Improvement of the Pharmacokinetic Profile of Antifungal Agent Using Nano-Lipoidal Technology

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Abstract

This work focuses on enhancing the pharmacokinetic profile of antifungal agents through nano-lipoidal technology. Nanoparticles have shown great potential in improving drug solubility, stability, bioavailability, and therapeutic efficacy. Among these, solid lipid nanoparticles (SLNs) have emerged as a promising approach for topical antifungal drug delivery. SLNs provide sustained release, biocompatibility, and reduced toxicity, making them ideal for targeting infection sites. Encapsulating antifungal agents in SLNs addresses challenges like poor tissue penetration, limited solubility, and suboptimal pharmacokinetics associated with conventional formulations. The study emphasizes the potential of nano-lipoidal technology in revolutionizing antifungal therapy by improving drug delivery efficiency and therapeutic outcomes. Key formulation parameters, such as lipid composition, particle size, surface charge, and drug loading capacity, are optimized to achieve desired properties for enhanced drug delivery. Comparative studies with conventional formulations highlight the advantages of SLNs in improving drug exposure and prolonging therapeutic effects. Physicochemical characterization techniques, including dynamic light scattering, transmission electron microscopy, and differential scanning calorimetry, are employed to assess the structural integrity and stability of the formulations. Future efforts will focus on understanding drug-lipid interactions, refining formulation parameters, and conducting preclinical and clinical evaluations to establish the efficacy of these nanoformulations.

Keywords: *nano-lipoidal technology, solid lipid nanoparticles, drug-lipid interactions, nanocarriers*

Introduction

In these couple of decades, nanotechnology is spreading their site of area in different fields when it comes to medicines which is called as nanomedicine. Nanoparticles through delivery such a novel idea to improve the pharmacokinetics properties of a different drugs. When it comes to medicine helpful to prevent different diseases such as cancer, heart, ocular diseases. Whenever, lipoidal drugs are incorporated. Whereas, cochleates are using for a new and precious alternative to liposomes for topical deliveryof drugs [1]. Nano lipoidal technology refers the use of nanoscale lipid-based particles in different applications. Lipids are organic molecules that are hydrophobic but soluble in organic solvents. At the nanoscale, through this technology it can exhibit unique properties or make improvement of different drug activity. Lipid nanoparticles, such as liposomes and solid lipid nanoparticles, can be used to encapsulate drugs [2]. These nanoparticles can improve the solubility, stability, and bioavailability of drugs, and they offer controlled release and targeted delivery to specific tissues or cells. It is actual important that the field of nano lipoidal technology is dynamic, and ongoing research continues to explore new applications and improve existing technologies. As with any arising technology, considerations regarding safety, efficacy toxicity, and regulatory approval are crucial for the successful translation of nano lipoidal technologies indifferent fields. Cochleates are prolonged microstructures made of lipid bilayers formed by the condensation of small unilamellarnegatively charged liposomes. These solid particles can encapsulate hydrophilic and hydrophobic moieties, making them versatile carriers for drug molecules, proteins, and peptides [3]. Nano cochleates entrapped by hydrophobic drug molecules effectively & remain unaffected by their surrounding environment, providing a reasonable solution for the problem. This present study helped to develop the antifungal properties and its stability testing. Comparing the performance of encochlated antifungal agent are also include in this study [4]. A ‘bioenhancer’ is a product that are used to improve bioavailability of a particular drug which it is incorporated. With the combining of bioenhancers the dosage is lowered and risks of drug resistance are minimized [5]. Combining nano-lipoidal technology with natural bioenhancers could provide valuable insights to show synergistic effects. To investigate how these two approaches work together to enhance the overall pharmacokinetic profile of antifungal agents [6]. Explore research that focuses on optimizing the formulation parameters of nano-lipid carriers, such as particle size, surface charge, and drug loading, to achieve the desired pharmacokinetic improvements. Studying the *in-vivo* studies that validate the efficacy of the developed formulations [7]. After reviewing the experimentation on animalor human trials will provide valuable information on the practical applicability and safety of the nano-lipoidal technology combined with natural bioenhancers [6]. Assessing the biocompatibility and potential adverse effects will be essential for clinical translation.

Antifungal Agents: Types Polyenes

Polyene antifungals, like amphotericin B, work by binding to ergosterol, acrucial component of fungal cell membranes, leading to membrane disruption and cell death [8].

Azoles: Azoles, suchas fluconazole, inhibit ergosterol synthesis, disrupting fungal cell membrane integrity. Examples include imidazole’s (clotrimazole) and triazoles (fluconazole).

Allylamines: Terbinafine (Lamisil) is an allylamine that interferes with ergosterol synthesis, affecting fungal membrane stability.

Echinocandins: Echinocandins, like caspofungin, target fungal cell wall synthesis by inhibiting β-glucan formation, leading to cell wall weakening and fungal cell death [9].

Others

Triterpenoids and synthetic antifungals (e.g., fluconazole) also have specific mechanisms of action against fungi.

Understanding these distinct classes of antifungal agents is crucial for tailoring treatment approaches to specific fungal infections [10].

Antifungal agent applications

Antifungal agents are drugs designed to selectively terminate fungal microorganism with minimum toxicity to the host. These drugs find applications in various medical and non-medical scenarios:

Advantage of antifungal agent in nano cochleates

Nanocochleates, a novel lipid-based drug delivery system, show promising advantages when used as carriers for antifungal agents:

Enhanced Delivery: Nanocochleates improve the deliveryofantifungalagents, such as Amphotericin B, both orally and parenterally, potentially enhancing therapeutic efficacy.

Improved Therapeutic Activity: Nano cochleates, a lipid-based drug delivery system, significantly enhance the therapeutic activity of various drug candidates, contributing to improved treatment outcomes.

GreaterStability: Nano cochleates exhibit greater stability than liposomes duet othelipid composition, making them less susceptible to oxidation [11].

Bio enhancer applications

Natural bio enhancers have various applications in improving the bioavailability and efficacy of drugs. These bioenhancers, obtained from natural sources, can be utilized in many administration routes, including various routes. The application of natural bio enhancers is particularly relevant for drugs that are toxic, expensive, scarce, or poorly bioavailable. They serve as agents to assist and enlarge the bioavailability of poorly absorbed drugs [12].

