Fungal infection caused by yeast (type of fungus) such as genus Candida and especially species Candida albicans. According to the recent reports approximately 75% of women are infected by Candida albicans and suffering from vaginal candidiasis. About 50% of women are infected a second time by Candida albicans. Therefore, we choose Curcumin an antifungal agent that had reported antifungal properties against the various fungal species. The Curcumin-containing emulgel based microemulsion system was prepared for greater retention time and penetration across the vaginal mucosa.

Methods: The screening of oil phase, surfactant, and cosurfactant for microemulsion formulation was selected based on the solubility study and followed by the construction of the pseudoternary phase diagram. The oil phase, surfactant and co-surfactant are selected from the pseudoternary phase diagram for the formulation of a stable microemulsion. The prepared Curcumin-loaded microemulsion was characterized by globule size, polydispersity index, Zeta potential, accelerated stability study, drug content, percent transmittance and antifungal assay by broth microdilution technique. The formulated microemulsion was converted into a vaginal emulgel by using Pluronic®F127. The formulated curcumin-loaded emulgel was characterized by different evaluation parameters and antifungal study by agar well diffusion method.

Results: The result showed that the average globule size of emulgel was 286.3 nm, polydispersity index was 0.241, Zeta potential was +19.20 mv, conductivity was 0.0390 mS/cm, and drug content was found to be 95.58%. The texture of formulated emulgel was found to be soft and smooth, with shear-thinning, pseudoplastic behavior, and easily spreadable. The in vitro permeability study of emulgel shows slow and complete release of curcumin in 10 h. The microemulsion and developed emulgel showed promising antifungal activity against Candida albicans.

Conclusion: The developed curcumin-loaded emulgel showed promising antifungal activity against Candida albicans as compared to the Fluconazole as an standard antifungal antibiotic. Our formulated Curcumin-containing emulgel can be a potential alternative as compared to the conventional dosage form for the treatment of vaginal candidiasis.

Keywords: Curcumin, Microemulsion, Emulgel, Candida albicans, Fluconazole, In vitro permeability, Antifungal assay

INTRODUCTION

Vaginal candidiasis is a fungal infection caused by yeast (type of fungus) such as genus Candida and especially species Candida albicans. According to the recent reports approximately 75% of women are infected by Candida albicans and suffering from vaginal candidiasis. About 50% of women are infected a second time by Candida albicans and 5-8% of adult women are again infected with Candida albicans and other Candidal species and suffering from recurrent vulvovaginal Candidiasis (RVVC) [1, 2]. Overall, from these reported epidemiological data findings show that successful women-dependent prophylactic strategies are desperately needed. The vaginal emulgel has one of the approach which provides greater retention time on vaginal mucosa and a physical, chemical barrier to avoid Candida infection to the vagina [3].

The Candida albicans species developed resistance against various synthetic drugs; thus, it is required in high doses, which causes severe adverse effects, allergic reactions, and develop tolerance. As a result, there is an urgent need for a safe and efficient vaginal candidal that is effective against Candida albicans. The curcumin, which is obtained from rhizomes of the herb Curcuma longa L, containing polyphenolic compounds and which show antifungal, antimicrobial, anti-inflammatory, and antibacterial activity with less toxicity, less resistance and higher efficiency [4]. The Curcumin shows antifungal activity by a mechanism such as prevents hyphae growth by targeting TUP1, inducing oxidative stress, decrease ergosterol biosynthesis, and reducing the fungal oxoenzymes aspartate proteases (SAP) [5].

The curcumin is a class II molecule that shows high permeability, low solubility and their by curcumin show poor availability, less solubility, and lower therapeutic effects. The micro emulsion-based system not only avoid the above problems but also provide additional lipophilicity, enhances penetration power through the mucosa, modified the biopharmaceutical and physicochemical properties of curcumin, target specificity, slow degradation, and enhances shelf-life. These properties of microemulsion provide an requisite rationale for the fabrication of curcumin in microemulsion [6]. Thus, the present research focuses on the formulation and development of microemulsion and further explores into the emulgel. The prepared microemulsion in Isopropyl myristate (oil phase) of average globule size 339.0 nm overcome the issue of solubility, stability, permeability, and poor availability and they further characterized for accelerated stability study, Scanning electron microscopy, percent transmittance, drug content, In vitro drug release, and In vitro antifungal assay by broth microdilution method.

After that, the curcumin-loaded microemulsion is fabricated into the emulgel by using gelling polymer Pluronic®F127, which increases adhesivity, retention time and provides sustained release over a long period. The emulgel is further characterized for globule size, polydispersity index analysis, rheological studies, spreadability studies, texture profile analysis, drug content, In vitro permeability study, antifungal activity, and stability study [7]. The developed emulgel showed promising antifungal activity against Candida albicans and provided a potential alternative to the conventional drug delivery system for prophylaxis of vaginal candidiasis.

