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FABRICATION AND OPTIMIZATION OF EMULSOMES CONTAINING TOPICAL GEL OF KETOCONAZOLE

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Abstract:

The topical route of administration for a therapeutic drug allows for direct contact with the intended area of the skin. Topical dosage forms such as ointments, creams, and gels are often inadequate in delivering medication to the desired spot, especially in higher concentrations. Advanced drug delivery systems such as liposomes, emulsomes, niosomes, dendrimers, and nanoparticles have the ability to specifically target tissues through the skin layer, offering improved therapeutic options for skin cancer. The findings indicate that when the concentration of the solid core changes and the quantity of lipid content increases, there is a corresponding rise in particle size. Consequently, this leads to an increase in both the PDI (polydispersity index) and the zetapotential action. The results of the dependent variables indicated that the KE4 formulation was chosen to optimize the impact of different surfactants at varying concentrations on the penetration rate or drug entrapment efficiency inside the solid lipid core. The gel basis is required to advance the formulation from emulsomes to emulsomal gel. The gel basis, consisting of a mix of guargum and xanthan gum, was chosen as the optimal concentration of gelling agent. The emulsomes were formulated and converted into an emulsomal gel, which was then assessed using several criteria. The physical assessment of the emulsomal gels revealed a bright yellow color, transparency, and a desirable consistency and smooth texture. The pH of the formulation does not disrupt the physiological balance of the skin. The analysis determined that the KEG4 formulation performed the best across all observations. The drug release perfusion profile and release kinetics of formulations KEG1 to KEG4 exhibited values of n> 0.5, indicating that they followed the Fickian diffusion and supercase II transport mechanism.

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INTRODUCTION:

Emulsomes are a newly developed lipid-based vesicular system consisting of a solid fat core enclosed by a double layer of phospholipids. Emulsome is an advanced nanocarrier technology designed specifically for medications that have low solubility in water. It exhibits characteristics of both emulsions and liposomes [1]. Emulsome refers to lipid-based drug delivery systems that have a wide range of therapeutic uses, especially for medicines that have low solubility in water. Emulsomes are composed of small lipid structures with a central region that is polar. This central region includes medications that are not soluble in water, but are in a solution form. Emulsomes do not need any surface active agent or co-solvent to hold the pharmaceuticals in this form [2]. The emulsomal formulations consist of a solid lipid core substance that is stabilized by cholesterol and soya lecithin. The medicine is loaded into the emulsomes by a process of sonication, resulting in the formation of small-sized emulsomes. The emulsomes-based method shown exceptional promise for targeted delivery. The formulations have the potential to greatly alter medication delivery by giving long-lasting effects at very low dosages. This may help reduce the toxicity associated with the medicine, since it is specifically targeted to the desired cells. They have a substantial impact on the pharmacokinetics of medicines. Additionally, they possess the ability to counteract the development of multi drug resistance, a phenomenon often linked to the excessive production of a cell membrane glycoprotein. This glycoprotein facilitates the removal of the drug from the cytoplasm, leading to an insufficient concentration of the drug inside the cellular compartment, rendering it useless. Lipid-based excipients can affect the absorption of drugs taken orally through various physiological mechanisms, including delaying gastric emptying, promoting bile flow, increasing the fluidity of membrane lipids, and directly influencing the transport and distribution of drugs in enterocytes [3]. Lipids with a low hydrophilic-lipophilic balance (HLB) and a high melting point are appropriate for achieving prolonged release. However, semi-solid excipients with a high hydrophilic-lipophilic balance (HLB) function as excipients that provide quick release and boost bioavailability. Triglycerides that are in a solid state at 25°C are suitable as core material since they reduce the allowable storage lifespan of oil-in-water emulsion. The triglycerides are used in the creation of emulsomes, which consist of straight-chain fatty acids [4]. The hand shaking approach generates multi lamellar vesicles (MLV), while the non-shaking method produces large unilamellar vesicles (LUVs) [5]. It is important to comprehend the attributes of different lipid formulations in order to create recommendations that enable the early identification of appropriate candidate formulations. Further study should include studies on human bioavailability, as well as more fundamental investigations into the mechanisms of action of this intriguing and varied collection of formulations [6]. Emulsomes enhance the solubility and bioavailability of medicines that have low water solubility. Triglycerides make up these structures, which may either form micelles or arrange themselves into lipid bilayers. In the bilayers, the hydrophobic tails are aligned with each other, while the hydrophilic head-groups face the water on both sides. Phospholipids has distinct characteristics that make them very ideal for usage as excipients in medications that have low solubility in water [7]. Emulsomes provide an efficient method for delivering drugs topically due to their ability to retain a high flux of medication and effectively retain the drug on the skin. This leads to improved antifungal action and less skin irritation. The advantages of emulsomes include protecting the medication from the harsh gastric environment, enhancing the solubility and bioavailability of the drug, reducing the required dosage, improving the pharmacological activity, and providing sustained release of the medication for up to 24 hours. Additionally, emulsomes enable targeted drug delivery. Emulsomes, which consist of phosphatidylcholine (soya-lecithine), cholesterol, and one of the solid lipids, were created and fine-tuned for the lipid ratios [9]. Therefore, there was a need to create formulations that would circumvent the issues of toxicity and quick medication removal. The objective of the research is to create a carrier system that may be used topically to control fungal infections, while also avoiding the harmful and unwanted effects of the medication [10].

