“DEVELOPMENT AND CHARACTERIZATION OF FLUCONAZOLE SOLID LIPID NANOPARTICLES”

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ABSTRACT

Solid Lipid Nanoparticles are first-generation colloidal carriers based on lipids, with their sizes ranging from 40-1000 nm. They are composed of biocompatible lipids, which are dispersed in an aqueous medium containing surfactant to form a lipid core. The active pharmaceutical ingredient is dispersed uniformly in the lipid matrix of the formulation. Water, along with lipids, is one of the major constituents in the preparation of solid lipid nanoparticles. Various active ingredients have adverse effects when taken by oral route and hence can be formulated as alternative drug delivery methods for the reduction of side effects. The objective of the present study was to develop and characterize solid lipid nanoparticles suitable for incorporating an anti-fungal drug, Fluconazole in solid lipid nanoparticles for formulation stability and reducing adverse effects associated with the oral administration of the drug.

Fluconazole (FLZ) was selected as a model drug for preparing solid lipid nanoparticles consisting of Stearic acid and Precirol ATO 5 as the lipids, Tween 80 as the surfactant, and Polyvinyl alcohol as the stabilizer. Hot homogenization and probe sonication techniques were used for the development of the fluconazole solid lipid nanoparticle formulation. Developed SLNs were characterized for the following: Particle size, polydispersity index, zeta potential, entrapment efficiency, and in-vitro drug release. The in-vitro release of the best formulation also showed promising results with 92.13% release in 24 hrs., with initial burst release followed by a sustained release. The particle size, PDI, and zeta potential of the prepared formulations were found to be in the range of 336.4 to 401.5 nm, 0.216 to 0.312, and -21.8 to -28.9 mV respectively.

DEDICATION

I would like to dedicate this thesis to my parents,

Narendra Kalaria, Sonal Kalaria for their support and sacrifices, and my brother

Nisar Kalaria without whom I could not have been at this place.

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LIST OF ABBREVIATIONS

FLZ – Fluconazole

SLN – Solid Lipid Nanoparticle

PDI – Polydispersity Index

PVA – Polyvinyl Alcohol

API – Active Pharmaceutical Ingredient

FDA – Food and Drug Administration

RPM – Rotations per minute

USP – United States Pharmacopoeia

SA – Stearic Acid

CM – Compritol 888 ATO

PC – Precirol ATO 5

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CHAPTER I: INTRODUCTION

Nanosystems

Nanoparticulate carrier technology is a significant part of the current revolutionary drug delivery methods. This technology is extensively used to deliver various agents ranging from small molecules to mRNA-based medications. There are numerous applications for delivery systems in this era including radiation, AIDS, cancer therapy, and gene therapy [3]. They exist in a size range between 100-500 nm, and the effectiveness of the particles is majorly dependent on their particle size [2]. The advantages of nanoparticles as drug vehicles are because of two main reasons: their tiny size and the use of biodegradable materials [3]. The small size of SLNs provides enhanced drug loading, improved drug release, increased bioavailability and increased stability over other conventional formulations. Various types of nanotechnological carriers are available in the market for drug delivery such as carbon nanotubes, nanoshells, liposomes, niosomes, polymeric micelles, polymeric nanoparticles, nanocapsules, solid lipid nanoparticles, nanoemulsions, nanosuspensions, nanostructured lipid carriers [3]. Polymers were one of the main components that were being used for the development of the SLNs. Although considering polymeric nanoparticles have many advantages, for example, versatile drug loading, and controlled drug release. They have the major disadvantage of using organic solvents like acetonitrile and tetrahydrofuran than can be hazardous to the environment and physiological systems. Drug leaking is also one of the major drawbacks of polymeric nanoparticles.

