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FORMULATION AND CHARACTERIZATION OF ANTIFUNGAL NANOEMULSION OF KETOCONAZOLE

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Abstract-

Nanoemulsions, also known as submicron emulsions, ultrafine emulsions and miniemulsions, are submicron sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions, which consist of two immiscible liquids like water and oil, stabilized by an interfacial film consisting of a suitable surfactant and co-surfactant to form a single phase.

The goal is to develop and test a nanoemulsion of the antifungal drug ketoconazole. The next challenge was to demonstrate that the system can administer medicine over healthy skin more consistently and uniformly over a longer period of time than is feasible with nonoemulsion. The oil phase of coconut oil, Tween 80 surfactants, and ethanol cosurfactants were chosen as the constituents for the nanoemulsion formulation. The aqueous titration method was used to build pseudo-ternary phase diagrams. The phase diagram showed various oil and surfactant concentrations, and the resulting nanoemulsions were chosen based on the thermodynamic stability and dispersibility tests. Based on factors such as reduced viscosity, minimal polydispersity value, optimal globule size, greater drug release, and overall lower surfactant concentration and co-surfactant, the optimized formulation was chosen for an in vivo investigation. It was discovered that the nanoemulsion formulation's t max differed.

Keywords- *Nanoemulsion, Microdomains*

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INTRODUCTION-

Topical drug delivery methods have advantages over other delivery methods, one of which is to avoid the metabolism of the first pass effect on the liver[1]. Physical-chemical characteristic of drugs and excipients is taken into consideration in designing formulas to produce a product that is stable, efficacious, attractive, easy to formulate, and safe. These characteristics affect several factors: Particle size distribution, drug dissolution rate, bioavailability, uniformity of content, taste, texture, colour, and stability. The particle size and solubility of the drug have an effect on the formulation because the drugs entering the blood circulation must be in solution form to produce the desired effect. Ketoconazole is included in Class II of Biopharmaceutical Classification System means, including a class of low solubility and high permeability, the increased solubility of this drug is of concern to pharmaceutical researchers [2].

The absorption of ketoconazole orally is not maximal due to the solubility and the side effects it causes to overcome the deficiencies of this conventional system a new drug delivery system is required. Topical ketoconazole available on the market today, such as cream has side effects such as rash, itching, irritation, pain, and redness, therefore, to overcome this problem, requires a new drug delivery system such as nanoemulsion. Nanoemulsion has been widely used as a vehicle in topical medicine and is an alternative to insoluble, topical, or oral drugs. Nanoemulsions are thermodynamically stable dispersions of two immiscible liquids (oil and water) which are stabilized using a surfactant and cosurfactant molecule. They may be either transparent or translucent and have a droplet size of 5–200 nm [3].

They are well tolerated orally on the skin and mucous membrane when used to deliver topically active drugs. Nowadays, increasing drug loading, enhancing drug solubility, and bioavailability are the most important advantages encouraging the usage of nanoemulsion as drug delivery carriers. A topical nanoemulsion is a form of delivery for a drug that is difficult to dissolve and has side effects when administered orally by increasing the penetration of the drug through the skin [4]. Nanoemulsions comprise safe surfactants with or without other emulsifiers to improve stability, oil (natural/synthetic/semi-synthetic) and cosurfactant [5].

The method of nanoemulsion formulation is divided into two methods that use high energy and low energy. High energy formulations require tools such as high-pressure homogenizers, microfluidizers, and sonicators, low energy formulation methods dependent on the solubility of the active substance so that it is more efficient to make a small droplet nanoemulsion. Nanoemulsions produced with low energy methods depend on the spontaneous formation of emulsions based on the phase behavior of certain surfactant, oil, and water systems.

There is interest in using lower energy techniques in the emulsion formation process due to the economic benefits and increasing amounts of research have been conducted to investigate the utility of different lowenergy approaches. Self-emulsifying systems offer a strategy for dealing with the low bioavailability of compounds (drugs and oils) that are not easily dissolved in water [6]. A low energy emulsification or spontaneous emulsification method used by the laboratory scale to achieve small droplet size using simple instruments [7].

The advantage of the low energy method is that it can use simple equipment such as a magnetic stirrer, which includes low energy manufacturing methods are phase inversion temperature and phase inverse composition (PIC). The nanoemulsion method of PIC which is often performed for laboratory scale is by spontaneous emulsification [9].