The MOA of natural bio enhancers include improving drug absorption, reducing metabolism, and enhancing cellular uptake. Various herbal compounds such as piperine, quercetin, and others have been identified as efficient bio enhancers, highlighting the potential of these natural substances in pharmaceutical applications [13].

Advantages of Bio enhancer in nano cochleates

Bio enhancers play a important values in improving the bioavailability and efficacy of drugs when used in nano cochleate formulations. Here are the advantages:

Increased Stability:- Nano cochleates, having a non aqueous structure, exhibit greater stability compared to other drug delivery systems, such as liposomes [14].

Enhanced Bioavailability:- Nano cochleates boost up the oral bioavailability of drugs, ensuring a higher quantity of the unchanged drug reaches the systemic circulation, thereby enhancing its therapeutic effect.

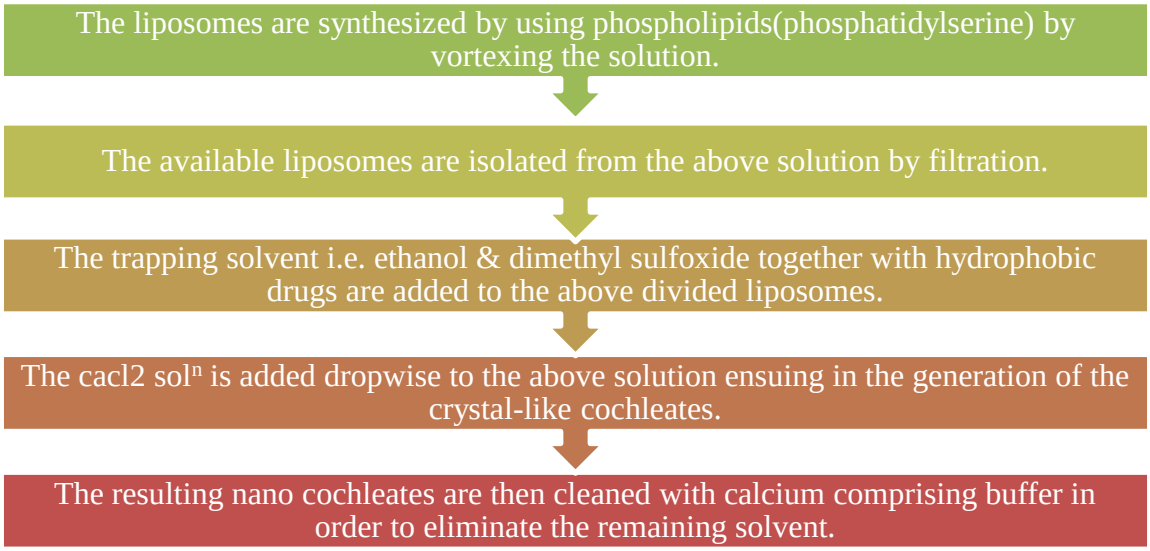

Fig 1: Trapping processing method

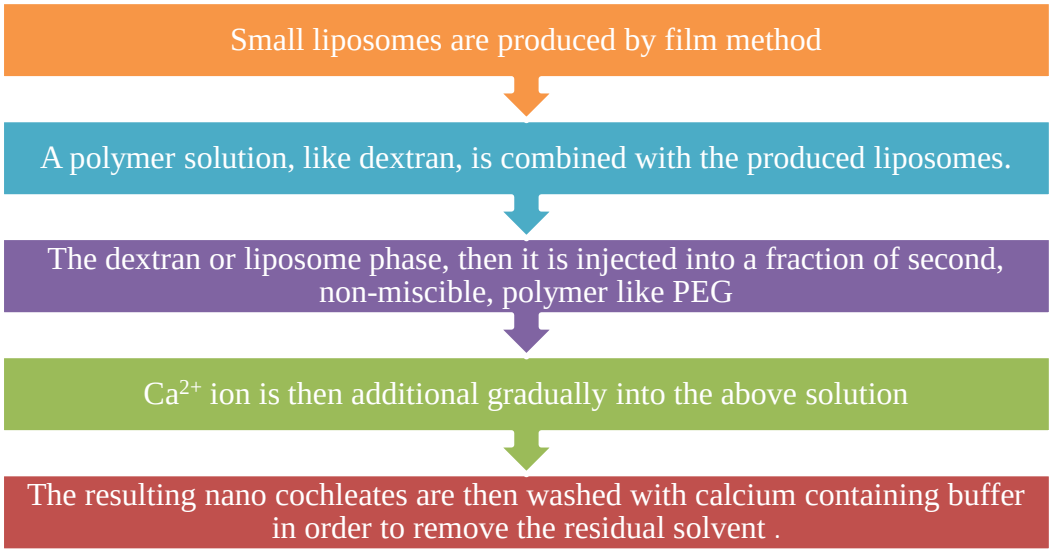

Fig 2 : Binary aqueous–aqueous emulsion system

Materials and Methods:

Name of the Instruments	Model & Manufacturer
Magnetic stirrer	Remi
Hot air oven	Unique Laboratory Machineries

Digital balance	Kern-Kb
Melting point apparatus	Chemi Lime
Ultra sonicator	HEXANE
UV spectrophotometer	UV -1800(Shimadzu)
Single pan balance	K Roy Single Pan Balance
Cooling centrifuge	REMI
Digital balance	Citizen
FTIR	Shimadzu
Rota evaporator	EYELA N-1200A
Vortex mixture	REMI

Table 1: List of the Instruments used

Name of the Chemicals	**Model & Manufacturer**
Ketoconazole	Yarrow chem products
Soya lecithin	Himedia
Cholesterol	Nice chemicals
Di sodium hydrogen phosphate	Nice Chemicals Pvt.Ltd
Sodium di hydrogen phosphate	Nice Chemicals Pvt.Ltd
Ethanol	S.D. Fine Chemicals
Calcium chloride	Yarrow chem products

Table 2: List of the Chemicals used

Methods

Pre-formulation Studies:-

Determination of Melting Point:–Firstly, the drug was sieved to decrease the size of the particles. Then the drug was loaded in a capillary tube. The capillary tube is kept in the melting point apparatus and check the melting point of the drug [12].