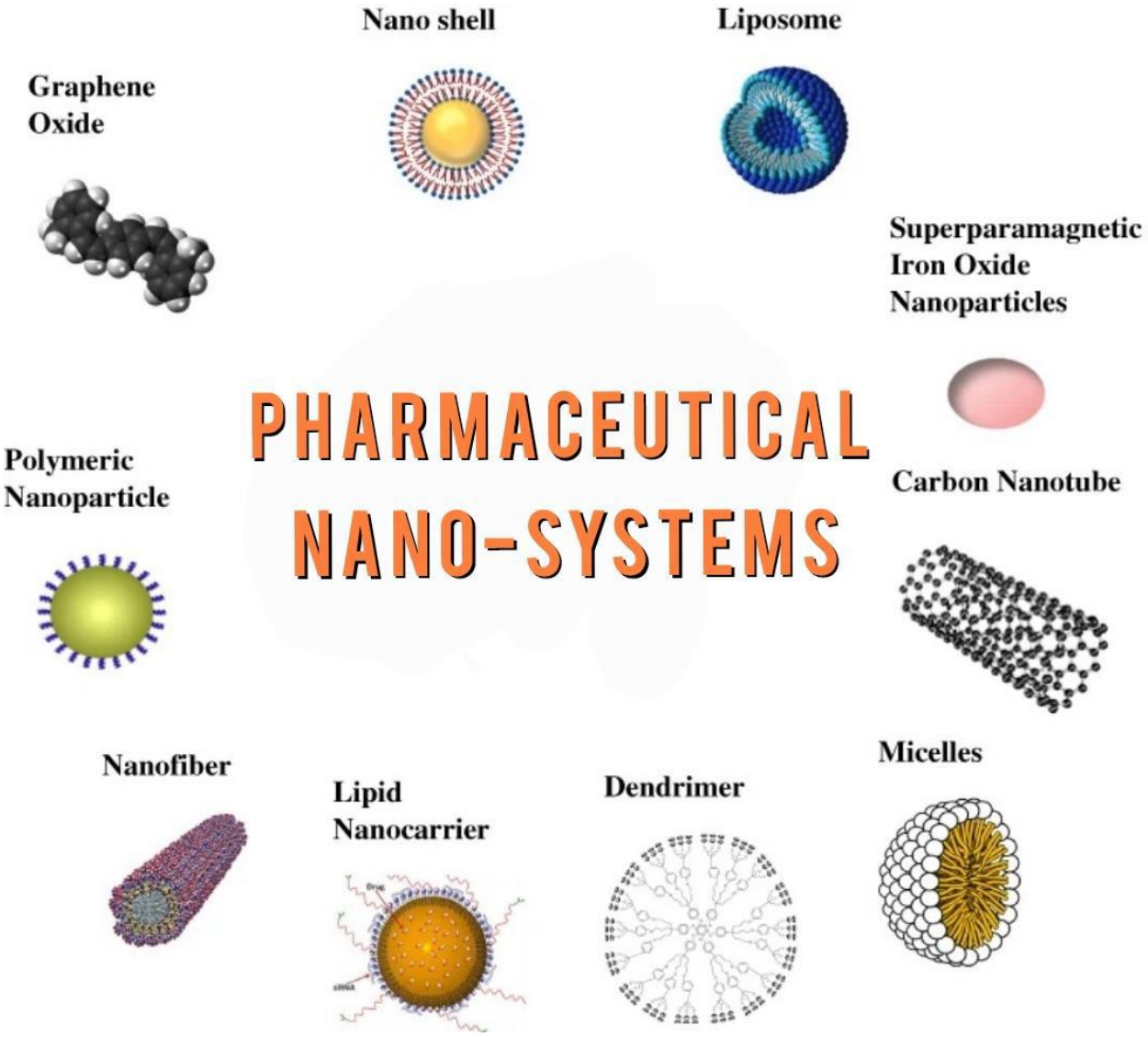

Figure 1 : Different types of Pharmaceutical Nano-system

Nanoshell

With a silica core and a metal outer layer, nanoshells are modified drug targeting models. These days, much consideration was acquired by these nanoshells. By altering the ratio of the core to the shell, these particles' characteristics can be altered. It is currently practical to plan these nanostructures in designated actual properties, similar to measure and morphology. Nanoshells are used to create new systems with a variety of morphologies because all of the materials cannot be formulated in the desired morphologies. In order to achieve the right morphology, particles with particular shapes could have a thin shell covering them. These shells enjoy the benefit of being cheap since valuable materials can be added to minimal expense centers. Consequently, nanoshell synthesis requires less precious material in smaller quantities. Immunological methods can be used to target nanoshells; Gold nanoshells, which were coated with antibodies on their outer gold surface to enhance their ability to target the cancer cell, serve as an illustration of this targeting strategy. The chemical stabilization of colloids, enhancement of luminescence characteristics, and drug improvement are just a few of the many applications for nanoshells [3].

Polymeric nanoparticles

Researchers are interested in biodegradable polymeric nanoparticles (PNPs) as a medication delivery strategy since they are typically biodegradable and biocompatible. PNPs are further separated into matrix systems (nanospheres) and vesicular systems (nanocapsules). Researchers recently investigated advanced modifications of natural polymers, including synthetic polyesters. Chitosan is one of the most well-known natural polymers. With artificial polymers, several polymers lessen hazardous problems. Natural PNPs won out over conventional distribution methods because of their greater efficacy and efficiency. They do, however, have significant shortcomings, such as low repeatability, issues with degradation, and possible antigenicity. The

manufacturing process regulates the drug's release behavior when it is encapsulated. Potential intracellular and site-targeting systems are known as PNPs [3].

Nanofibres

Nanofibers are very thin continuous fibers with widths ranging from nanometers to a few micrometers. They may be created using a number of techniques, including electrospinning, thermal-induced phase separation, drawing, self-assembly, and template approaches. These nanofibers are endowed with characteristics including big, high surface-area-to-volume ratio porous networks, high porosity, and variable pore size options. In order to boost the drug's solubility in water and improve therapeutic effectiveness, nanofibers can facilitate a larger surface-area-to-volume ratio.

Dendrimer

A unique class of polymers known as dendrimers are multi-branched and have variable size and form. These dendrimers' degree of branching, which may be controlled, determines their size. Additionally, dendrimer spherical branching produces gaps that can be used for drug delivery and trapping. Dendrimers, on the other hand, may be modified to allow for conjugation to other molecules. These nanostructures provide distinct drug delivery opportunities due to their enhanced surface functionalization and stability. Core, branches, and surface are the three primary fundamental building blocks. These networks aid in the distribution of bioactives like as drugs, DNA, and vaccines to certain tissues [3].

Micelles

A form of micelle known as polymeric micelles is composed of block copolymerized units of lipophilic and lipophobic monomers. They consist of a core of lipophilic polymeric chains stabilizing a center of lipophilic blocks. Utilizing PEG blocks that create corona, a lipophilic

center-forming block's length is comparable to a hydrophilic block's. A micellar system provides a number of benefits over traditional systems as a drug carrier. A medication that is only mildly water-soluble is made more soluble by the use of micelle-forming surfactants. They also make drugs more permeable to physiological barriers, increasing their bioavailability. Changes in medication biodistribution arise as a result. They lessen the adverse effects of important drugs. Due to their small size and lipophilic shell, polymeric micelles are less readily absorbed by the reticulo-endothelial system and last longer in the circulation following intravenous administration. Micelles can also be chemically modified to chemically bind a targeting component to their surface, making them target selective. The drug is adequately protected from any degradation brought on by biological environments since it is in a micellar form. It will make its way to the target organ or tissue since it is in a micellar form [3].

Carbon nanotube

These are carbon-based tubular constructions. These tubes have a length range of 1 to 100 nm and are composed of graphite sheet cylinders that are sealed at one or both ends by bucky balls. There are two designs that have recently grown in popularity: multiwalled nanotubes (MWNTs) and single-walled nanotubes (SWNTs). Common combinations also contain C60-fullerenes. They are noted for being hollow and cage-like and come in a range of graphite cylinder forms (nanotubes and fullerenes). Because of their size and surface characteristics, as well as important physical characteristics, they are appropriate for drug encapsulation. The diameter of the DNA helix is one-half that of SWNTs. Contrarily, depending on how many walls they have in their construction, MWNTs have widths that range from a few nanometers to tens of nanometers. In order for these structures to be used as reliable drug transporters, their strength and stability are used to describe them. Nanotubes enter cells through endocytosis or insertion across the cell membrane. The

architectures of fullerenes were able to target mitochondria both intracellularly and in tissues. They also exhibit antioxidant and antibacterial properties [3].