MATERIALS AND METHODS

Materials

Curcumin was kindly gifted by Konark Herbals and Healthcare, Mumbai, India. Glycerol monolaurate gifted by Mohini Organic Ltd.,
Mumbai, India. The other supporting materials like Isopropyl myristate, Sesame oil, Castor oil, olive oil, Tween-20, Tween-80, ethanol, isopropyl alcohol were procured from Modern Industries, Nashik, India. Tea tree oil, Polyethylene glycol-400, Butanol Disodium Hydrogen Phosphate, Methylparaben, Propyl paraben, Potassium Hydrogen Phosphate, Hydrochloric acid, Triethanolamine were obtained from Fine Chemical Industries, Mumbai, India. Oleic acid, Span-20, Span-80 purchased from Croda India Company Pvt. Ltd., Mumbai, India. Pluronic®F127 was purchased from Sigma-Aldrich, SAFC, Bangalore, India. All reagents were used were of analytical grade.

Reagents, media, and fungal strains
DMSO cell culture grade, Resazurin, RPMI-1640 medium supplemented with glutamine and phenol red, without bicarbonate, 3-(N-morpholino)propanesulfonic acid (MOPS), Sabouraud broth and Sabouraud agar medium purchased from Hi-Media Pvt. Ltd., Mumbai. Fluconazole was obtained as a gift sample from Cadila Pharma, Ahmedabad, Gujarat, India. The fungus strains (Candida albicans) were obtained from the Microbial Type Culture Collection (MTCC, India).

Methods
Solubility study
The solubility of curcumin in various oils (Tea tree oil, Isopropyl myristate, Glycerol monolaurate, Sesame oil, olive oil, Castor oil, and oleic acid), surfactants (Tween-20, Tween-80, Span-20, and Span-80) and co-surfactants (Polyethylene glycol-400, Butanol, Butanol, Isopropyl Alcohol) was determined by shaking flask method. This method was performed by adding an excess amount of curcumin was added to each vial containing 10 ml of suitable vehicle i.e. either oil, surfactant, and co-surfactant [8]. The mixture was vortexed or sonicated for 15 min in order to promote proper mixing of curcumin with the vehicles. After that, the mixtures were kept in an orbital shaking incubator for 48 h at 25 °C to facilitate the solubilization and achieve equilibrium. The mixtures were centrifuged at 5000 rpm for 15 min and the supernatant layer was filtered through a 0.45 mm membrane filter. The filtered solution was diluted with ethanol and the concentration of curcumin was determined by taking absorbance at 438 nm by using a UV spectrophotometer (UV-1800, Shimadzu Kyoto Co., Japan) [9].

Construction of pseudoternary phase diagram
The surfactants/co-surfactants that show better solubility were selected for the construction of the pseudoternary phase diagram by performing the water titration method. A uniform mixture of selected oil (Isopropyl Myristate), with selected surfactant (Tween-80) and co-surfactants (Isopropyl Alcohol) was titrated with the double-distilled water and a pseudoternary phase diagram was constructed. The selected surfactant to cosurfactant (Smix) was taken in different ratios 1:1, 2:1, 3:1, 1:2, and 1:3 and the mixture was shaken properly [10]. The mixtures of selected oils with selected surfactants/co-surfactant were prepared at weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 in different vials. After that, a small amount of double-distilled water drop wise by using a micropipette to the above mixtures and all the mixtures were stirred or vortexed continuously until homogenous dispersion was obtained and equilibrate at 25 °C for 30 min. Afterward, the mixtures were visually examined for transparency, phase separation, and flow properties. The end of the water titration was the point at which the mixture became turbid and shows phase separation. The ternary phase diagram was designed by CHEMIX School Ver. 9.00 and the microemulsion area (1 phase) and turbid (2 phase) area was plotted [11, 12].

Screening of Smix (Km value)
The Km value (ratio) for stable microemulsion was found out by the water titration method at various ratios of surfactant and cosurfactant [1:1, 2:1, 3:1, 1:2, and 1:3] and thereafter, the pseudoternary phase diagram was designed. The optimized value is selected from the pseudoternary phase diagram which shows the highest microemulsion (1 phase) area and it is further used for microemulsion preparation [13].