The current research work aims to Formulate and evaluate the Antifungal nanoemulsion of ketoconazole at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer period of time from nonoemulsion.

MATERIALS AND METHODS:

Ketoconazole was gifted by Mankind pharma limited paonta sahib, H.P.), Coconut oil – National chemical (Baroda), Tween 80 – National chemicals (Baroda), Ethanol – Suvidhinath laboratories (Baroda). All other chemicals used were of analytical grade.

Screening of oils, surfactants and cosurfactants for nanoemulsion formation:

The most important criterion for the screening of oil for nanoemulsion is the maximum solubility and compatibility with drug. Basing on the biocompatibility profile of oils from literature review, drug solubility was determined. Excess amount of drug (100mg) in 3mL of selected oils (Olive oil, Castor oil, Sesame oil, olive oil, Coconut oil,) was

taken in stopper vials and was then mixed by vortex mixer [10].

The mixture vials were then kept at 37±2.0 ° C in an isothermal orbital shaker for 72 hr to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 5000 rpm for 15 min. The solubility profile of drug in oil was determined from the supernatant using UV-VIS spectrophotometer at 257 nm. Insoluble drug from the settled material was determined and mass balance was then found out[11].

Optimization of oils, surfactants, and cosurfactants Optimization of surfactant concentrations, cosurfactant and oil were using different ratio The ratio of surfactant mixture to cosurfactant (smix) varies from 4:1, 3:1, 2:1, and 1:1. The ratio of oil and smix concentration is 1:9, 2: 8, 3:1, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Aquades is added with titration and stirred using a magnetic stirrer until it becomes translucent and no phase separation.

Pre-formulation studies of Ketoconazole

Solubility determination

The solubility of the Ketoconazole was determined in methanol, chloroform and phosphate buffer of pH 7.4 using standard method.

Melting point determination

Melting point of the drug was determined by taking a small amount of the drug in a capillary tube closed at one end and was placed in Thiel's melting point apparatus and the temperature at which the drug melts was noted.

Partition coefficient

The partition coefficient of drug was determined by taking equal volume of 1-octanol and aqueous solution in a separating funnel. A drug solution of 1mg/ml was prepared in n- octanol. 25ml of this solution was taken in a separating funnel and shaken with an equal volume of phosphate buffer of pH 7.4 (aqueous phase) for 10 minutes and allowed to stand for two hrs. Then aqueous phase and organic phase were collected separately and centrifuged at 2000 rpm. Both the phases were analyzed for the drug concentration using U.V. spectrophotometer. Partition coefficient was calculated by taking the ratio of the drug concentration in n-octanol to drug concentration in aqueous phase[12-13].

Permeability coefficient:

The permeability coefficient of drug was calculated by "Potts and Guy equation",

log Kp = -2.7 + 0.71 x log Ko/w – 0.0061 x Molecular weight

Where, log Kp = Permeability coefficient Ko/w = Partition coefficient

Infrared (IR) absorption spectroscopy

To investigate any possible interaction between the drug and the utilized polymers, IR spectrum of pure drug ketoconazole and its physical mixture was carried busing FT- IR (Perkin elmer 1600 series USA) the range selected was from 400cm^{-1} to 4000 cm^{-1}

Formulation of Nanoemulsion

Nanoemulsion formulations were prepared using selected oil, surfactant, co-surfactant along with aqueous phase by water titration method. Required quantity of Drug was added to adequate amount of oil in a cleaned and dry beaker having a small magnetic bead and was mixed completely by magnetic stirrer and mixed by gentle stirring. The above mixture was finally allowed to titrate by distilled water under constant stirring on a magnetic stirrer. Store the Prepared nanoemulsion in sealed container at room temperature till further use [14].

Table no. 1 Formulation of Nanoemulsion

Batch No	Drug Ketoconazole	Coconut Oil (%)	Tween 80 + Ethanol (%)	Water (%)
F1	2	30	58%	68%
F2	2	35	53%	63%
F3	2	40	58%	58%
F4	**2**	**45**	**52%**	**53%**
F5	4	30	62%	66%
F6	4	35	61%	61%
F7	4	40	56%	56%
F8	4	45	50%	51%

Characterization of nanoemulsion

Viscosity:
The viscosity was measured to determine rheological properties of formulations. Brookfield Rheometer viscometer at 30˚C with a CPE 61 spindle at 30 rpm was used to serve this purpose. Results were taken in triplicate and the average was taken in to consideration[15].

pH:
Another important parameter of nanoemulsion is pH. The change in the pH may affect the zeta potential of the formulation which in turn can affect the stability of preparation. The pH of the formulations was measured using digital pH meter. Results were taken in triplicate and the average was taken in to consideration [16].