Superparamagnetic iron oxide nanoparticles

Small, magnetic nanoparticles may be manipulated by a magnetic field and have a diameter of less than 100 nm. These particle materials are created using magnetic components. These nanoparticles are categorized based on their sensitivity to magnetic fields. Paramagnetic nanoparticles have a greater magnetic susceptibility than conventional contrast forms. These nanoparticles are used in therapy and diagnosis plans. Targeting of magnetic nanoparticles is useful for identifying certain organs [3].

Liposomes

Amphiphilic phospholipids are used to create synthetic liposomes, which self-assemble. The size of the aqueous core domain can vary from 50 nm to several micrometers, and they are composed of spherical, double-layered vesicles that surround it. Biological properties of liposomes that are attractive include their general biocompatibility and biodegradability. The most often used nanosystems as drug delivery systems in clinical studies are liposomes. They can be used to lessen systemic effects and toxicity as well as drug clearance. Nanoscale modified liposomes offer good pharmacokinetic properties for the delivery of DNA, siRNA, proteins, and cancer therapies. The drawbacks of liposomes are their low loading capacity, quick drug release, and lack of programmable drug release patterns. Because liposomes cannot enter cells, drugs are also released into the extracellular fluid [3].

Solid Lipid Nanoparticles

Solid Lipid Nanoparticles (SLNs) are a new generation of sub-micron-sized lipid emulsions where solid lipids and aqueous media exist together as a system in the presence of stabilizers and

surfactants [25]. The solid lipids form a lipid matrix in which the active pharmaceutical agent can be encapsulated, which is stabilized by biocompatible surfactants such as poloxamers. They are ideal for optimizing drug delivery and reducing toxicity related to drugs by providing a sustained release. SLNs have various advantages over conventional drug delivery mediums and other systems like low toxicity, biocompatibility because of the use of biodegradable lipids, protection of the drug from harsh environments, avoiding organic solvents in the manufacture, controlled release of the drug via the lipid matrix, better drug stability in the nanometer size range, and efficient large-scale production [25]. They have the ability to overcome the solubility and bioavailability of drugs that have poor solubility by increasing the drug surface area for solubility Surfactants used may contribute to increasing permeability [28]. They are in a solid state at physiological temperatures and room temperatures. They have been proven to be better than other nanoparticles like liposomes which has disadvantages like complex manufacturing, lower entrapment efficiency, lower physical stability and problematic large-scale production [26]. The ultimate goals of nanoparticle development are to control particle size, surface properties, and API release to the target site [3].

Fungal Infections

Globally, the prevalence of superficial fungal infections has increased; almost 40.0 million people, both in developed and developing nations, are affected by this infection. As fungus infections worsen, the immune system may also suffer [15].

Fluconazole

The triazole family, one of the most popular classes of antifungal drugs, includes fluconazole. A fluorine-substituted bis-triazole antifungal agent is called fluconazole. It is a medication that the FDA has approved for the treatment of systemic Candida infections, such as candidemia,

disseminated candidiasis, pneumonia, and cryptococcal meningitis, as well as vaginal, oropharyngeal, and esophageal candidiasis, urinary tract infections, peritonitis, etc. In patients having bone marrow transplantation who concurrently receive cytotoxic chemotherapy or radiation therapy, prophylaxis is known to reduce the incidence of candidiasis [4].

For the treatment of blastomycosis, histoplasmosis, and coccidioidomycosis, fluconazole is not FDA-approved. Fluconazole administration has increased recently to treat conditions such meningitis, pneumonia in immunocompromised patients, and pneumonia as the predominant infection in people with HIV or who are severely disabled [4].

Fluconazole is a slightly water-soluble drug. It has a log P of 0.4 which indicates that it is a lipophilic drug [27]. It has a bioavailability of about 90% when administered via oral and parenteral routes, but FLZ has oral adverse effects such as nausea, vomiting, diarrhea, abdominal pain, and gastrointestinal disturbance. It may also cause hepatotoxicity in some patients [17]. The effectiveness of antifungal therapy using nanoparticles has been researched, and the results clearly demonstrate advantages in drug properties such as solubility and stability in water, enhanced bioavailability, and tissue penetration, which lead to increased efficacy and decreased toxicity. Additionally, compared to conventional antifungals, drug-loaded nanoparticles can enhance the fungal inhibitory profile even at lower doses [29]. SLNs can be used to reduce adverse effects associated with anti-fungals[29]. The objective of the study was to develop fluconazole solid lipid nanoparticles as dry powder as an alternative oral delivery system with sustained release to reduce the adverse effects associated with the drug and the possibility of achieving therapeutic effects at lower doses.

Structure of Fluconazole

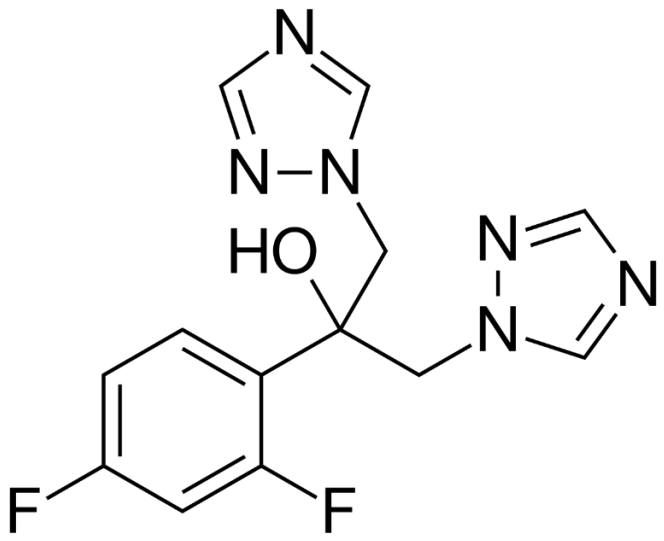

Figure 2: Structure of Fluconazole

Mechanism of action of Fluconazole:

Fluconazole has a broad spectrum anti-fungal activity. It impairs ergosterol synthesis by inhibiting fungal cytochrome P-450 mediated enzyme, 14-demethylase, responsible for catalyzing the conversion of lanosterol to ergosterol. As ergosterol forms a critical part of the fungal cell membrane and provides stability to the membrane, inhibition of the synthesis of ergosterol causes the fungal cell membrane to disrupt and hence increases cellular permeability.