Physical compatibility study:-100mg of each soya lecithin, albendazole, and cholesterol were weighed. Individuals soya lecithin and cholesterol with admixture of drug in airtight screw cap amber-colored vials were kept at room temperature as well as 40ºC±2 ºC / 75% ± 5% RH for 30 days. The color change was observed after 10, 20, 30 days [15].

FTIR Study of the sample drug: -Firstly, KBr was heated at 60°c for 3 hours. Then blank KBr pellet was prepared and scan in FTIR. After that pinch of the drug sample was added into KBr & pellet was scan. The transmittance (%) and wave number (cm^{-1}) was plotted for the purpose of determining functional groups [16].

Preparation of standard curve of the sample drug:–firstly, 100 mg of the drug is weighed and dissolved in a 100 ml of distilled water. From the solution different concentrations was prepared (2-20 ppm) by diluting from the solution. The absorbance is checked in U.V spectrophotometer in the range of 225 nm [17].

Solubility study of pure ketoconazole :-Solubility of ketoconazole pure drug was tested in distilled water, phosphate buffer pH 7.4. An excess amount of ketoconazole pure drug was added in the pertinent media. The mixtures were stirred in a mechanical shaker at speed of 100rpm for 24 hours and the temperature was maintained at 37ºC±0.5 ºC. Visual inspection was carefully made to ensure excess ketoconazole solids were in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm filter. The absorbance of the solution was measured at 225nm in UV-Visible spectrophotometer [18].

Nanococleates preparation

Preparation of Liposome –It is done by film hydration method by using cholesterol & soya lecithin through vortexing for 15 min. Cholesterol & lecithin was added by different ratio 7.5:1, 9:1. Sodium phosphate dibasic & monobasic are used here. Soya lecithin & cholesterol was mixed in ethanol solution. Next the mixed solution ethanol was removed by using the rotary evaporator.Next the small amount of pH 7.4 was added the formulation certain period latter liposome are formed [19]. The formulation are store 4-8◦C.

Preparation of Nanococleates

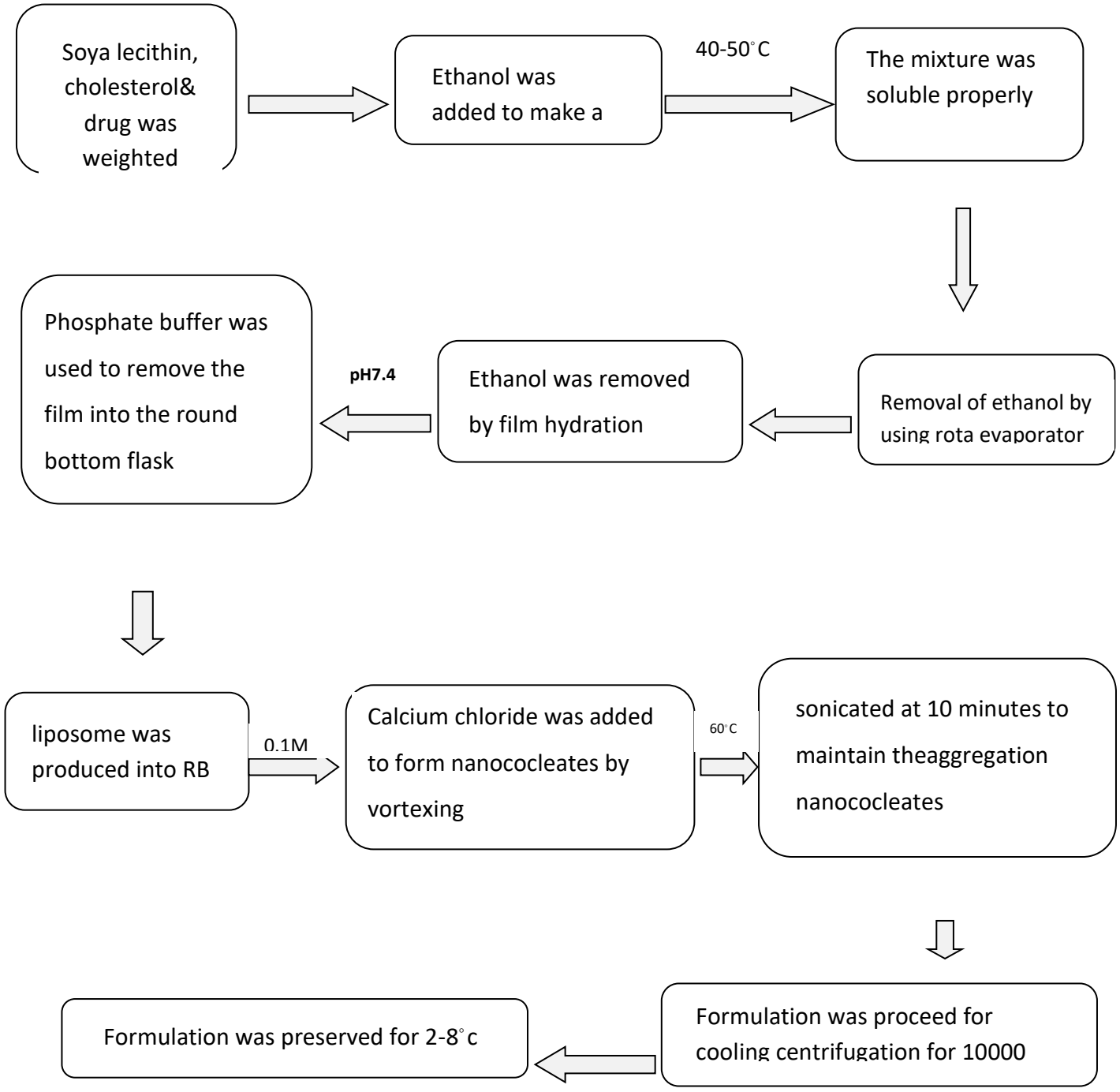

Fig 3: Preparation process of Nanococleates

Characterization of Nanococleates

Drug encapsulation efficiency

The1ml formulation was taken and subjected for cooling centrifuge to maintain 10000 g for 10 min. Supernatant & sediment vesicles were separated ethanol was added to relish the drug then volume was make up & observed the absorbance [20].