Materials and Methods

Preparation of Emulsomes: Emulsomes with drug in different combinations prepared by the high-speed homogenization method. The various formulations were prepared using varying amount of gelling agent and penetration enhancers. The composition of the formulation was prepared with nutmeg oil as a carrier, surfactant Tween 40 and glycerol in purified water by high-speed homogenization. Accurately weighted quantities of surfactants and cholesterol were taken to give the desired ratio and were dissolve in 50 ml of ethanol in a round bottom flask. Then, accurately weighted amount of drug was added to the solvent (ethanol). The solvent was evaporated in a rotary flash evaporator at temperature of 60°C at 120 rpm until the smooth, dry lipid film was hydrated with 20 ml of PBS 7.0 was added and shaking on the

water bath. The emulsome suspension was formed at this kept at 2 to 8°c for 24 hrs [11].

Preparation and characterization of emulsomal gel: Preparation and characterization of emulsomal gel: The emulsomal gel formulation was created using a gel basis to get KEG1 – KEG8 and tested according to the previously mentioned criteria. The amounts of gel components were measured. Propyl paraben (75 mg) and Glycerol (5 ml) were dissolved in about 35 ml of water in a beaker. The mucilaginous ingredients were agitated vigorously using a mechanical stirrer. Subsequently, 2 grams of Guargum and 1 gram of Xanthan gum were gradually introduced into the beaker holding the aforementioned liquid dispersion, all the while stirring. After a duration of 2 hours, the hydrogel solution containing the medication was rendered neutral by the gradual addition of 5 ml of the alkali triethanolamine, which acted as a gelling agent. This process was carried out while continuously stirring the solution until a transparent gel structure with the highest possible viscosity was achieved. The emulsomal gel was then put into an aluminium collapsible tube and appropriately labelled [12].

Table 1: A various composition of different emulsomes formulations

Formulation Code	Lecithin (LC) (mg)	Phosphatidylcholine (PC) (mg)	Coconut oil (CO) (mg)	Tween 40
KEG1	200	0	200	10
KEG2	0	200	200	10
KEG3	200	100	150	10
KEG4	100	200	150	10

Characterization of emulsomes: The optimized formulation will undergo characterization to determine its size and size distribution, shape and surface morphology, entrapment efficiency, and zeta potential.

Measurement and analysis of vesicle dimensions and their distribution: The stability of emulsomes is shown by their capacity to maintain a consistent size and size distribution over an extended period. Electron microscopy is extensively used to evaluate the surface characteristics, dimensions, and distribution of emulsomes. In addition to normal laboratory procedures such as gel chromatography, it is necessary to use techniques based on light scattering and electron microscopy for a statistically meaningful examination of the size and size distribution of the carriers. The mean vesicle size and size distribution were assessed using photon correlation spectroscopy with a zeta sizer. The dispersion sample was diluted by a factor of 1:9 with distilled deionized water.

Zeta potential: Zeta potential determination involves measuring the net charge acquired by particles in a certain medium. Understanding the zeta potential of a preparation may aid in predicting the behavior of the preparation in living organisms and evaluating the stability of colloidal systems. The Zeta potential of emulsomes formulations was evaluated using p-hoton correlation spectroscopy using a Zetasizer Nanoseries instrument equipped with a flow-through cell [13].