CHAPTER 2: MATERIALS AND METHODS

2.1. Materials

Fluconazole was purchased from Spectrum chemical corporation (Gardena, CA, USA). Compritol 888 ATO and Precirol ATO 5 were obtained from Gattefosse SAS (France). Kolliwax S (Stearic Acid), Kolliphor P407, and Polyvinyl alcohol were procured from BASF. Tween 80, Tween 20, microcentrifuge tubes, and Millipore Sigma amicon Ultra Centrifugal Filter Units were obtained from Fisher Scientific. All the solvents and water used were analytical grade.

2.2. Methods

2.2.1 Standard Calibration Curve of Fluconazole

50 mg of fluconazole was dissolved in phosphate buffer pH 6.8 in a 100 ml volumetric flask to prepare a stock solution of 500 μg/ml. Various dilutions of 80-400 μg/ml were prepared. Absorbance was measured at 260 nm in a UV spectrophotometer (Thermofischer scientific). The standard calibration curve of Fluconazole was obtained by plotting Absorbance vs concentration graph.

2.2.2. Screening of solid lipids

Three solid lipids, stearic acid, Precirol ATO 5 (PC), and Compritol 888 ATO (CM) were studied to estimate the maximum amount of fluconazole that they can dissolve in them to find the drug loading capacity of the lipids. A fixed amount of solid lipid (200 mg) was added into a 20 ml glass

scintillation vial. The heat was applied via a water bath for uniform heating at 10^{o}C greater than the melting temperature of the chosen lipid. FLZ was then added with gradual increments of 1 mg to the molten lipid until FLZ was completely dissolved. The maximum solubility was determined by checking the solubility of FLZ in melted lipids by visual observation with the naked eye under normal lights. A mix of lipids, CM and PC in the ratio 1:1, SA and PC in the ratio 1:1 and 1.5:1 was also tested for maximum drug loading.

2.2.3. Screening of surfactants

Emulsifying agents (Tween 80, tween 20, and Kolliphor P407) were studied for their suitability for the formulation. Placebo formulations were made using solid lipids, PVA as the stabilizer for the SLN formulation, and different surfactants using a hot homogenization technique followed by ultra-sonication. Different concentrations of surfactants were studied to get the best results. The amount of lipids and PVA used, and process parameters were kept constant. The particle size, zeta potential, and polydispersity index (PDI) of the placebo formulations were recorded, and based on the results, the one that gave the best results was selected for the development of SLNs with the API.

2.2.4. Optimization of homogenization time

To investigate the effect of homogenization time on the formulation, placebos were made keeping all the ingredients the same except for the homogenization time. The formulations were homogenized for 5,10 and 15 minutes to check their effect on the reduction of particle size. It was then characterized for particle size, zeta potential, and PDI.

2.2.5. Optimization of sonication time

Similar to the above process, placebos were made keeping all the excipients the same and in the same quantities. The sonication amplitude was kept constant at 40% and with pulses of 10 seconds.

The placebo was sonicated for 10, 15, 20, and 25 minutes. It was then characterized for particle size, zeta potential, and PDI.

2.2.6. Preparation of Fluconazole Solid Lipid Nanoparticles (FLZ-SLNs)

FLZ-loaded SLNs were prepared by using hot homogenization and ultra-sonication/ probe sonication technique. All the ingredients were weighed accurately. The aqueous and lipid phases were prepared differently in 20 ml glass vials. A water bath was used to regulate temperature to ensure uniform heating and prevention of charring of lipids on direct heat. The water bath containing the temperature probe was turned on and maintained at 10 degrees Celsius above the melting point of the lipids. The lipid phase was taken in a glass vial and placed in the water bath to melt the lipid. Simultaneously aqueous phase containing 0.5% w/w PVA, the choice of surfactant, and milli Q water quantity sufficient to 10 ml was taken in a separate glass vial and placed in the water bath. The temperatures of both lipid and aqueous phases were maintained equally. FLZ was added to the lipid phase with constant stirring via a magnetic stirrer on complete melting. After the complete incorporation of the drug in the molten lipid, the aqueous phase which was maintained at the same temperature as the lipid phase was added dropwise to the lipid phase with constant stirring using a magnetic stirrer. The RPM of the magnetic stirrer was gradually increased as the aqueous phase was added to the lipid medium to ensure a uniform emulsion formation. After complete mixing of the lipid and aqueous phases, the lid of the vial was closed, the RPM was set at 2000 and the prepared mixture was allowed to emulsify for five minutes. Post emulsification, the mixture was homogenized using T25 ultra Turrax digital homogenizer at 14,000 RPM for five minutes at 10°C lower than the temperature set for developing SLNs. This was done to maintain the temperature as the temperature of the system increases while rigorous agitation during homogenization After homogenization, the mixture was allowed to cool down for

10 minutes before placing it in the probe sonicator for 20 minutes. The pulses were maintained at 10 seconds. The prepared SLNs were allowed to sit for 24 hours before their characterization.

2.2.7. Particle size, polydispersity index, and zeta potential

The particle size analysis of fluconazole solid lipid nanoparticles formulation was performed using a zeta sizer which provides mean particle size referred to as the Z-average of the nanoformulation as well as the polydispersity index which measures the width of the distribution. For particle size analysis, 990 microliters of Milli Q water were taken in a cuvette and 10 microliters of the prepared SLN solution were added. The mixture was shaken to distribute the product with the water uniformly. The cuvette was then placed in the zeta sizer and particle size along with the polydispersity index was measured.

The zeta potential is an important surface characterization technique employed for determining the potential stability and surface charge. Large negative or positive values of zeta potential is crucial for SLNs dispersion as it prevents aggregation of particles by electrostatic repulsion.

For zeta potential measurement, the same dilution was made with the prepared formulation and water, and it was placed in a zeta cell which was then placed in the zeta sizer to get the zeta potential of the system.