Drug release study

The formulation was taken and subjected for cooling centrifuge to maintain 10000 g for 10 min.The rest of the formulation excluding supernatant was taken .The mixture was poured into dialysis bag & dipped into phosphate buffer pH 7.4 .At the certain time of interval the drug was release,check the absorbance in uv spectrophotometer [21].

Drug content

The formulation was taken and subjected for cooling centrifuge to maintain 10000 g for 10 min.

The supernatant was taken and checked the absorbance in uv spectrophotometer.

Morphology analysis

The prepared ketoconazole loaded liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 10x, 40x&100$_X$magnification. Photographs were taken to prepared slides using digital camera [22].

Results and Discussion

Pre formulation study

Determination of Melting Point

Melting point of pure drugs

DRUG	REPORTED	OBSERVED
KETOCONAZOLE	148-156°C	152-158°C

Table 3: Melting point of pure drug

Seeing the above reported value and observed value, we can conclude that the ketoconazolewe have taken is pure.

Standard curve of ketoconazole drug [23].

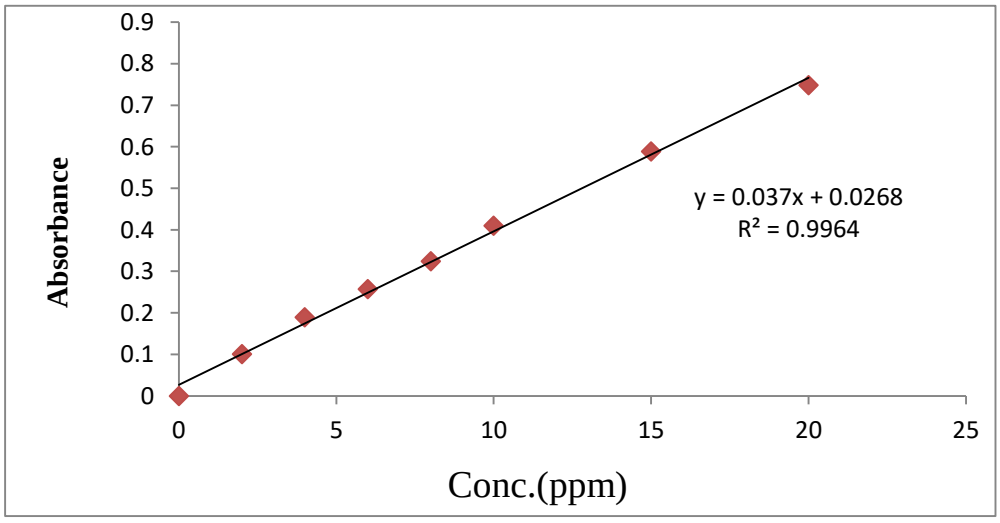

Fig 4: Standard curve of ketoconazole

Concentration(ppm)	Absorbance	Reported wavelength
0	0	225 nm
2	0.101	
4	0.189	
6	0.257	
8	0.324	
10	0.410	
15	0.589	

Tale 4: Standard curve value of ketoconazole

From the graphical representation we obtained a straight line of ketoconazole with the equation y = 0.037x and the R^2 value is 0.9964.

FTIR Study of API and Excipients and Incompatibility study [24]

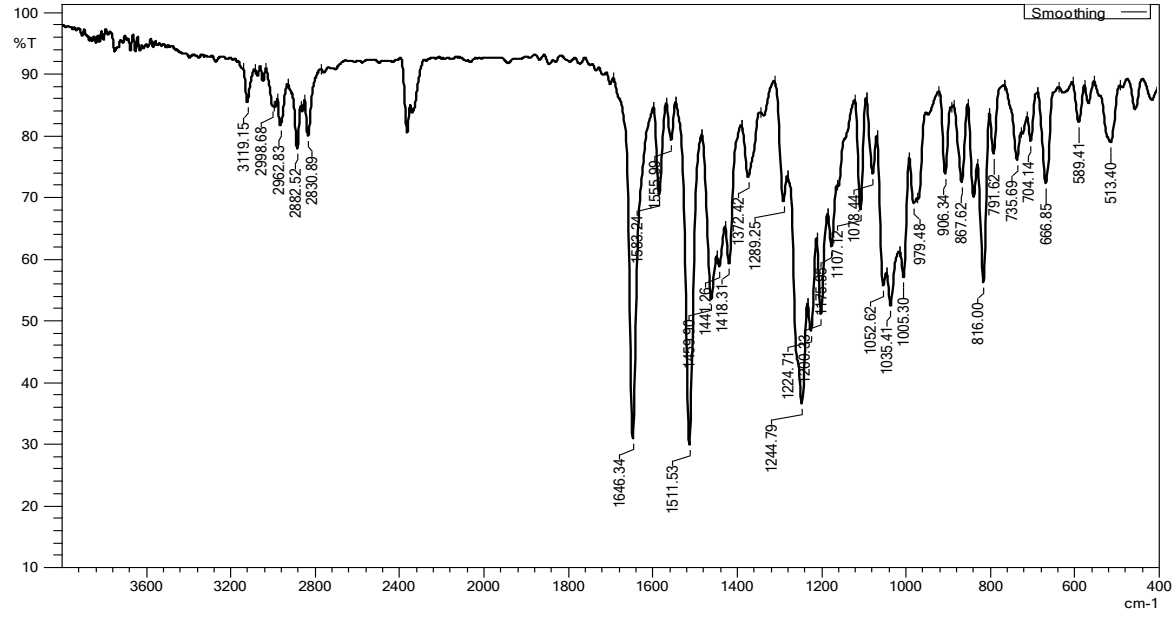

Fig 5: FTIR analysis of ketoconazole

From the above graph shows that peaks around 1600-1550 cm^{-1} (C=C stretching), absorption peak around 1650-1700 cm^{-1} (C=O stretching). 1600-1550 cm^{-1} shows imidazole ring. The presence of aliphatic chains in absorption bands in the range of 3000-2800 cm^{-1},shows methyl and methylene group in there.