Analysis of shape and surface morphology: The shape and surface morphology of emulsomes were assessed using the Transmission Electron Microscope (TEM) method. The 10µl sample was applied onto the grids and left at room temperature for 90 seconds. Any surplus fluid was eliminated by gently brushing the edge of a filter paper. The samples were analyzed using a Transmission Electron Microscope (Tecnai G2, Hillsboro Oregon, USA) with an acceleration voltage of 100 kV. Photomicrographs were captured at a magnification of 1400X.

Measurement of Entrapment Efficiency: The entrapment efficiency was assessed by using the minicolumn centrifugation technique to separate the unentrapped drug, as described by Fry et al. (1978) and New (1990). Sephadex G-50 (1.2gm) was hydrated in 20 ml of 0.9% NaCl solution for 5 hours at room temperature with intermittent agitation. The gel was created and thereafter kept at a temperature of 4ºC. In order to create the minicolumn, the barrel of a 1mL disposable syringe was filled to the top with hydrated gel and then sealed with a whatman filter pad. The barrel was put in the centrifuge and spun at a speed of 2000 revolutions per minute for a duration of 3 minutes in order to eliminate the saline solution. The eluted volume was extracted from the centrifuged tubes, and a precise amount of 0.2mL of undiluted emulsomal solution was carefully poured drop by drop onto the gel bed in its center. The columns were once again subjected to centrifugation at a speed of 2000 rpm for a duration of 3 minutes in order to eliminate and extract the void volume that included emulsomes, which were then transferred to

the centrifuge tubes. The elute was removed and 0.25 mL of saline was added to each column, followed by centrifugation as before. The quantity of drug enclosed inside the vesicle was then measured by breaking down the vesicle using 1 ml of 0.1%v/v triton-X 100, filtering it, and analyzing the drug content using UV-Vis spectroscopy at a wavelength of 254 nm.

The percentage efficiency was determined by followimg equation:

$$\text{Percentage drug entrapment} = \frac{\text{Amount of entrapped drug efficiency}}{\text{Total amount of drug}} \times 100$$

Evaluation of emulsomal gel formulation:

Physical appearance: The created gel was visually inspected to assess its color, look, and texture upon application.

pH determination: The pH of the emulsomal gel was measured using a digital pH meter. A solution was prepared by dissolving 1 gram of gel in 100 ml of distilled water, which was then kept for a duration of two hours. The pH electrodes were fully immersed in the formulations and the pH was recorded. The pH of each formulation was measured in triplicate and the average results were determined.

Extrudability assessment: The emulsomal gel was injected into flexible metal tubes. The tubes were subjected to equal pressure by manual compression, and the extrudability of the formulations was assessed. The extrudability of the formulation was assessed by measuring the amount of weight in grams needed to extrude a gel ribbon with a thickness of 0.5 cm during a time frame of 10 seconds.

Viscosity determination: The viscosity of the emulsomal gel that was formed was tested using a Brookfield viscometer. Adequate amount of gel base was placed into a wide-mouth jar, ensuring that there is enough space for the spindle to be dipped. The spindle's revolutions per minute (RPM) were set to 2.5. The measurements of the viscosities of the formulations were recorded [14].

Spreadability: It refers to the ability of an emulsomal gel to easily cover a large area when applied to the skin or damaged region of the skin. The medicinal efficacy of a formulation is also influenced by its spreading capacity. The spreadability of a formulation is measured by the time it takes for two slides to slide off from a gel base, which is sandwiched between the slides and subjected to a certain load. The shorter the period required for the separation of two slides, the greater the spreadability. The calculation is performed using the formula.

$$S = M * L / T$$

where, M = Weight tied to upper slide; L = Length of glass slide; T = Time taken to separate the slides

Homogeneity: The emulsomal gel has been placed in the container; all manufactured gels were assessed for homogeneity by visual examination. The individuals underwent testing to assess their physical appearance and to detect the existence of any abnormalities such as lumps, flocculates, or aggregates.

Grittiness: The emulsomal gel was examined using a light microscope to see whether there were any noticeable particles present. The preparation should be devoid of particles and any rough texture that may be present in any topical medication should be eliminated.