2.2.8. Entrapment efficiency of FLZ-SLNs

For the presence of the free drug in the fluconazole solid lipid nanoparticle formulation, 400 microliters of the formulation were taken in Amicon ultra centrifugal filter unit which was then fitted over a microcentrifuge tube, and it was centrifuged at 14,000 RPM for 90 minutes at 4 degrees Celsius. The filtrate was measured at 260 nanometers by using a UV spectrophotometer after dilution with phosphate buffer pH 6.8. The entrapment efficiency of FLZ-SLNs was calculated using the formula.

$$EE\ \% \ = \ \left[\left(W_{\text{initial drug}} - \ W_{\text{free drug}}\right) / W_{\text{initial drug}}\right] \ \times \ 100$$

Where the $W_{\text{initial drug}}$ is the mass of the initial drug used for the assay and the '$W_{\text{free drug}}$' is the mass of free drug detected in the filtrate after centrifugation.

2.2.9. In-vitro release study

An in-vitro release study of FLZ-SLNs was performed in phosphate buffer pH 6.8. The study was carried out in 900 ml dissolution media using United States Pharmacopoeia (USP) Dissolution apparatus II by adding SLNs containing 20 mg of FLZ in the media. A stirring speed of 75 rpm and temperature of 37±0.5 ºC was maintained throughout the process. Sink conditions were maintained by collecting 2 ml samples and replacing them with the same volume of freshly prepared media at pre-decided time intervals (0,0.5,1,2,4,8,12,24 h). The collected samples were passed through 0.45 μm syringe filters and then centrifuged at 14,000 rpm for 10 minutes. The supernatant was then analyzed in UV spectrophotometer at 260 nm.

2.2.10. Stability studies

Sometimes during the storage of lipid nanoparticles, problems such as particle agglomeration and drug leaking from nanoparticles are observed [30]. To ensure that the prepared SLNs were stable, a stability study on the optimized formulation was performed to measure any change in the particle size, PDI, entrapment efficiency, and zeta potential on the storage of the formulation. A 20 ml scintillation glass vial containing FLZ-SLNs was placed at room temperature for 30 days. The particle size, PDI, zeta potential, and entrapment efficiency of the formulation were measured just after formulation and after 30 days of storage.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Standard calibration curve of Fluconazole

The plotted graph in Figure 4 shows the standard calibration curve of FLZ. A correlation coefficient of 0.997 was obtained from the curve. The equation thus obtained was used to determine concentrations in unknown samples in further studies.

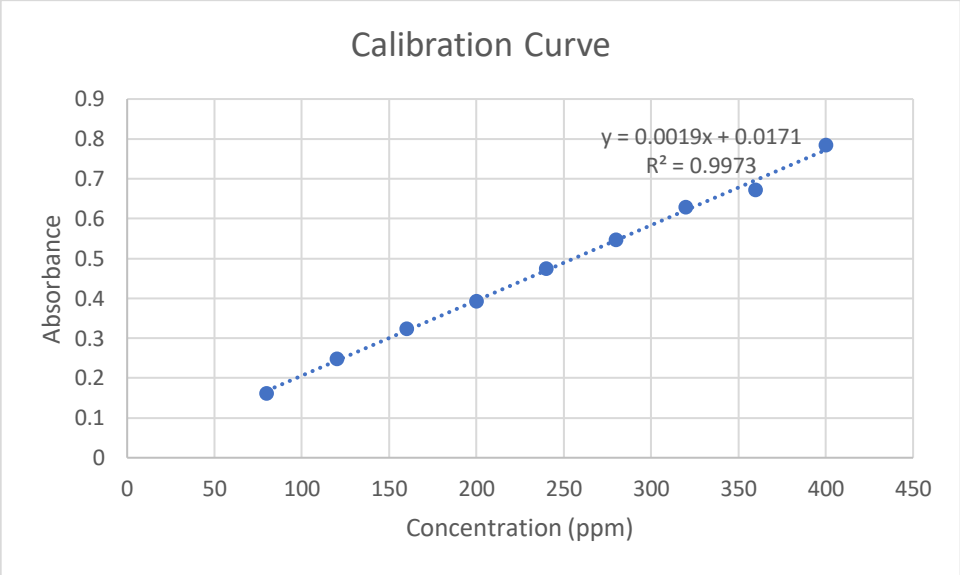

Figure 3: Calibration Curve of Fluconazole

3.2 Screening of Solid Lipids

Solubility of FLZ was observed in stearic acid, Precirol ATO 5, and Compritol 888 ATO

individually. Solubility was also measured using a mix of two lipids to ensure the highest solubility. The highest solubility of FLZ was found in a mix of stearic acid and Precirol ATO 5 in a ratio of 1:1. The lipids were also selected based on their non-irritancy and pharmaceutical acceptance. Based on the above results, stearic acid and SA and Precirol ATO 5 in the ratio 1:1 was selected for the formulation of FLZ-SLNs as they showed the maximum drug solubility.

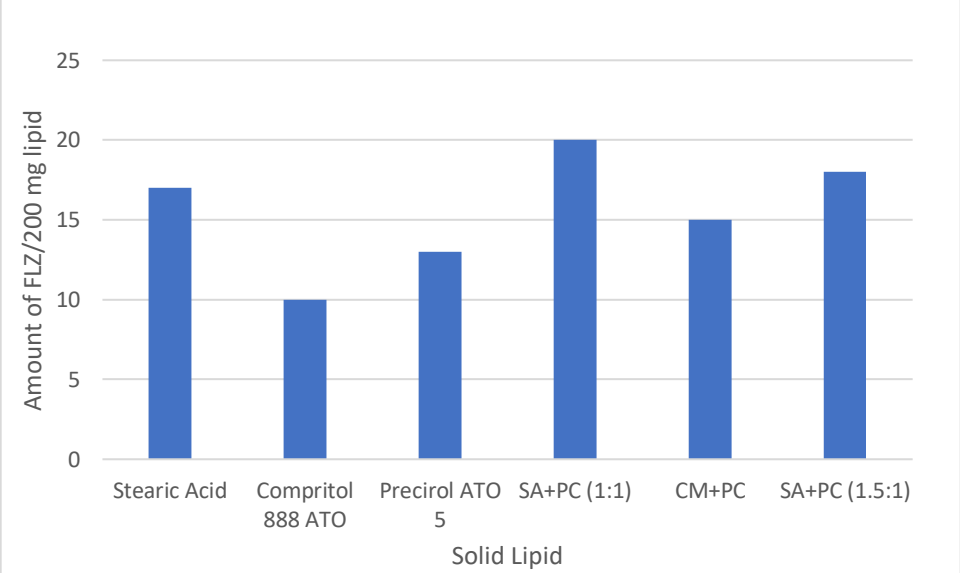

Figure 4: Solubility of FLZ in different lipids

3.3 Screening of surfactants

In this development of FLZ-SLNs, three surfactants were studied: Kolliphor P407, tween 80, and tween 20. Surfactant mixes were also studied to check for better results. Surfactant screening was carried out to check its impact on particle size, PDI, and zeta potential. The surfactant that gave the best results i.e., lowest particle size, lowest PDI value, and high value of zeta potential was preferred for the development of FLZ-SLNs. With the increase in surfactant concentration, it was found that particle size was reduced, and a narrow distribution was observed. Tween 80 was found to give the best results keeping all the other parameters of the process the same.