Functional Group	**Standard spectra**	**Observed spectra**

(C=C stretching),	1600-1550 cm^{-1}	1555.99 cm^{-1}
C=O stretching	1650-1700 cm^{-1}	1646.34 cm^{-1}
aliphatic chains	3000-2800 cm^{-1},	2830.39 cm^{-1}

Table 5: FTIR data of pure ketoconazole

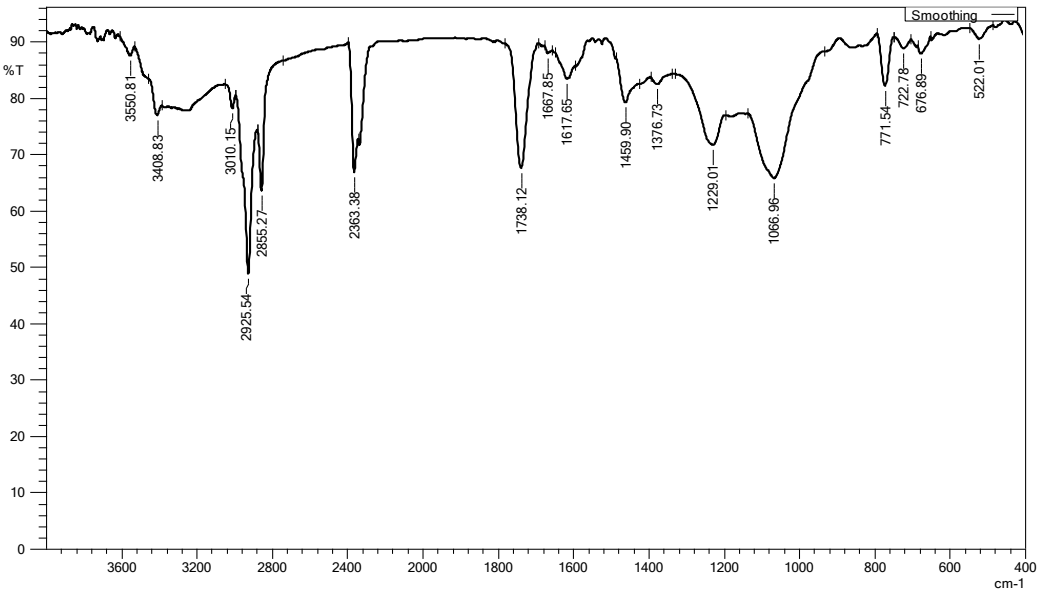

Fig 6: FTIR analysis of Lecithin

From the above graph shows that peaks around peaks in the range of 3000-2800 cm^{-1}: Correspond to C-H stretching vibrations in the fatty acid , peaks near 1100-1000 cm^{-1} corresponds to C-O stretching vibrations in the glycerol backbone of the phospholipids [25].

Functional Group	**Standard spectra**	**Observed spectra**
C=H stretching	3000-2800 cm^{-1}	2855.27 cm^{-1}
C-O stretching	1100-1000 cm^{-1}	1066.96 cm^{-1}

Table 6: FTIR data of pure Lecithin

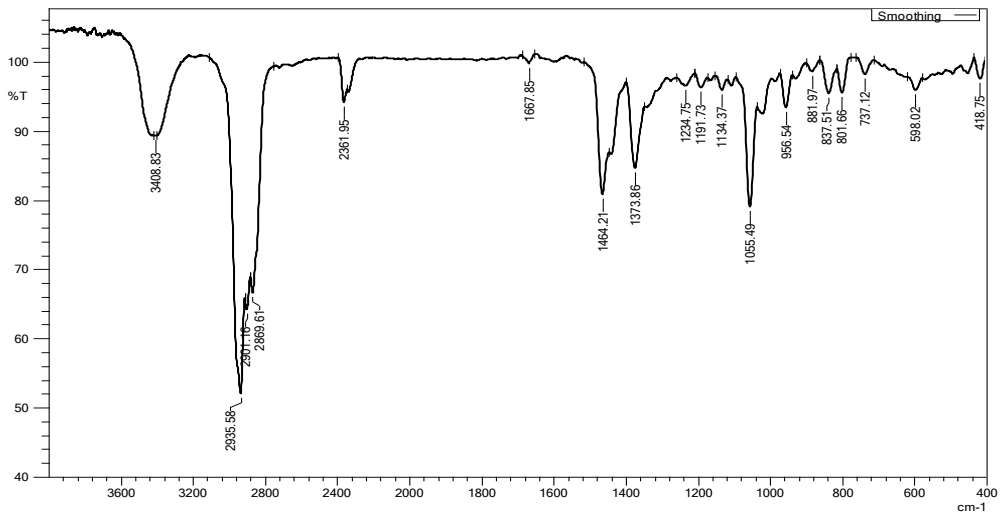

Fig 7: FTIR analysis of cholesterol

From the above graph corresponds 3400-3300 $cm^{-1}$for O-H stretch (alcohol) 3000-2800 cm^{-1}: C-H stretches (alkanes) 1650 cm^{-1}: C=O stretch (carbonyl group) 1465 cm^{-1}: C-H bend (methyl group) 1375 cm^{-1}: C-H bend (methylene group) 1100-1000 cm^{-1}: C-O stretch (alcohol).

Functional Group	**Standard spectra**	**Observed spectra**
C=O stretch (carbonyl group)	1465 cm^{-1}	1464.21 cm^{-1}
C-H bend (methylene group)	1375 cm^{-1}	1373.86 cm^{-1}
C-O stretch (alcohol)	1100-1000 cm^{-1}	1066.96 cm^{-1}

Table 7: FTIR data of pure cholesterol

Formulation study

Sl no.	**Formulation**	**Soya - lecithin(mg)**	**Cholesterol (mg)**	**Drug (mg)**	**Ethanol (ml)**	**Buffer (ml)**	**Calcium - chloride(µl)**
1.	**F_1**	300	40	150	10	10	400
2.	**F_2**	300	40	75	10	10	400
3.	**F_3**	450	50	150	15	15	400
4.	**F_4**	450	50	75	15	15	400

Table 8: Different types of formulation

Trinocular Microscopic Visulization Of Liposome Tonanococleates [26].

