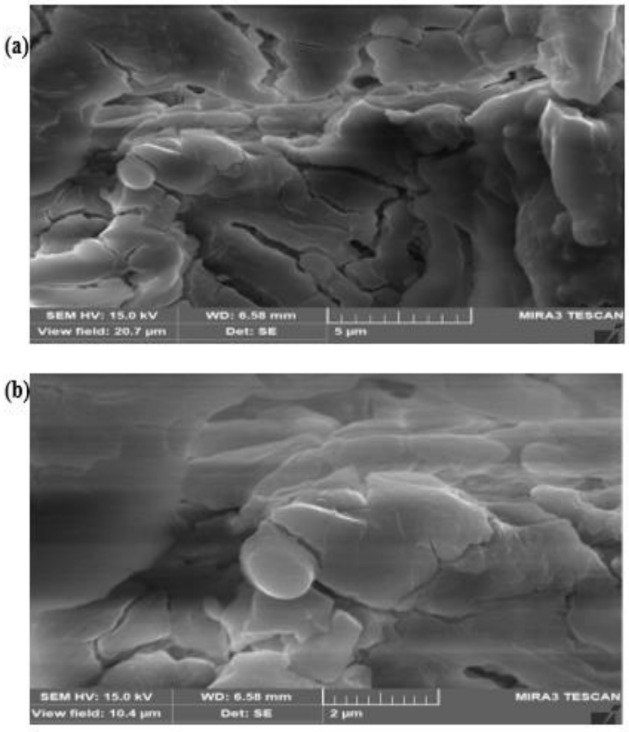

Figure 5: Scanning Electron Microscopy pictures of Doxycycline solid lipid nanoparticles after Freeze-drying (a), before freeze-drying in primary preparation (b).

The structural characteristics of nanocomposites can be determined via X-ray diffraction (size and crystallinity index of cellulose crystallite). Following the addition of nanoparticles, XRD is used to assess any changes in the matrix's crystal structure. Using an X-ray diffractometer (XRD) with a copper target and nickel filter, an X-ray diffractometer (XRD) was used to determine the crystallinity of the drug in nanoparticle formulation. On aluminium stages with glass bottoms, powders were flattened to a level surface. Each sample's XRD pattern was measured from 10 to 79 degrees. 2-theta with a 0.1 2-theta degree step increment and a 1 sec rest time between each step. The result is shown in the Table 6, Figure 6.

Table 6: Determined Value of X-Ray Powder Diffraction.

Pos. [°2Th.]	Height [cts]	FWHM left [°2Th.]	D- spacing [Å]	Rel. Int. [%]
10.5998	274.90	0.0502	8.34629	8.59
15.1976	270.62	0.0669	5. 83003	8.45
20. 1966	372.21	0.0836	4.39687	11.63
25.1384	278.45	0.0502	3.54260	8.70
30.6042	251.99	0.0502	2.92123	7.87
35.8062	99.37	0.4015	2.50786	3.10

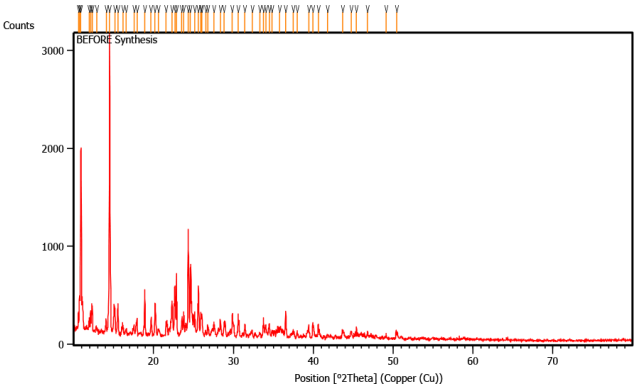

Figure 6: XRD Measurement from 10[°2Th] to 79[°2Th] at a 25°C of Freeze-Dried of Doxycycline SLNs.