Surfactant and its concentration	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
1% Tween 20	558.2	0.415	-17.6
2% Tween 20	531.5	0.365	-21.4
3% Tween 20	502.6	0.385	-24.5
2% Tween 80	431.6	0.347	-19.2
3% Tween 80	405.6	0.236	-22.6
4% Tween 80	365.2	0.234	-25.4
5% Tween 80	340.2	0.212	-27.4
1% Kolliphor P407	648.6	0.512	-12.8
2% Kolliphor P407	540.5	0.398	-14.4
3% Kolliphor P407	531.7	0.375	-11.2

Table 1: Effect of surfactants and their concentrations on the formulation

3.4 Optimization of homogenization time

The homogenization time didn't prove to have much effect on the formulation's particle size. There was no significant difference in the particle size of the SLNs made with varying homogenization times. It reduced the formulation's PDI, indicating it helps make the system uniform rather than reducing the particle size. The homogenization time was thus set at 5 minutes for each formulation.

Homogenization time (min)	Amount of Lipid (mg)	Concentration of surfactant (%w/w)	Concentration of PVA (%w/w)	PDI	Particle Size (nm)	Zeta Potential (mV)
5	400	5	0.5	0.541	423.4	-19.6
10	400	5	0.5	0.406	421.8	-20.4
15	400	5	0.5	0.312	420.6	-18.9

Table 2: Effect of homogenization time on SLNs

3.5 Optimization of sonication time

Sonication is the main process through which the reduction of particle size takes place. It was observed that particle size was reduced when sonication time was increased from 10 minutes to 20 minutes but after that when the formulation was sonicated for more time, for example, 25 minutes, it didn't seem to have a significant effect on the particle size or other parameters. Thus, the sonication time was set at 20 minutes with 10-second pulses.

Homogenization time (min)	Amount of Lipid (mg)	Concentration of surfactant (%w/w)	Concentration of PVA (%w/w)	PDI	Particle Size (nm)	Zeta Potential (mV)
10	400	5	0.5	0.312	419.2	-21.6
15	400	5	0.5	0.298	402.5	-19.4
20	400	5	0.5	0.245	395.6	-23.2
25	400	5	0.5	0.306	393.2	-23.4

Table 3: Effect of sonication time on SLNs

3.6 Preparation of Fluconazole Solid Lipid Nanoparticles (SLNs)

Three formulations F1, F2, and F3 were prepared with stearic acid, the combination of stearic acid, and Precirol ATO 5 in a ratio of 1:1 and ratio of 1.5:1. Total lipid was kept at 400 mg in each formulation. The amount of PVA and Tween 80 was kept constant at 0.5% w/w and 5% w/w respectively for each formulation. Each formulation was hot homogenized for 5 minutes followed by probe sonication for 20 minutes. The best characteristics were found to be with the formulation containing SA: PC in the ratio 1:1.

Formulation	Amount of FLZ (mg)	Amount of stearic acid (mg)	Amount of Precirol ATO 5 (mg)	Amount of PVA (%w/w)	Amount of Tween 80 (%w/w)
F1	34	400	0	0.5	5
F2	40	200	200	0.5	5
F3	36	240	160	0.5	5

Table 4: FLZ-SLN formulation table

3.7 Particle size, PDI, and zeta potential

The particle size, PDI, and zeta potential of the prepared formulations were found to be in the range of 336.4 to 401.5 nm, 0.216 to 0.312, and -21.8 to -28.9 mV respectively. The particle size thus obtained was found to be in the optimal range for SLNs. The smallest particle size was observed with the system containing equal amounts of stearic acid and Precirol ATO 5.

The PDI value of all the formulations was found to be less than 0.5 which indicates a homogenous/narrow size distribution of prepared FLZ-SLNs. It reflects the suitability of the method of preparation used.

The zeta potential of each formulation was found to be in negative and was also found to be satisfactory for a stable nanoparticle formulation.

Formulation	Amount of Lipid (mg)	Particle size (nm)	PDI	Zeta potential (mV)
F1	400	401.5	0.312	-21.8
F2	400	336.4	0.216	-28.9
F3	400	369.8	0.254	-24.6

Table 5: Formulations of FLZ-SLN and their characteristics

3.8 Entrapment efficiency of FLZ-SLNs

The entrapment efficiency of the 3 formulations was found to be in the range of 77.4% to 90.1%. It can be attributed to the lipids' structure, which has fewer perfect crystals with defects that can entrap high amounts of the drug.

Formulation	Entrapment efficiency (%)
F1	77.4
F2	90.1
F3	85.4

Table 6: Entrapment efficiency of developed FLZ-SLNs

3.9 In-vitro drug release of SLNs

The drug release of the formulations was performed in phosphate buffer pH 6.8. The FLZ-SLNs formulation was subjected to the drug release media for 24 hrs. For the formulation with just stearic

acid, F1, the formulation showed a release of 73.18% but it was not in a sustained release manner. This may be attributed to a more crystalline nature of stearic acid from which the dissolution of drug is easier from the lipid matrix. In 4 hours, about 60% of the drug was released and it showed very less release from the formulation in the next hours. For the formulation having stearic acid and Precirol ATO 5 in the ratio 1.5:1, F3, the drug release was 82.65% in 24 hrs. The results were found to be better than individual stearic acid, but the formulation didn't show a complete release of the drug. The best release was found to be in the formulation developed with formulation F2, stearic acid and Precirol ATO 5 in the ratio 1:1. There was a burst of drug release from the nanoparticles in the first four hours, which may have been caused by some drug residue on the surface of the lipid nanoparticles. As a result of the medication being homogenously entrapped in the SLNs, the formulation then demonstrated a sustained rate of drug release. The inclusion of Tween 80 in the formulation aids in the drug's dissolution by lowering the surface tension between SLNs and the dissolving medium. Over the course of 24 hours, a 92.13% drug release was observed.