Formulation of curcumin-loaded microemulsion
The curcumin-loaded microemulsion was prepared by dissolving the selected concentration of curcumin in accurately weighed oil. The resultant oil phase mixture was mixed uniformly and heated up to 45-50 °C to form an oil phase [14]. The aqueous phase were prepared by adding an accurately weighed quantity of surfactant and cosurfactant (at selected ratio 3:1 from pseudoternary phase diagram) to the accurately weighed quantity of water in a vial and shake vigorously and prepared aqueous phase kept for heating at 45-50 °C [15]. The temperature of both the oil and aqueous phases was maintained in the range of 45-50 °C and the oil phase was added drop wise to the aqueous phase with continuous stirring using a magnetic stirrer and vortexed for 2 min to form a homogenous microemulsion. The formed microemulsion was sealed in a glass vial and the sealed vial stored at room temperature before further evaluations [16].

Effect of curcumin loading
The curcumin was added at various concentrations (0.6%, 0.89%, and 1.6% w/v) to the selected composition of oil, surfactant, and cosurfactant from an optimized pseudoternary phase diagram to form W/O microemulsions. The prepared microemulsion instantly analyzed for globule size, polydispersity index (PDI), and also examined for phase separation and drug precipitation for 24 h [13].

Evaluation of microemulsion
Measurement globule size, polydispersity index, and zeta potential
The average globule size, polydispersity index, and zeta potential of the microemulsion were measured by using the Zetasizer nano ZS: Malvern Instruments (UK) [13]. The microemulsion sample was loaded into the cylindrical cuvettes and placed in a thermostated scattering chamber. The light scattering was measured at a fixed 90 ° angles and temperature 25 °C. The small amount of microemulsion sample (1 or 0.1 ml) was diluted to 10 ml of double-distilled water (in a test tube and gently mixed) to make sure that light scattering intensity was within the sensitivity range of the instrument. The sample analysis performed triplicate for the confirmation of reproducibility in results [17, 18].

Accelerated stability study
The accelerated stability study of optimized curcumin loaded microemulsions was performed by subjected the microemulsion formulation to the centrifugation and freeze-thaw cycle.

Centrifugation: The microemulsion was centrifuged at 5000 rpm for 30 min. The microemulsion formulation was observed visually for phase separation and drug precipitation (Creaming) [17].

Freeze-thaw cycle: The microemulsion sample subjected to-20 °C for 24 h and then another 24 h at 40 °C. The physical stability of the microemulsion was examined by measuring globule size and polydispersity index before and after the freeze-thaw cycle and centrifugation [19].

Scanning electron microscopy
The morphology of Curcumin-loaded microemulsion was studied by using a scanning electron microscope (SEM) [13]. The microemulsion was diluted with double distilled water 100 times. After that, approximately 10 μl samples were deposited on the porous carbon grid and allow it to dry. Thereafter the sample was subjected to analysis under a scanning electron microscope (SEM) [20].

Percent transmittance
The % transmittance of the microemulsion was determined by using a UV-Visible spectrophotometer (UV-1800, Shimadzu Kyoto Co., Japan) [20]. The microemulsion was diluted 10 and 100 times with distilled water and % transmittance measured at 650 nm and keeping the distilled water as blank [21].

% Transmittance = Antilog (2-Absorbance)
Drug content

The drug content from the microemulsion was determined by 1.2 ml (equivalent 10 mg of curcumin) of curcumin loaded microemulsion was diluted with 100 ml of ethanol in 100 ml volumetric flask and the resultant mixture stirred for 30 min and centrifuged at 1000 rpm at 25 °C for 15 min [17, 19]. The resultant mixture is filtered through Whatman filter paper. The 1 ml filtrate was diluted with 10 ml ethanol and the drug content was measured by UV-Visible Spectrophotometer at 438 nm [21].

In vitro drug release profile

The in vitro permeability study of curcumin-loaded microemulsion was conducted by Franz diffusion cell. The cellophane membrane and phosphate buffer solution of pH 5 was used for in vitro permeability study. The cellophane membrane was placed between donor and receptor compartments and it has a diffusion area of 1.77 cm². The 10 ml phosphate buffer (pH 5) was filled in the receptor compartment and this phosphate buffer in the receptor compartment of the Franz diffusion cell was kept under magnetic stirring at 500 rpm at 37±0.5 °C to avoid the stagnant layer formation. The 1 gm of curcumin-loaded microemulsion was filled in the donor compartment and the temperature of the system was maintained at 37±0.5 °C. At the specific time interval, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h, 1 ml aliquots were withdrawn from the receptor compartment through a side tube and replaced with a fresh medium. The concentration or amount of curcumin permeated across vaginal mucosa was determined by suitable dilution using a UV-Visible spectrophotometer at 438 nm [13, 22].