Antimicroial Activities[16-17]

Preparation of the bacterial inoculum

The antibacterial activity of freeze-dried doxycycline SLNs was investigated using a good diffusion test. *E. coli* (ATCC 25922), Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 6633), and Pseudomonas aeruginosa were among the microbes used (ATCC 27853). Stock cultures were kept at 4°C on nutritional agar and potato dextrose agar slopes. To prepare active cultures for experiments, a loop full of cells from stock cultures was transferred to test tubes containing 50ml nutrient broth. Bacterial cultures were incubated at 37°C on a shaking incubator for 24 hr with agitation, while fungal cultures were kept at 27°C for 3-5 days.

The test organism suspensions were then brushed out on nutritional agar medium and potato dextrose agar. Bacterial cultures were then incubated at 37°C for 24 hr, whereas fungal cultures were kept at 27°C for 3-5 days. A single colony was transferred to nutritional agar medium slants and cultured for 24 hr and 3-5 days at 37°C and 27°C, respectively. These stock cultures were kept at a constant temperature of 4°C. For use in research, a loop of each test organism was placed in 50ml nutrient broth and incubated separately for bacterial culture at 37°C for 18-20 hr. The result is shown in the Table 7, Figure 7.

Table 7: Antimicrobial Activities of Freeze-Dried Doxycycline SLNs.

Sl. no	Name of the microorganism	Zone inhibition (cm) band	Zone inhibition (cm) standard
1	*E. coli*	1.6 cm	1.5 cm
2	*Staphylococcus aureus*	1.2 cm	0.8 cm
3	*Bacillus subtilis*	1.0 cm	1.3 cm
4	*Pseudomonas aeruginosa*	0.9 cm	1.2 cm

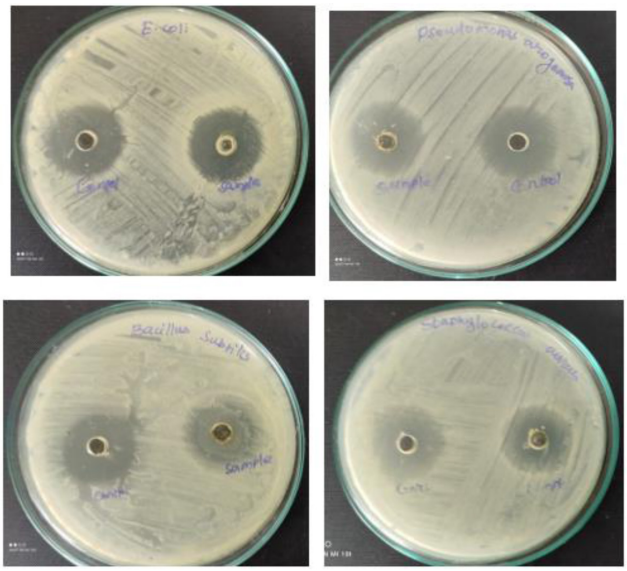

Figure 7: Photographs of the zone of inhibition produced by free Doxycycline and its SLNs in Freeze-dried SLNs against E. Coli, P. Aeruginosa, bacillus subtilis and staphylococcus aureus.

Disc Diffusion Method

The antibacterial activity of the Green Herbal Mask was tested using the Disc Diffusion method. 20ml of molten media was poured into sterilised petriplates to make MHA plates. A 20-25 l suspension of bacterial inoculums was uniformly swabbed after the media had hardened. After being soaked in the proper solvents, the sterile paper discs were put onto agar plates. The 1 cm broad test cloth was used to fill the plates. After that, the plates were incubated at 37°C for 24 hr. The assay was done in triplicates, and control plates were retained as well.

Agar diffusion was employed to assess antibacterial activity and the zone of inhibition was measured in millimetres from the well's border to the zone. By inoculating bacteria (*Staphylococcus*, *E. coli*) in nutritional broth media and growing them at 37% for 18 hr, the

stock culture of bacteria (*Staphylococcus*, *E. coli*) was obtained. The above media's agar plates were made. The bacteria were swabbed in the sterile plates after each plate was inoculated with 18-hr-old cultures.