Drug content:

The drug content of Drug nanoemulsion formulation was measured as 278.2 nm using UV- VIS spectroscopic method. Results were taken in triplicate and the average was taken in to consideration[17].

Centrifugation:

This parameter was characterized to check the physical stability at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation.

Conductivity:

Electrical conductivity of formulated samples was measured using a digital conductometer at ambient temperature.

Dilution Test:

If the continuous phase is added in nanoemulsion, it will not crack or separate into phases. Here 50- and 100-times aqueous dilution of the formulation were visually checked for phase separation and clarity[18].

Globule size and Zeta potential analysis:

Nanoemulsion formulation was diluted 50 times and 100 tomes with distilled water. The resultant samples were prepared by gentle agitation for 5 min using a magnetic stirrer. In addition, globule size distribution (PSD) and zeta potential of the final nanoemulsion were determined using dynamic light scattering technique by Malvern zetasizer (NANO ZS)[19].

Stability of Drug nanoemulsion:

Samples of Drug nanoemulsion formulations was sealed in ampoules and then placed in Stability chambers at different temperature conditions i.e., room temperature (25°C) and accelerated temperature (40±2°C) for 2 months. The physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation), and a globule size analyzer was used to determine the mean globule size and zeta potential after dilution with water. Chemical stability was expressed as the content of Drug determined by UV visible spectroscopic method at 257 nm [20].

RESULTS AND DISCUSSION:

Determination of Solubility in various solvent:
The solubility of Ketoconazole was determined in various solvents. The drug was found soluble in chloroform and methanol, sparingly soluble in ethanol and almost insoluble in water represented in Table no.2

Table no.: 2 Ketoconazole Solubility Characteristics

S. No.	Solvent	Standard Solubility	Solubility (ppm)	Observed
1.	Dichloromethane	Freely Soluble	1-10	+++++
2.	Chloroform	Soluble	10-30	++++
3.	Methanol	Soluble	10-30	++++
4.	Ethanol	Sparingly Soluble	30-100	+++
5.	Water	Insoluble	>1000	-
6.	Ether	Insoluble	>10000	-

Determination of Partition Coefficient:

Partition coefficient of ketoconazole was shown in Table No.3

Table no: 3 Partition Coefficient of ketoconazole

S. No	Solvent \ drug	Reported value	Observed value
1.	n-octanol \ Chloroform: water+ ketoconazole	4.35	4.01

According to be observed value of partition coefficient ketoconazole was found to be acidic and lipophilic in nature.

Determination of Melting point:
The melting point was taken as mean of the three values i.e., 150℃,147℃,150℃. Therefore, the melting point of ketoconazole was calculated to be 149℃.

Table no.: 4 Melting Point of Ketoconazole

S. No	Drug	Reported melting range	Observe melting range
1.	Ketoconazole	148-152℃	149℃

FT-IR SPECTRUM

Infrared spectrum of drug was recorded over the KBr disc method and obtained spectra was shown in Figure no. 1. The FT-IR spectrum of Ketoconazole show the characteristic peaks at 1718.30 confirmed the presence of C= O functional group (α, β- unsaturated esters), characteristic peaks at 1507.71 confirmed the presence of N-O functional group (Nitro Compounds), characteristic peaks at 1457.39 confirmed the presence of C-H functional group (Alkane), characteristic peaks at 1384.71 confirmed the presence of C-H functional group (Aldehyde), characteristic peaks at 1114.47 confirmed the presence of C-N functional group (Aliphatic amines), characteristic peaks at 615.01 confirmed the presence of C-Br functional group (Alkyl halides). The observed FT-IR Spectra confirmed and identify the presence of functional group and purity of drugs. FT-IR Spectrum of Ketoconazole shown in Figure No.1.