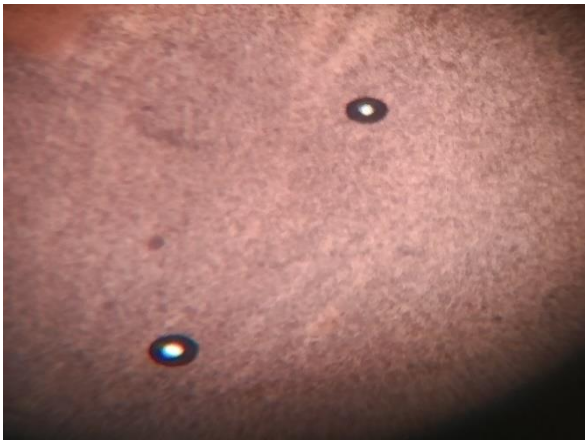

Fig 8: Liposomes with 40x zoom

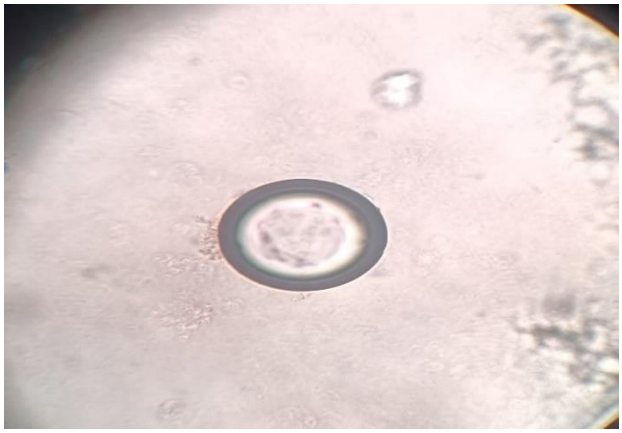

Fig 9: Liposomes with 100x zoom

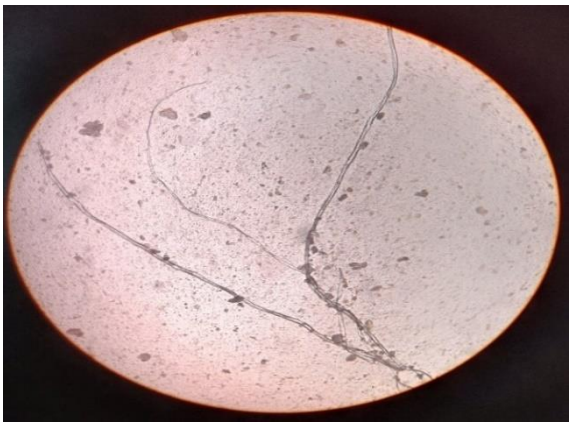

Fig 10: Nanococleates with 40x zoom

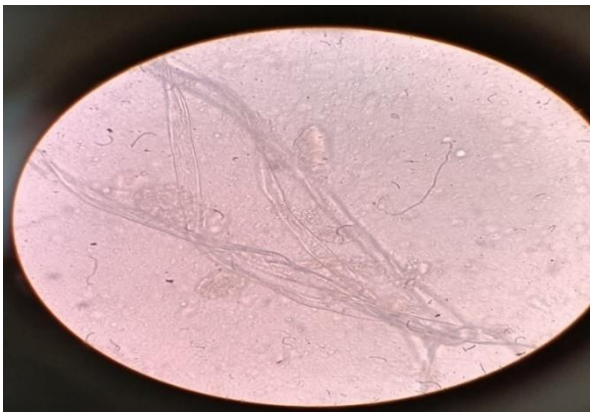

Fig 11: Nanococleates with 100x zoom

FTIR Study of API and Excipients and Incompatibility study

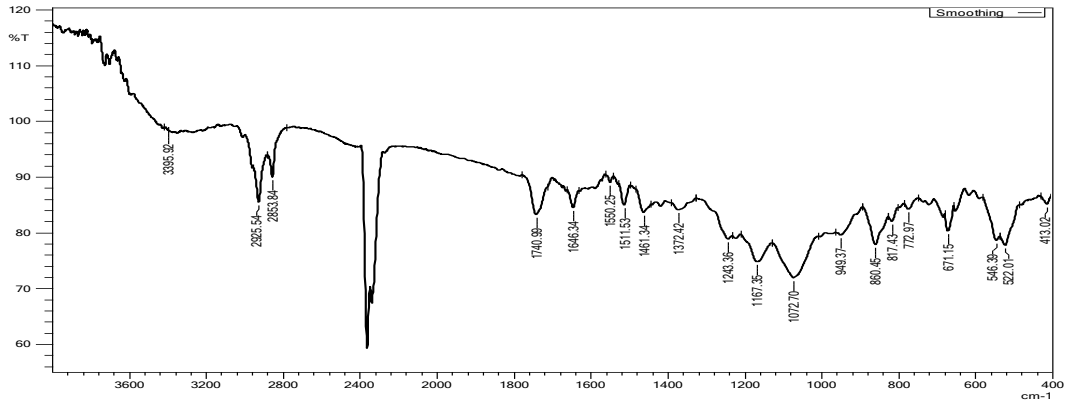

Fig 12: FTIR data of prepared formulation

From the above graph shows that peaks around 1600-1550 cm^{-1} (C=C stretching), absorption peak around 1650-1700 cm^{-1} (C=O stretching). 1600-1550 cm^{-1} shows imidazole ring. The presence of aliphatic chains in absorption bands in the range of 3000-2800 cm^{-1},shows methyl and methylene group in their peaks near 1100-1000 cm^{-1} corresponds to C-O stretching vibrations in the glycerol backbone of the phospholipids [27].