The plates were filled with the 1cm wide test cloth. All of the plates were incubated at 37°C for 24 hr, and the inhibitory zone's diameter was measured in centimetres. The antibacterial activity and minimum inhibitory concentrations of plant extracts against Gram positive and Gram negative bacteria were determined using the agar well diffusion method. Doxycycline SLNs that had been freeze dried had antibacterial activity against the microorganisms that had been examined.

In-vitro Anticancer Activity[18-19]

Cell Line

The human cancer cell line was donated by the National Centre for Cell Science (NCCS) in Pune, and it was grown in Eagles Minimum Essential Medium with 10% foetal bovine serum (FBS). The cells were held at a temperature of 37°C, with 5% CO_2, 95% air, and 100% relative humidity. The maintenance cultures were passaged once a week and the culture medium was refreshed twice a week.

Cell Treatment Procedure

The monolayer cells were detached using trypsin-EDTA to create single-cell suspensions, and viable cells were counted with a hemocytometer before being diluted with a medium containing 5% FBS to achieve a final density of $1x10^5$cells/ml. 100 microlitres of cell suspension per well were seeded into 96-well plates at a plating density of 10,000 cells per well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air, and 100% relative humidity. After 24 hr, the cells were given multiple doses of freeze-dried doxycycline SLNs. After dissolving them in DMSO, an aliquot of freeze-dried doxycycline SLNs was diluted in a serum-free medium to twice the intended final maximum test concentration. A total of five freeze-dried SLN concentrations were achieved by doing four further serial dilutions. The required final sample concentrations were obtained by adding 100µl aliquots of each of these different sample dilutions to wells already containing 100µl of the medium. The plates were then incubated for a further 48 hr at 37°C, 5% CO_2, 95 percent air, and 100 percent relative humidity after the sample was added. As a control, a medium without samples was utilised, and all concentrations were done in triplicate. The result is shown in the Table 8,9.

Table 8: Different concentrations of Anticancer activity.

Conc	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	Cont
ABS	0.022	0.067	0.127	0.203	0.281	0.405
	0.025	0.069	0.129	0.205	0.283	0.402
	0.023	0.069	0.129	0.207	0.283	0.404
Avg	0.023333	0.068333	0.128333	0.205	0.282333	0.403667

Table 9: Concentrations and Cell inhibiton of Anticancer activity.

Conc (µg/ml)	% Cell inhibition
6.25	5.780347
12.5	16.92816
25	31.79191
50	50.78448
100	69.9422

MTT Assay

3-[4,5-dimethylthiazol-2-yl] The yellow tetrazolium salt 2,5-diphenyltetrazolium bromide (MTT) dissolves in water. Succinate-dehydrogenase, a mitochondrial enzyme found in living cells, breaks down the tetrazolium ring, converting MTT to an insoluble purple formazan. As a result, the number of viable cells determines the amount of formazan generated. 15µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well after 48 hr of incubation and incubated for 4 hr at 37°C. After that, the MTT medium was turned off, and the generated formazan crystals were dissolved in 100µl of DMSO before being quantified with a microplate reader at 570 nm.

In comparison to the control, the percentage cell viability was calculated as follows:

[A] Test / [A]control × 100 = Cell Viability Percentage

The formula below was used to compute the % cell inhibition.

Abs (freeze-dried doxycycline SLNs)/Abs (control) × 100 = percent Cell Inhibition

Using GraphPad Prism software, a nonlinear regression graph between percent Cell inhibition and Log concentration was created, and the IC_{50} was calculated. The result is shown in the Figure 8.

Thermogravimetric Analysis (TGA/DTA)[20]

While the temperature of a freeze-dried doxycycline SLNs is altered over time, a thermogravimetric analyser continually measures mass. In thermogravimetric analysis, mass, temperature, and time are considered