Permeation tests conducted in vitro: The produced gels were tested for permeation using a Franz-diffusion cell. The dialysis membrane (Himedia, with a thickness of 0.025 mm) was divided into identical segments measuring 6 cm by 2.5 cm. These segments were then immersed in distilled water for a duration of 12 hours prior to their use. The drug release experiments of the salicylic acid emulsomalgel were conducted in a 10 ml solution of phosphate buffer with a pH of 6.8 and saltwater, which was kept at a temperature of 37±2°. The solution was stirred using a magnetic stirrer and heated consistently. 2 ml of salicylic acid emulsomalgel was put in the receptor compartment. 1 ml aliquot samples were periodically taken and replaced with an equal amount of new buffer. If required, the aliquots were diluted using new medium. The quantity of medication that permeated the membrane was quantified using a U.V. spectrophotometer at a wavelength of 254 nm, with phosphate buffer (pH 7.4) serving as the reference solution [15].

RESULTS AND DISCUSSION:

The lipid content was optimized over the solid lipid content to determine its impact on the particle size, zeta potential, polydispersity index, and drug % entrapment effectiveness of several formulations ranging from KE1 to KE8. The solid lipid core triglycerides (coconut oil; CO) were encapsulated with a single lipid layer and a double layer consisting of lecithin (LC) and phosphatidylcholine (PC). The different combinations of phosphatidylcholine, lecithin, and coconut oil are optimized with a

constant concentration of surfactant to maximize the penetration of the medicine. The penetration enhancer influences the incorporation of the medication (ketoconazole) into the solid lipid core (CO) by traversing either a single or double layer of lipid membranes. The sonication period should remain constant throughout the formulation process to ensure the production of unilaminar vesicles. This procedure is also effective in addressing the issue of vesicle agglomeration. The evaluation of all dependent variables resulted in the selection of KE4 as the optimal formulation, which has a ratio of phosphatidylcholine:lecithin:coconut oil of 0.5:1.5:1.0. The emulsomes were created with varied ratios of lipid layers, ranging from 0.5 to 1.5, using solid lipid coconut oil (CO). This resulted in the formation of a hydrophobic core. The findings indicate that the particle size increases as the concentration of the solid core fluctuates and the quantity of lipid content increases. Consequently, this leads to an increase in the PDI and zetapotential action. The optimization of single or double layer also resulted in differences in all parameters. By optimizing the sonication period, the particle size of KE1 and KE2 was enhanced due to the presence of a single layer of emulsomes, resulting in lower sizes compared to previous formulations. Therefore, the particle size distribution, PDI (Figure 1), and zetapotential action (Figure 2) of KE1 and KE2 were comparable to the formulations KE4. This similarity may be attributed to the presence of a solid lipid core (CO) with higher levels of PC and lower levels of LC. The variation in vesicle size and drug entrapment effectiveness is also associated with each other, as a result of the lipid layer's thickness. The KE4 formulation achieves drug entrapment of over 80% when tween 40 is added at a concentration of 10%. The results of the dependent variables indicated that the KE4 formulation was chosen to optimize the impact of different surfactants at varying concentrations in determining the rate of penetration or drug entrapment efficiency inside the solid lipid core. The sonication period was also assessed to optimize the detection of vesicular size and shape. The physical assessment of the manufactured emulsomal gels revealed a color ranging from light yellow to pale yellow. The emulsomal gel exhibited a clear and translucent look, and it felt smooth when applied to the skin. The individual characteristics, such as the uniformity of the formulations and the smoothness of the created emulsomal gel, were satisfactory. The pH of the gel formulation was measured at room temperature and ranged from 7.03 to 8.21. According to the literature, the pH of the skin rises from the epidermis to the dermis and reaches a neutral value. Gel formulations with a pH range of 7.0 to 7.6 are considered beneficial for the skin since they do not disrupt the skin's physiology. The emulsomal gel formulations were assessed for their extrudability, which was attributed to their favorable viscosity characteristics.