Functional Group	**Standard spectra**	**Observed spectra**
C=C stretching	1600-1550 cm^{-1}	1511.53 cm^{-1}
C=O stretching	1650-1700 cm^{-1}	1646.34 cm^{-1}
Aliphatic chains	3000-2800 cm^{-1}	2925.54 cm^{-1}
C-O stretching	1100-1000 cm^{-1}	1072.70 cm^{-1}

Table 9: FTIR data of prepared formulation

Comparison with Different Formulation Drug Release (Zero order kinetic, First order kinetic, Korsemeyer peppas, Higuchi reaction) [28].

Zero orderkinetic

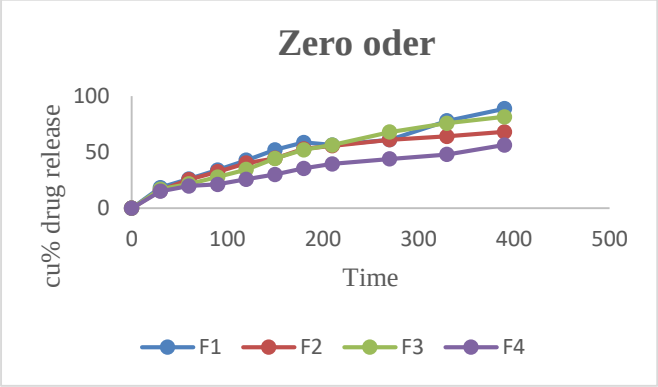

Fig 13: Zero order kinetic of various formulation (F-1-F-4)

First order kinetic

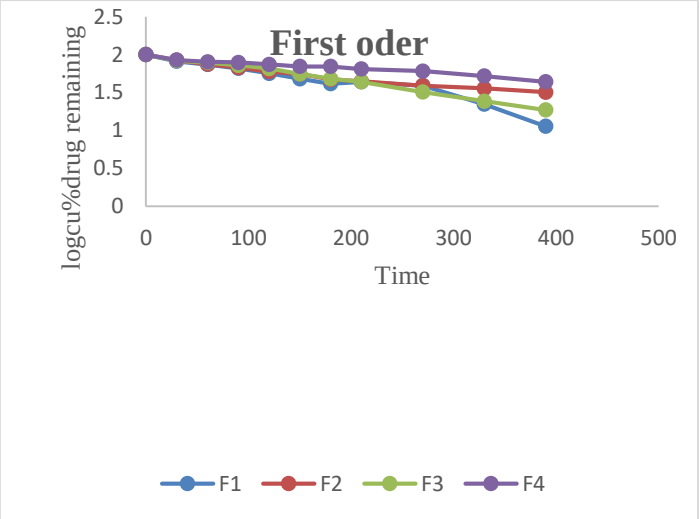

Fig 14: First order kinetic of various formulation (F-1-F-4)

Korsemeyer peppas model

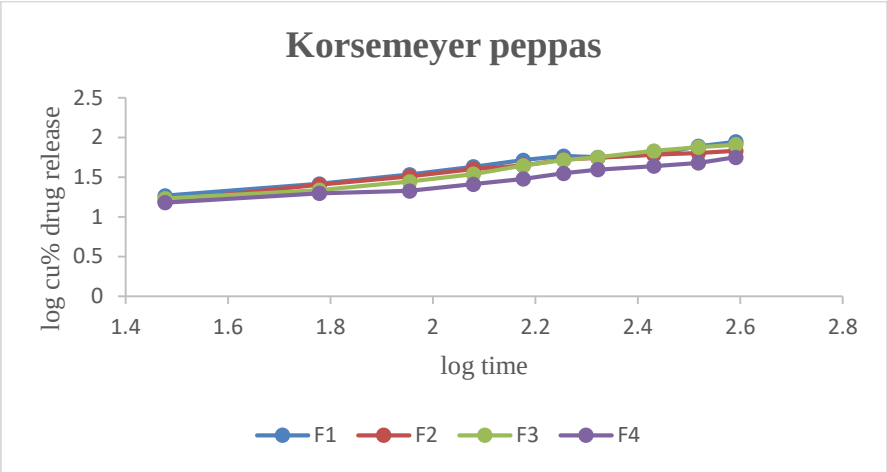

Fig 15: Korsemeyer peppas model of various formulation (F-1-F-4)

Higuchi reaction

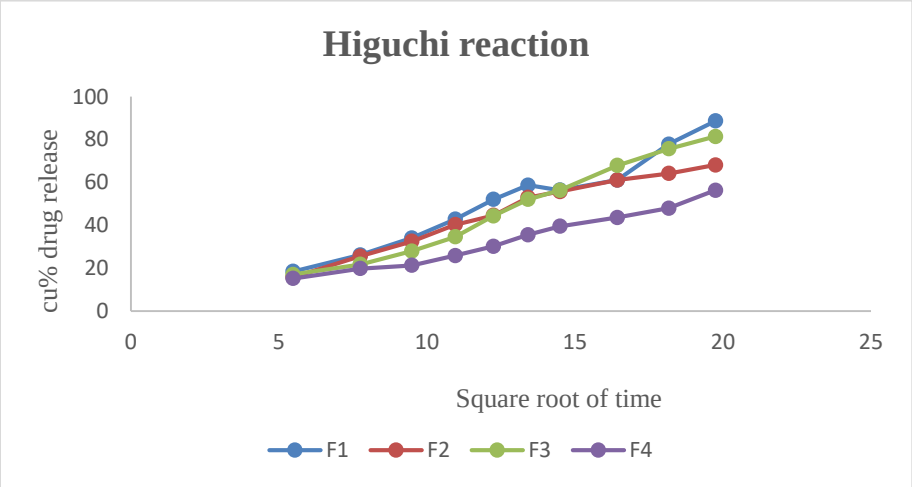

Fig 16: Higuchi reaction of various formulation (F-1-F-4)

Fig -10,11,12,13The drug release study of the formulations was studied by using dialysis bag. The formulation F_1,F_2,F_3& F_4 has shown release of about 88.69%, 68.09%,81.41% &56.31% at 24 hr respectively. This is maybe the amount of drug are essential the formulation. The in vitro release data of F_1,F_2,F_3&F_4 formulations were applied on Zero-order, first-order, and Higuchi model to test the release mechanism. R^2 values are higher for the **zero order kinetics** for all formulations, hence the drug release from all batches follows a diffusion rate-controlled mechanism [29].