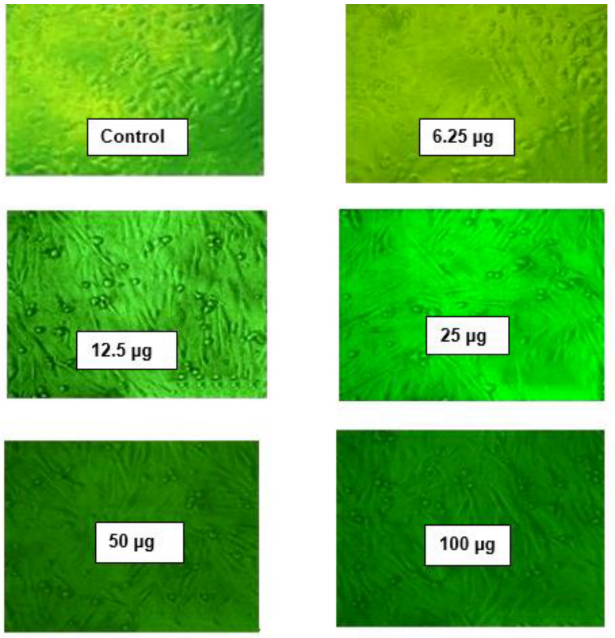

Figure 8: MTT assays Revealed that the Doxycycline SLNs Decreased the Percent Viability of all the Cells in Different Concentrations.

foundation measures from which a variety of additional data may be extracted. Thermogravimetric Analysis establishes a test sample's per cent weight loss when heated at a consistent rate in an appropriate environment, as well as oxidative stability, thermal stability, and the estimated lifespan of a product.

DTA is a technique for detecting and quantifying the chemical composition of substances by watching the thermal response of a 12mg sample while it is heated at 26°C to 900°C within 45min. Heat causes reactions and phase changes in a substance, including heat absorption and emission. In DTA, the temperature of the test sample is compared to the temperature of a nearby inert substance. The result is shown in the Table 10, Figure 9 and Figure 10.

RESULTS AND DISCUSSION

FT-IR Spectroscopy

The results of FT-IR study for doxycycline hyclate and doxycycline SLNs are shown in Figure 1, 2.

Drug Loading Efficacy

Drug loading effectiveness was found to be 78% in our study. The drug release profile was also calculated using the proportion of drug loading.

Antimicrobial Activities

After redispersion in water, lyophilized doxycycline SLNs showed antibacterial action against Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus (Figure 7).

Table 10: Thermogravimetric Analysis of Freeze-Dried Doxycycline SLNs.

Roll.no	Time min	Temperature °C	TGA/DTA (%)
1.	5min	84.80	99.33
2.	10min	186.80	97.90
3.	15min	296.06	90.65
4.	20min	403.34	88. 80
5.	25min	506.69	87.17
6.	30min	606.90	85. 56
7.	35min	705. 53	84. 35
8.	40min	803. 91	82.96
9.	45min	900. 89	80.93

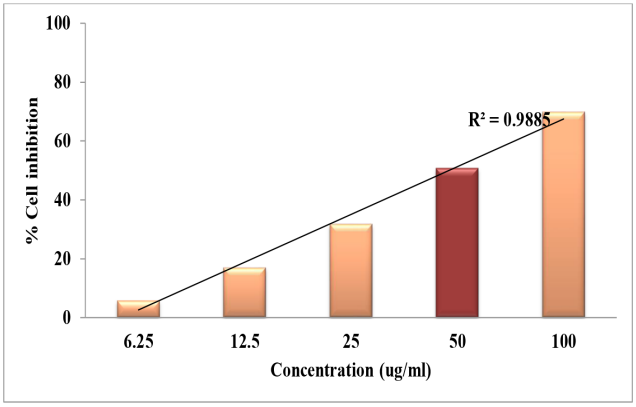

Figure 9: Concentration and Cell inhibition of Anticancer activity Freeze-dried Doxycycline SLNs.

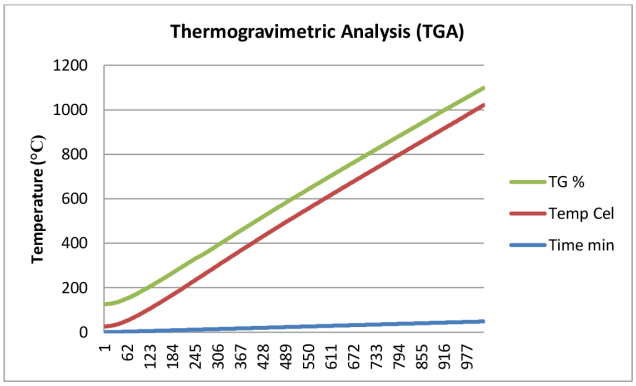

Figure 10: Thermogravimetric Analysing the Freeze-Dried of Doxycycline SLNs.