Table 1: Characterization of ketoconazole containing emulsomal gel

Formulation Code	Particle size (nm)	Layers	Zeta potential (mV)	PDI	Drug Entrapment (%)
KEG1	122.01±1.02	Single	-20.12±1.02	0.216±0.08	76.53±1.3
KEG2	123.03±1.04	Single	-20.18±1.05	0.215±0.02	79.78±1.1
KEG3	129.03±0.08	Double	-22.91±1.03	0.221±0.27	71.86±1.1
KEG4	127.21±1.11	Double	-24.21±1.09	0.216±0.05	81.37±0.8

Table 2: Evaluation of emulsomal gel formulation

Parameters	Formulations			
	KEG1	KEG2	KEG3	KEG4
Colours	Pale yellow colour	Pale yellow colour	Pale yellow colour	Pale yellow colour
Appearance	Translucent	Translucent	Translucent	Translucent
Odour	Pleasant odour	Pleasant odour	Pleasant odour	Pleasant odour
Feel of application	Smooth	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	7.4	8.9	12.5	8.4
Consistency	Poor	Poor	Good	Poor
pH	6.88	6.88	6.43	6.18
Viscosity (cps)	0.88	0.93	0.91	0.94
Extrudibility	Poor	Poor	Good	Poor
Drug content (%)	99.37	99.51	99.82	99.41

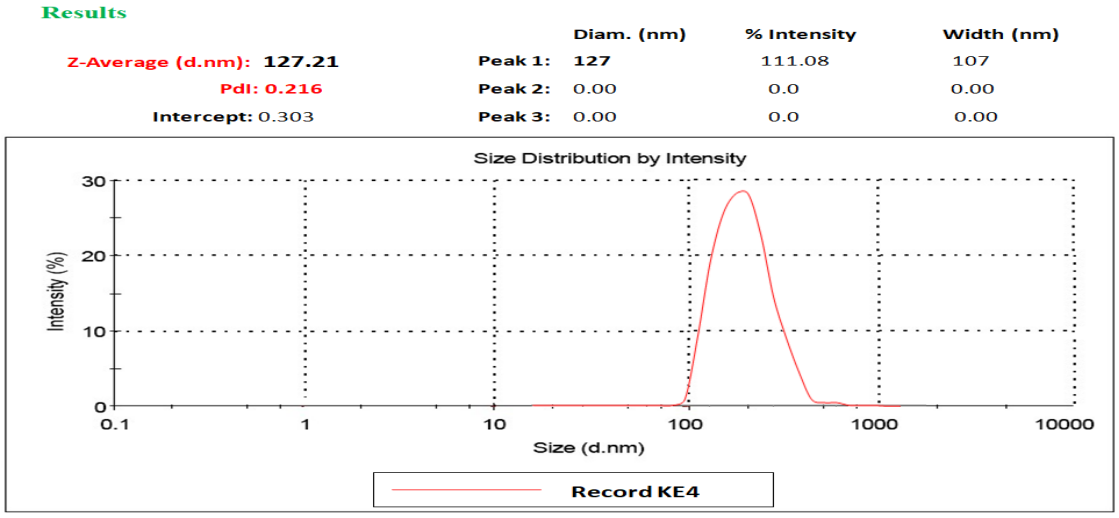

Figure 1: Particle size distribution & Polydispersity Index (PDI) of emulsomes (KE4)

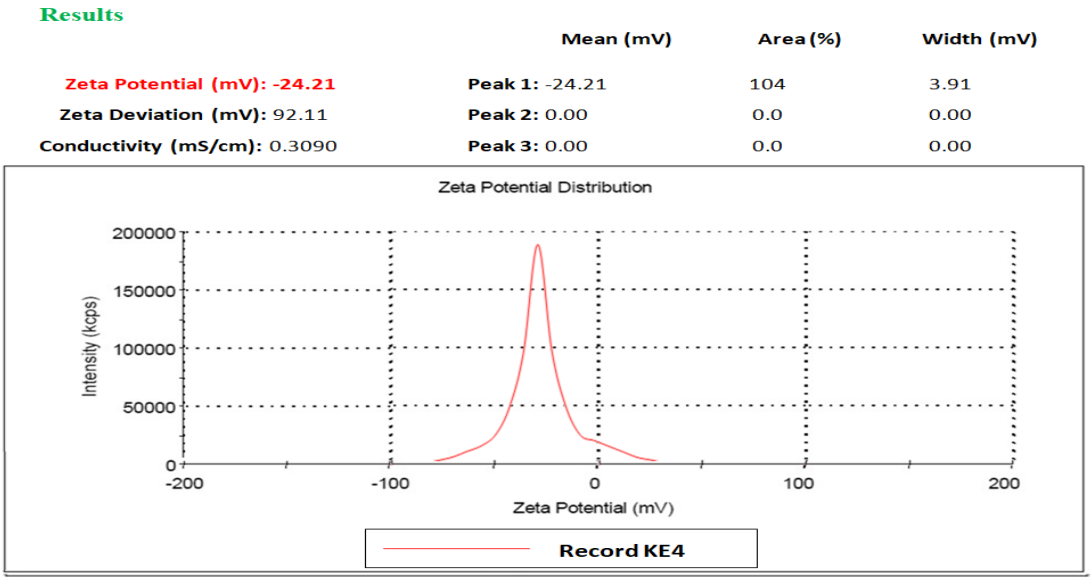

Figure 2: Zeta potential (mV) of emulsomes (KE4)