Comparison with Different Formulation Drug Release [30].

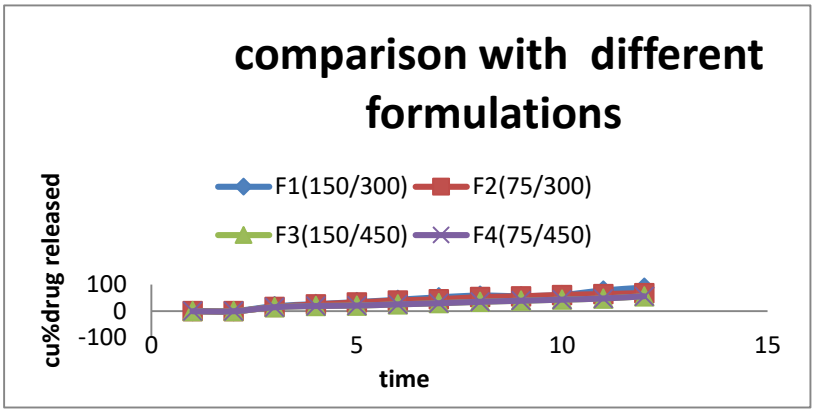

Fig 17: Comparison with different formulation drug release

DRUG CONTENT STUDY [31]

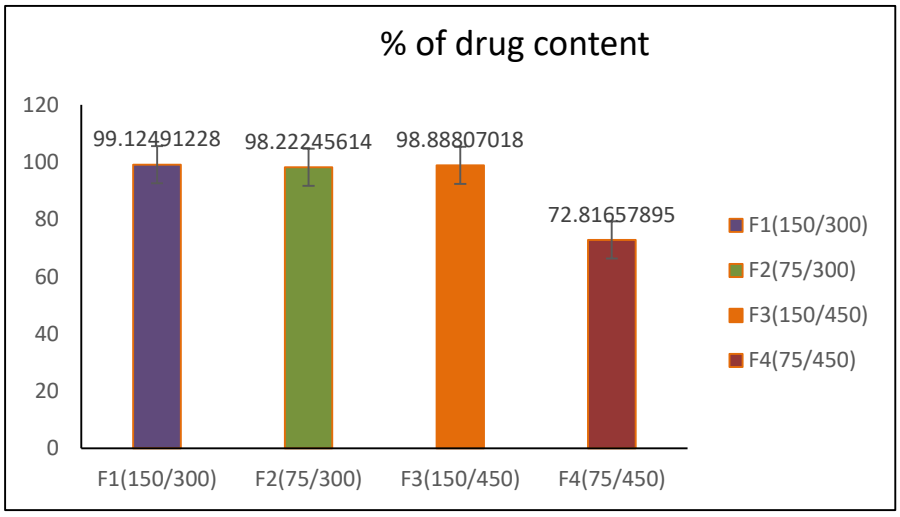

Fig 18: Drug content study of various formulations (F-1-F-4)

The formulation F_1, F_2, F_3& F_4 has shown the percentage of drug content 99.1249%, 98.2224%, 98.888%&72.8163% respectively. While, F1 shows the highest percentage of drug content [32].

DRUG ENCAPSULATIONSTUDY [33].

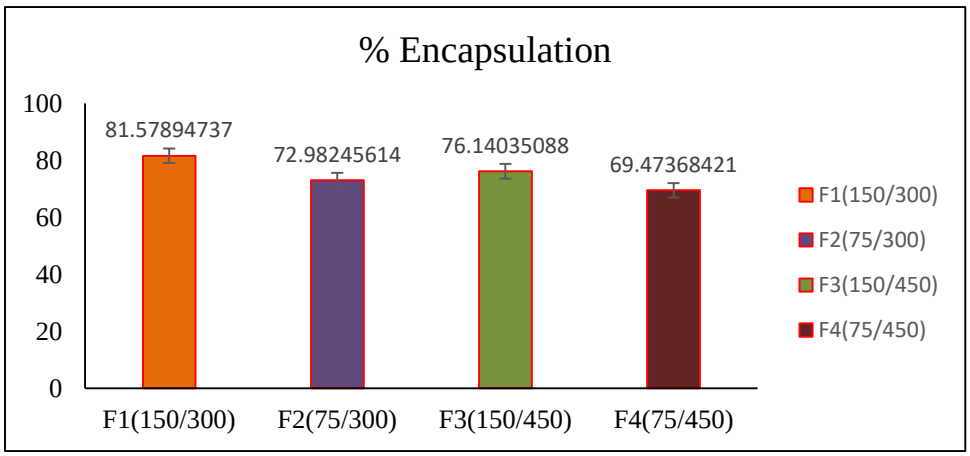

Fig16: Drug encapsulation study of various formulation (F-1-F-4)

The formulation of F_1, F_2, F_3& F_4 has shown the percentage of encapsulation 81.5789%,72.982456%,76.1403%& 69.47365% respectively. While, F1 shows the highest percentage of encapsulation [34].

Conclusion

The Formulation Number F_1 was found to have the most co-relatable properties followed by F_3, F_2 and F_4 in decreasing order. There is scope of further studies like SEM, TEM, Particle Size Analysis, Zeta Potential which is currently under process but beyond the scope of submission for evaluation. In conclusion, the highlights the potential of nanococleates as a promising drug delivery system to enhance pharmacokinetic properties properties of Ketoconazole, particularly for oral and topical administration, by enhancing bioavailability, solubility, stability, and targeted delivery. Nanocochleates are a lipid-based system that can deliver genes, proteins, antigens, and vaccines. Nanocochleates possess unique multi-layered structure, and this system has the ability to protect the drug over rigorous environmental conditions. Resultsin lesser toxicity, increasing bioavailability, improving therapeutic efficacy, ultimately improve the quality of treatment of various ailments. In recent years, research in major diseases like cancer, diabetes increased its significance as a drug delivery system. The major challenge in this field is to overcome the aggregation of nano-cochleates and the scale-up feasibility.

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