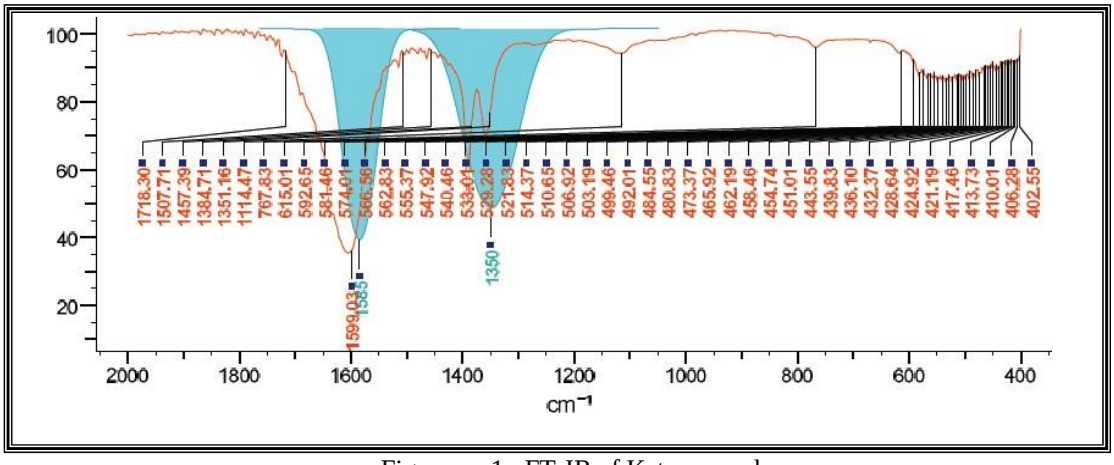

Figure no.1: FT-IR of Ketoconazole

Table no:5 Interpretation of Fourier Transform Infrared Spectrum of Ketoconazole

S. No.	Reference Peaks	Observed Peaks	Functional Group
1.	1730-1715	1718.30	C= O (α, β- unsaturated esters
2.	1550-1500	1507.71	N-O (Nitro Compounds)
3.	1470-1450	1457.39	C-H (Alkane)
4.	1390-1380	1384.71	C-H (Aldehyde)
5.	1250-1020	1114.47	C-N (Aliphatic amines)
6.	690-515	615.01	C-Br (Alkyl halides)

Solubilities studies of drug oil surfactant and co-surfactant:
Result of solubility study of drug in oil, surfactant and co-surfactant are shown in Table no.6

Table no.6- Ketoconazole Solubility Study Data

Excipients	Solubility (mg/ml)
Oil	
Seasame Oil	8.88±0.4
Coconut Oil	32.42±0.6
Olive Oil	24.91±0.4
Castor Oil	17.54±0.2
Arachis Oil	9.41±0.2
Surfactant	
Tween 20	12.09±0.6
Tween 80	22.75±0.3
Co-surfactant	
Methanol	68.5±0.4
Ethanol	120.5±0.5

D	4:1	+++

Formulation of nanoemulsion: Eight formulation were prepared by using different concentration of oils, S mix (3:1) and water and further evaluated for phase separation by centrifugation,

Table no.7 Optimization of Drug Loaded Nanoemulsion

Batch No.	Oil (%)	S mix (%)	Water (%)	Centrifugation
F1	2	30	68%	√
F 2	2	35	63%	√
F 3	2	40	58%	√
F 4	**2**	**45**	**53%**	**X**
F 5	4	30	66%	√
F 6	4	35	61%	√
F 7	4	40	56%	√
F 8	4	45	51%	√

(√- phase separation, ×- no phase separation)

Selected Nanoemulsion –After formulating eight nanoemulsion formulations, when we go for centrifugation of all formulation, we found that only F4 formulation is able to maintain its stability and didn't go for phase separation so we selected F 4 formulation composition for further study.

Table no-8 Composition of final Nanoemulsion

S. No	Ingredient	% w/w
1	Coconut oil	2
2	S mix (Tween 80: Ethanol) (4:1)	45
3	Water	53

Characterization of Nanoemulsion:

After characterization study of nanoemulsion we get pH, viscosity particle size globule size within limits shown in Table no- 9

Table no- 9 : Characterization of Nanoemulsiion

Characterization of Nanoemulsion		
S. No	**Test**	**Optimized drug loaded nanoemulsion**
1	% Assay	84.5 % ± 0.4%
2	pH	5.5 ± 0.2
3	Zeta potential(mV)	-2.06
4	Globule size (nm)	900.5
5	Weight/ml of ME	1.03 gm/ml
6	Viscosity (cp)	560.9
7	Particle size	955 nm
8	PDI	0.033

The release profile of Ketoconazole:

The percentage *in vitro* release of liposomal hydrogel was carried out after 1 hour of interval up to 6 hours. The results were shown in below Table No.10 and 11 and graphically represented in figure no.2 and 3.