In-vitro Anticancer Activity

Anticancer Results: Liver cancer (HepG-2)

Figure 9 shows the anticancer activity curves of the optimized freeze-dried of doxycycline SLNs.

DISCUSSION[21-22]

FT-IR Spectroscopy

The results of FT-IR study for doxycycline hyclate and doxycycline SLNs show that there is no interaction between drug and excipients.

Particle Size

The size of solid lipid Nanoparticles is found to be 3.66, 6.58, 9.96, 4.97,17.36 and 25.48mm respectively at a different magnification of the analysis surface activation of nanoparticles enhanced its size by forming a layer on the doxycycline SLNs. This study revealed that lyophilizing doxycycline SLNs had no effect on the particle size of the produced particles. After lyophilization, the particle size of SLNs demonstrates that 10% mannitol as a cryoprotectant yields the smallest particles.

Drug Loading Efficacy

The Drug loading efficacy study showed that the drug was uniformly distributed in the prepared formulations of solid lipid nanoparticles.

Drug Release Studies

The doxycycline release profile from SLNs before and after lyophilization, which lasts around 24 hours and releases roughly 98% of the doxycycline in a sustained manner. No significant difference was observed between the two profiles. No burst effect was established for lyophilized SLNs and the reported burst effect after freeze-drying of doxycycline SLNs.

Sem Study

According to the results, The freeze-dried formulations morphologies in comparison to nonlyophilized particles. After freeze-drying, there was no substantial growth of particle size.

X-Ray Diffraction

The XRD study confirmed the conversion of amorphous form of the drug into crystalline form.

Antimicrobial Activities

As a result, the antibacterial activity of SLNs as doxycycline carriers was unaffected by the lyophilization procedure.

In-vitro Anticancer Activity

The freeze-dried doxycycline SLNs decreased the % viability of all the cells, but at varying concentrations, according to MTT experiments. The cytotoxicity of doxycycline SLNs was observed to be higher in cancer cell lines. At a concentration of 100g/mL of leaf crude extract, the highest inhibition of cell growth (69.9422) was recorded, with an IC_{50} value of 48.69g/mL. These findings demonstrated that the extracts caused morphological alterations and cell shrinkage in prostate cancer cell lines, leading to cell death. Plant extracts' IC_{50} values against the cancer cell line. As a result, the optimised SLNs created in this study could be an effective vehicle for delivering doxycycline to the target region without causing any side effects. The therapeutic content of these lipidic nanoparticles is mostly released inside cancer cells, leading to improved efficacy and fewer adverse effects.

Thermogravimetric Analysis (TGA/DTA)

While the temperature of the sample is regulated in a specific atmosphere, a group of procedures in the freeze-drying of doxycycline SLNs is monitored against time or temperature. To evaluate a material's thermal stability and proportion of volatile components, track the weight change that happens when a sample is heated at a constant pace.

CONCLUSION

Our goal was to study the lyophilized doxycycline SLNs and optimise the lyophilisation process with cryoprotactant so that their physiochemical, antibacterial, and anticancer characteristics were not compromised. The best condition for avoiding particle aggregation was achieved by freezing SLNs doxycycline dispersion at -20°C and adding 10% mannitol. The release profile, SEM of lyophilized SLNs, TGA, antibacterial, and anticancer efficacy were all investigated, and the results revealed that there was no burst effect on the doxycycline release profile. This might be concluded that freeze-drying doxycycline SLNs increase stability and reduces side effects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DMSO: Dimethyl Sulfoxide; **DOX-SLN:** Doxycycline Solid Lipid Nanoparticles; **FTIR:** Fourier Transform

Infrared; **LE:** Loading Efficency; **MMT:** Manual Muscle Testing.

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