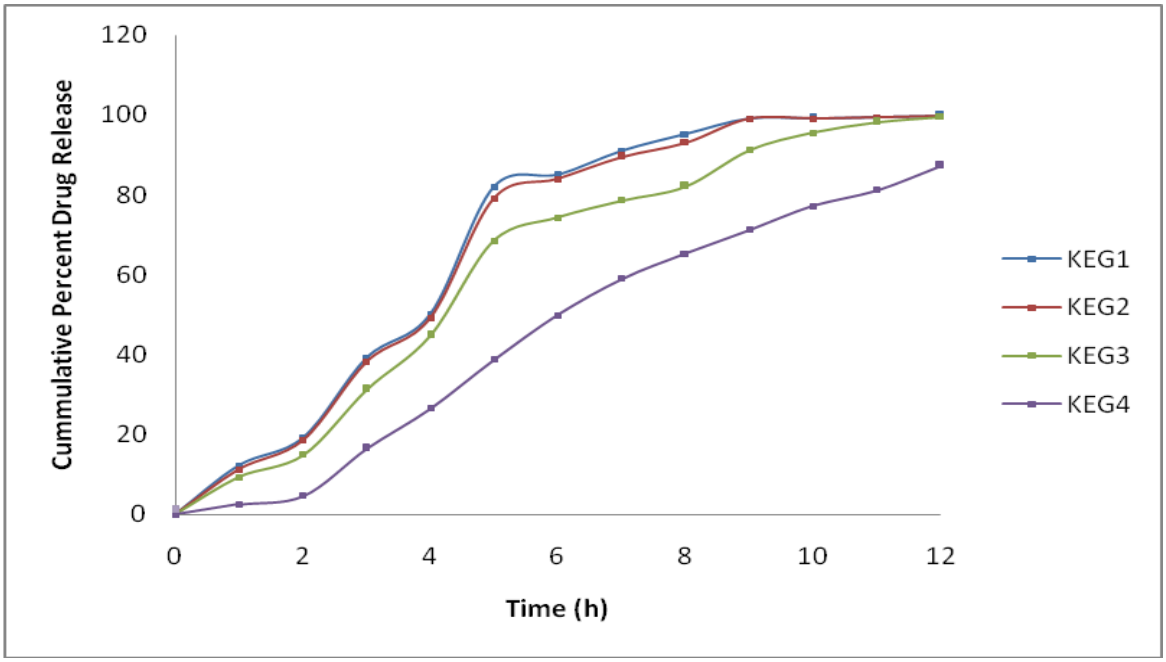

Figure 3: Zero-order in-vitro drug diffusion release kinetic study of emulsomal gel formulation (KEG1-KEG4)

SUMMARY AND CONCLUSION:

The emulsomes were prepared by optimizing the lipid ratio and solid lipid content. The variables considered for optimization were particle size, zeta potential, poly-dispersity index, and drug percentage entrapment efficiency. Various formulations were prepared from KE1 to KE8 using a 3^2 factorial design. The solid lipid core consists of a mixture of two layers of lecithin (LC) and phosphatidylcholine (PC). The different combinations of phosphatidylcholine, lecithin, and coconut oil are optimized with a constant concentration of surfactant to increase the penetration of drugs. The penetration enhancer influences the incorporation of the medication (Ketoconazole) into the solid lipid core (CO) by traversing the single or double lipid layers. The laminar character of the vesicles was not affected by the sonication period, since the same sonication time was used for all. Emulsomes provide a viable method for formulating therapeutic molecules that have low solubility in water and varying levels of oral bioavailability. Emulsomes have shown significant enhancement in the oral bioavailability of hydrophobic medicines, enabling their effective distribution by oral administration.

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