Table no: 10 In-vitro drug release study

Sr. No	Time (min)	Nanoemulsion (%) ± SD	Marketed Product (%) ± SD
1	0	0	0
2	30	15.46 ± 0.3	8.08 ± 0.2
3	60	18.46 ± 0.5	14.23 ± 0.2
4	90	20.45 ± 0.2	18.66 ± 0.4
5	120	24.65 ± 0.1	21.45 ± 0.4
6	150	31.78 ± 0.6	26.03 ± 0.3
7	180	39.54 ± 0.4	34.4 ± 0.3
8	210	45.67 ± 0.5	39.89 ± 0.6
9	240	53.32 ± 0.5	47.8 ± 0.2
10	270	61.5 ± 0.3	55.76 ± 0.2
11	300	69.78 ± 0.4	64.5 ± 0.1
12	330	77.8 ± 0.6	71.32 ± 0.5
13	360	85.54 ± 0.5	79.34 ± 0.3

*n = 3

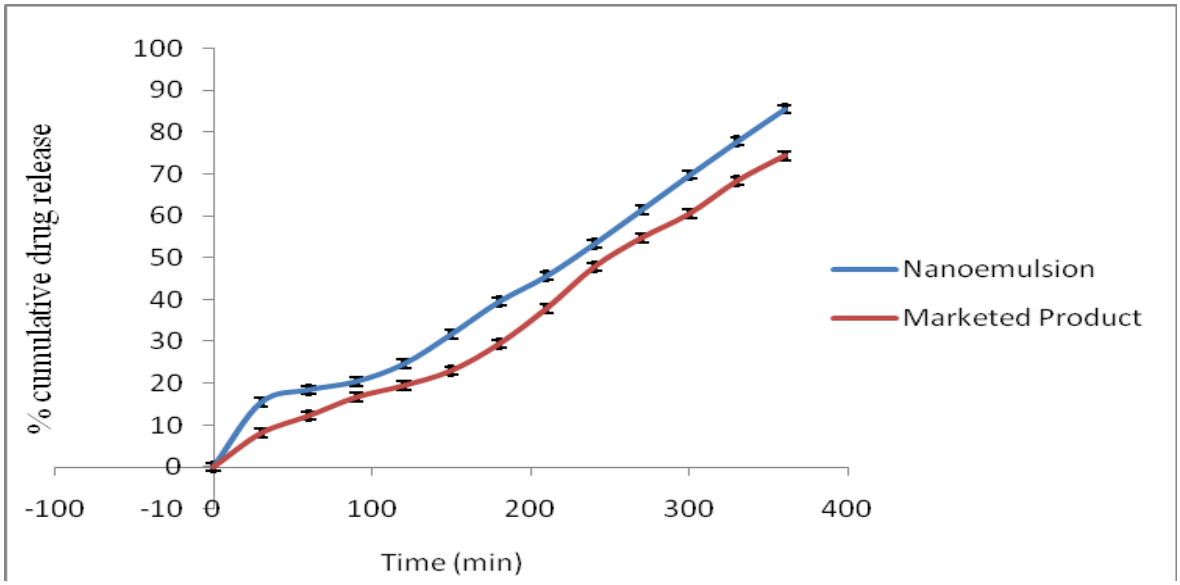

Figure no. :2 % Cumulative Drug Permeation

Table no: 11 Drug Permeation Study

Sr. No	Time (min)	Nanoemulsion (%) ± SD	Marketed Product (%) ± SD
1	30	6.33 ± 0.1	5.12 ± 0.2
2	60	12.48 ± 0.5	10.08 ± 0.3
3	90	16.36 ± 0.4	14.55 ± 0.6
4	120	22.15 ± 0.5	18.32 ± 0.4
5	150	27.07 ± 0.4	23.03 ± 0.3
6	180	32.92 ± 0.3	27.78 ± 0.4
7	210	37.5 ± 0.4	31.67 ± 0.6
8	240	43.38 ± 0.2	38.86 ± 0.1
9	270	50.67 ± 0.2	44.12 ± 0.3
10	300	58.87 ± 0.5	49.07 ± 0.2
11	330	65.8 ± 0.2	55.98 ± 0.4
12	360	72.45 ± 0.4	63.75 ± 0.4