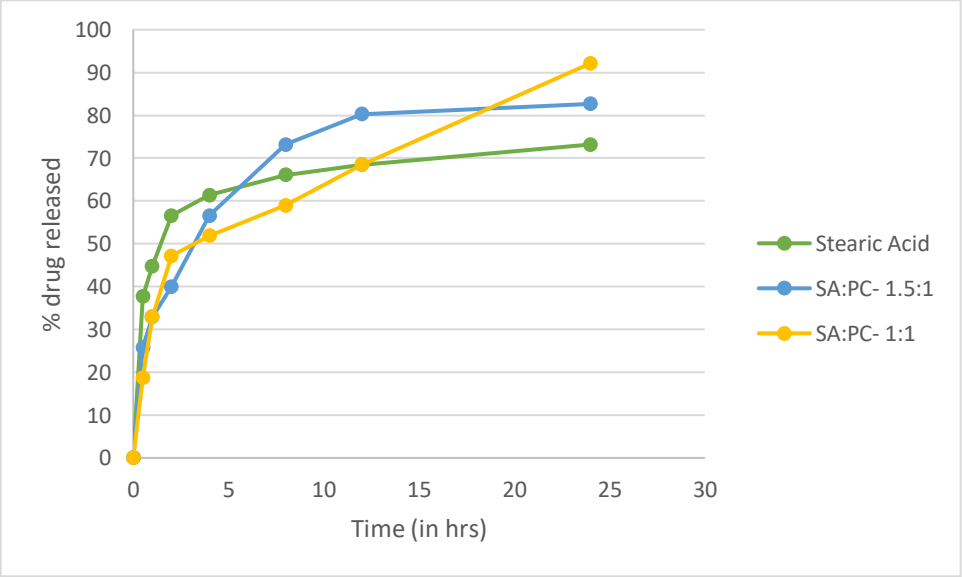

Figure 5: In-vitro release of FLZ from FLZ-SLN

3.10 Stability of FLZ-SLNs

The SLN formulations were kept at room temperature 666t for 30 days to check the effect of storage on particle size, PDI, zeta potential, and entrapment efficiency. F2 was found to be the most stable formulation among the three prepared formulations as it had the least change in the above parameters on storage.

Before storage

Formulation	Particle size	PDI	Zeta potential	Entrapment efficiency
F1	401.5	0.312	-21.8	77.4
F2	336.4	0.216	-28.9	90.1
F3	369.8	0.254	-24.6	85.4

Table 7: Characteristics of FLZ-SLNs before storage

After storage

Formulation	Particle size (nm)	PDI	Zeta potential (mV)	% Entrapment efficiency
F1	411.6	0.336	-24.1	72.8
F2	341.7	0.232	-26.8	88.3
F3	380.9	0.295	-27.1	82.1

Table 8: Characteristics of FLZ-SLNs after 30 days storage

CHAPTER 4: CONCLUSION

Fluconazole Solid Lipid Nanoparticles were successfully developed as a novel drug delivery method using the ultrasonication technique with stearic acid and Precirol ATO 5 as solid lipids, Polyvinyl alcohol as the stabilizer, and Tween 80 as the emulsifier. Stable SLNs were developed and evaluated. The evaluated parameters, particle size, zeta potential, PDI, and entrapment efficiency were found to be satisfactory. The in-vitro release of the optimized formulation also showed promising results with 92.13% release in 24 hrs., with initial burst release followed by a sustained release. The prepared FLZ-SLNs showed good physical stability on storage at room temperature. Thus, the developed SLNs may be employed as a dry powder for oral administration for Fluconazole delivery for fungal infections.

LIST OF REFERENCES

1) Mishra, B., & Singh, J. (2020). Novel drug delivery systems and significance in respiratory diseases. In *Elsevier eBooks*(pp. 57–95). https://doi.org/10.1016/b978-0-12-820658-4.00004-

2) Rizvi, S. S., & Saleh, A. M. (2017). Applications of nanoparticle systems in drug delivery technology. *Journal of the Saudi Pharmaceutical Society*, *26*(1), 64–70. https://doi.org/10.1016/j.jsps.2017.10.012

3) Mazayen, Z. M., Ghoneim, A. A., Elbatanony, R., Basalious, E. B., & Bendas, E. R. (2022). Pharmaceutical nanotechnology: from the bench to the market. *Future Journal of Pharmaceutical Sciences*, *8*(1). https://doi.org/10.1186/s43094-022-00400-0

4) Govindarajan, A. (2023, February 11). *Fluconazole*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK537158/

5) Moazeni, M., Saeedi, M., Kelidari, H. R., Nabili, M., & Davari, A. (2019). An update on the application of nano-scaled carriers against fluconazole-resistant Candida species: nanostructured lipid carriers or solid lipid nanoparticles? *Current Medical Mycology*. https://doi.org/10.18502/cmm.5.4.1965

6) Almehmady, A. M., El-Say, K. M., Mubarak, M. A., Alghamdi, H. A., Somali, N. A., Sirwi, A., Algarni, R., & Ahmed, T. (2022). Enhancing the Antifungal Activity and Ophthalmic Transport of Fluconazole from PEGylated Polycaprolactone Loaded Nanoparticles. *Polymers*, *15*(1), 209. https://doi.org/10.3390/polym15010209

7) Ramu, B. G., Spandana, K. A., Divith, K., Nagashree, A., Patel, A. R., & Preethi, S. (2021). A Review on SLN and NLC for Management of Fungal Infections. *Journal of*

Pharmaceutical Research International, 65–81. https://doi.org/10.9734/jpri/2021/v33i59a34250

8) Harde, H., Das, M., & Jain, S. (2011). Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. *Expert Opinion on Drug Delivery*, *8*(11), 1407–1424. https://doi.org/10.1517/17425247.2011.604311

9) Weng, J., Tong, H. H., & Chan, H. (2020). In Vitro Release Study of the Polymeric Drug Nanoparticles: Development and Validation of a Novel Method. *Pharmaceutics*, *12*(8), 732. https://doi.org/10.3390/pharmaceutics12080732

10) Üner, M. (2007, September 1). *Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives*. PubMed Central (PMC). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2676658/

11) Solid Lipid Nanoparticles for Drug Delivery: Pharmacological and Biopharmaceutical Aspects.