*n = 3

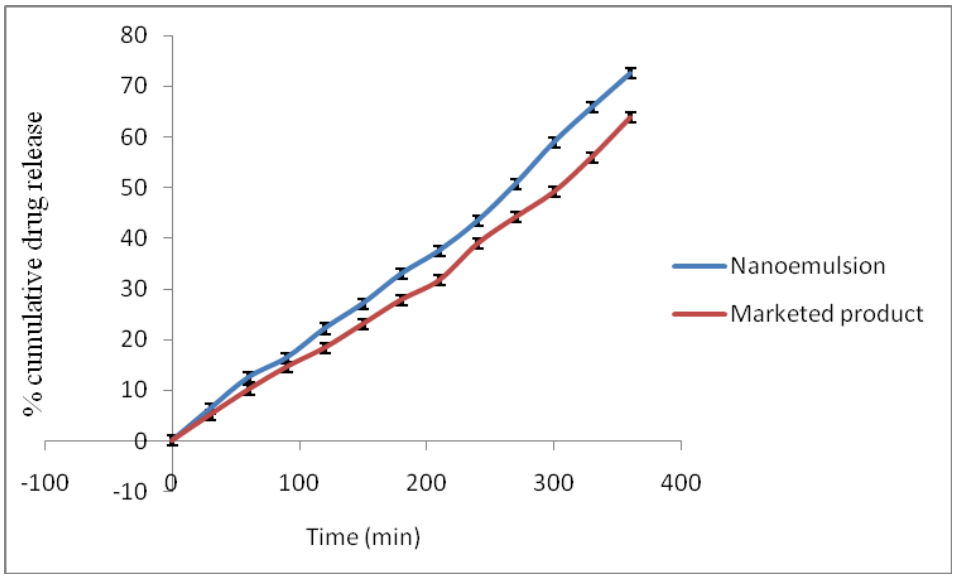

Figure no. :3 % Cumulative drug permeation

From the above graph, it can be concluded that the drug released form nanoemulsion was characterized by initial burst release in first few hours and later on providing a sustained release drug profile.

Stability study: Optimize preparation were checked Table no. 12 and 13, for

stability study for 2 months.

Table no: 12 Stability of Nanoemulsion at Room Temperature:

Data from the stability study revealed that no significant changes in % assay, and pH for both formulations at room temperature. Stability of nanoemulsion at accelerated 40˚C ±2˚C temperature:

Test	**Time Period**		
	Initial	1 Month	2 Month
% Assay	89.98%	89.98%	89.98%
Ph	5.55	5.55	5.55

Stability Study of Nanoemulsion

Data from the stability study revealed that no significant changes % assay, and pH for both formulations at accelerated 40˚C ± 2˚C temperature.

Table no: 13 Stability Study of Nanoemulsion

Test	**Time Period**		
	Initial	1 Month	2 Month
% Assay	89.98%	89.98%	89.98%
Ph	5.55	5.55	5.55

CCONCLUSION:

The present study was an attempt to develop nanoemulsion of Ketoconazole using by different concentration of surfactant and co surfactants agent by phase inversion temperature method. From above result concluded that Coconut oil based nanoemulsion drug delivery system can be effective for topical application in the treatment of fungal dieses and further study on a on animal being need to perform before its commercial use[21].

A number of surfactants with diverse characteristics (ionic or non-ionic) had been used with such nanoemulsions. Most widely used among them were nonionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide). Early nanoemulsions were oil-in-water (O/W) type emulsions with average droplet diameter ranging from 50 to 1000 nm[22].

Nanoemulsions more recently are classified into three categories such as O/W type (oil is dispersed in aqueous phase), water-in-oil (W/O) type (water is dispersed in oil phase), and bi-continuous (microdomains of water and oil are interspersed within the system). Transformation among these three types can be attained by altering the components of the emulsions. Multiple emulsions are also a type of nanoemulsions, where both O/W and W/O emulsions present simultaneously in one system[23]. For stabilizing these two emulsions, both hydrophilic and lipophilic surfactants are used simultaneously.

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Author's contributions All the authors have contributed equally.

Conflicts of interests Declared none

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