(2020). *Frontiers*. https://www.frontiersin.org/articles/10.3389/fmolb.2020.587997/full

12) PubChem. (n.d.). *Hazardous Substances Data Bank (HSDB) : 7420*. PubChem. https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7420#section=Metabolism-Metabolites-(Complete)

13) Kraisit, P., Hirun, N., Mahadlek, J., & Limmatvapirat, S. (2021). Fluconazole-loaded solid lipid nanoparticles (SLNs) as a potential carrier for buccal drug delivery of oral candidiasis treatment using the Box-Behnken design. *Journal of Drug Delivery Science and Technology*, *63*, 102437. https://doi.org/10.1016/j.jddst.2021.102437

14) Attia, D. A. (2017b). Development of Fluconazole Controlled Release Formulations Based on Solid Lipid Nanoparticles for Topical. . . *ResearchGate*. https://www.researchgate.net/publication/315620054

15) Fernandes, A. D. S. A., Pydi, C. B., Verma, R., Jose, J., & Kumar, L. (2020). Design, preparation and in vitro characterizations of fluconazole loaded nanostructured lipid carriers. *Brazilian Journal of Pharmaceutical Sciences*, *56*. https://doi.org/10.1590/s2175-97902019000318069

16) *Kopparam MANJUNATH | Professor | M. Pharm., Ph.D | Sree Siddaganga College of Pharmacy, Tumkūr | Department of Pharmaceutics | Research profile*. (n.d.). ResearchGate. https://www.researchgate.net/profile/Kopparam-Manjunath/publication/7903634_Solid_lipid_nanoparticles_as_drug_delivery_systems/links/544b2a760cf2d6347f41dd0c/Solid-lipid-nanoparticles-as-drug-delivery-systems.pdf

17) El-Housiny, S., Eldeen, M. a. S., El-Attar, Y. A., Salem, H. A., Attia, D. A., Bendas, E. R., & El-Nabarawi, M. A. (2018). Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. *Drug Delivery*, *25*(1), 78–90. https://doi.org/10.1080/10717544.2017.1413444

18) Sawant, B., & Khan, T. (2017). Recent advances in delivery of antifungal agents for therapeutic management of candidiasis. *Biomedicine & Pharmacotherapy*, *96*, 1478–1490. https://doi.org/10.1016/j.biopha.2017.11.127

19) Helal, S., Abdel-Aziz, H. M. M., El-Zayat, M. M., & Hasaneen, M. N. A. (2022). Preparation, characterization and properties of three different nanomaterials either alone or loaded with nystatin or fluconazole antifungals. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-26523-1

20) Akbari, J., Saeedi, M., Ahmadi, F., Hashemi, S. A., Babaei, A. H., Yaddollahi, S., Rostamkalaei, S. S., Asare-Addo, K., & Nokhodchi, A. (2022). Solid lipid nanoparticles and nanostructured lipid carriers: a review of the methods of manufacture and routes of administration. *Pharmaceutical Development and Technology*, *27*(5), 525–544. https://doi.org/10.1080/10837450.2022.2084554

21) Dudhipala, N., & Ay, A. A. (2020). Amelioration of ketoconazole in lipid nanoparticles for enhanced antifungal activity and bioavailability through oral administration for management of fungal infections. *Chemistry and Physics of Lipids*, *232*, 104953. https://doi.org/10.1016/j.chemphyslip.2020.104953

22) Xu, L., Wang, X., Liu, Y., Yang, G., Falconer, R. J., & Zhao, C. (2021). Lipid Nanoparticles for Drug Delivery. *Advanced nanoBiomed Research*, *2*(2), 2100109. https://doi.org/10.1002/anbr.202100109

23) Khairnar, S. V., Pagare, P., Thakre, A., Nambiar, A. R., Junnuthula, V., Abraham, M. C., Kolimi, P., Nyavanandi, D., & Dyawanapelly, S. (2022). Review on the Scale-Up Methods for the Preparation of Solid Lipid Nanoparticles. *Pharmaceutics*, *14*(9), 1886. https://doi.org/10.3390/pharmaceutics14091886

24) Wikipedia contributors. (2023). Fluconazole. *Wikipedia*. https://en.wikipedia.org/wiki/Fluconazole

25) Mukherjee, S., Ray, S. K., & Thakur, R. B. (2009). Solid lipid nanoparticles: A modern formulation approach in drug delivery system. *Indian Journal of Pharmaceutical Sciences*, *71*(4), 349. https://doi.org/10.4103/0250-474x.57282

26) Koynova, R., Bird, R. C., Curtze, A. E., & Zhou, Q. (2021). Lipid Nanoparticles─From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and

Advancement. *ACS Nano*, *15*(11), 16982–17015. https://doi.org/10.1021/acsnano.1c04996

27) PubChem. (n.d.-a). *Fluconazole*. PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/Fluconazole

28) Hu, L., Xing, Q., Meng, J., & Shang, C. (2010). Preparation and Enhanced Oral Bioavailability of Cryptotanshinone-Loaded Solid Lipid Nanoparticles. *Aaps Pharmscitech*, *11*(2), 582–587. https://doi.org/10.1208/s12249-010-9410-3

29) De Almeida Campos, L., Fin, M., Santos, K. A., De Lima Gualque, M. W., Cabral, A. K. L. F., Khalil, N. M., Fusco-Almeida, A. M., Mainardes, R. M., & Mendes-Giannini, M. J. S. (2023). Nanotechnology-Based Approaches for Voriconazole Delivery Applied to Invasive Fungal Infections. *Pharmaceutics*, *15*(1), 266. https://doi.org/10.3390/pharmaceutics15010266

30) Mirchandani, Y., Patravale, V. B., & Brijesh, S. (2021b). Solid lipid nanoparticles for hydrophilic drugs. *Journal of Controlled Release*, *335*, 457–464. https://doi.org/10.1016/j.jconrel.2021.05.032

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