Antifungal assay of curcumin-loaded microemulsion

Antifungal assay of Curcumin-loaded microemulsion was performed by using broth microdilution technique by using 96-well microplates under the protocol of the National Committee for Clinical Laboratory Standards. The RPMI-1640 medium whose pH 7 was adjusted by 0.1 mol/l sodium hydroxide at 25 °C used as broth medium for dilution of fungal inoculum suspension and final dilution of the test sample (microemulsion) and standard sample. The fungal strain inoculum was sub-cultured into the broth medium (RPMI-1640 medium) and incubated at 25-30 °C for 48 h. The prepared suspension of fungal stain was added to the 5 ml distilled water and further diluted to match the turbidity of a 0.5 McFarland standard to achieve concentration 1×10³ CFU/ml–5×10³ CFU/ml. To achieve final concentration 0.5×10³ –2.5×10³ CFU/ml. The standard antifungal drug used was fluconazole and DMSO solution was also included. The prepared microplate covered with a plastic bag to avoid evaporation and to maintain sterility and further incubated for 48 h at 37°C. The prepared suspension of fungal stain was added to each well in the microplate. Starting with a final concentration of 100 mg/ml. After that, they further diluted with RPMI-1640 medium (broth medium) to 1:50. The important part to perform this assay is the preparation of a 96-well microplate (clear and flat bottom). To prepare a microplate 50µl of RPMI-1640 medium is added to each well in the microplate. Starting with a final concentration of 0.05µg/ml, sample solutions (50µl) were serially diluted two-fold in the microplate with the RPMI-1640 medium. The sample analysis performed using Brookfield viscometer (Brookfield Synchro-Lectic Viscometer (Model RVT)) with helipath stand. The 30 gm of micro emulsion-based gel sample was placed in a beaker and was allowed to equilibrate for 5 min. The viscosity of the emulsion determined using spinning-rotating spindle at different rpm (0.5, 1, 2.5, and 5 rpm) by using spindle no 62 [29]. Placed the spindle in a gel formulation and noted the dial reading on the viscometer at each rpm of spindle or speed. The spindle speed was successively lowered and the equivalent dial reading was noted. The measurements were carried in triplicate ambient temperature. The viscosity in centipoises is finding out by the direct multiplication of the factors given in the Brookfield Viscometer catalogue with dial readings [30, 31].

Spreadability studies

The spreadability of Curcumin-loaded emulgel was determined by Brookfield Texture Analyzer CT 3 in TPA mode. The formulated emulgel was transferred into the lower cone. Care should be taken to avoid introducing air in the gel samples. A conical analytical probe (45°) was forced down into the gel sample at a defined depth (12 mm) and at a defined speed (2 mm/second) [35]. From the resulting force-time plot, compressibility (the work required to deform the product during the first pass of probe), hardness (the force required to attain given deformation), and the adhesiveness (the work required to overcome the attractive forces between the surface of a sample and the surface of the probe were derived) were calculated [32, 33].

Texture profile analysis

The texture profile analysis of the curcumin-loaded emulgel formulation was determined using Brookfield Texture Analyzer CT 3 in TPA mode. The formulated emulgel was transferred into the lower cone. Care should be taken to avoid introducing air in the gel samples. A conical analytical probe (45°) was forced down into the gel sample at a defined depth (12 mm) and at a defined speed (2 mm/second) [35]. From the resulting force-time plot, compressibility (the work required to deform the product during the first pass of probe), hardness (the force required to attain given deformation), and the adhesiveness (the work required to overcome the attractive forces between the surface of a sample and the surface of the probe were derived) were calculated [32, 33].

Drug content

The drug content from the curcumin-loaded emulgel was determined by 1.7 gm of (equivalent to 10 mg of curcumin) curcumin-loaded emulgel diluted with 100 ml of ethanol, 100 ml volumetric flask and the resultant mixture stirred for 30 min at 25°C for 2 h to get homogeneous and smooth emulgel [28].

Evaluation curcumin-loaded emulgel

pH

The pH of prepared curcumin loaded emulgel was measured by using Eutech Digital pH meter at room temperature (25 °C) and it is calibrated or standardized by using pH 4 and 7 buffers before use [29]. The measurements of pH were carried out in triplicate for reproducibility of the result [30].

Globule size and polydispersity index, and zeta potential analysis

The average globule size, polydispersity index, and zeta potential of the curcumin-loaded emulgel were determined by dispersed gel into distilled water, and kept for sonication (20–30 s) and after that for vortexed for 1 min to make sure that to minimize the aggregation if present in microemulsion gel [19]. After that, the sample was analyzed for average globule size, polydispersity index, and zeta potential by using Zetasizer (Nano ZS: Malvern Instruments, UK), with an angle of 90 °C at 25 °C. The sample analysis performed triplicate for the confirmation of reproducibility in results [27].

Rheological studies

The viscosity of curcumin-loaded emulgel was determined by Brookfield viscometer (Brookfield Synchro-Lectic Viscometer (Model RVT)) with helipath stand. The 30 gm of micro emulsion-based gel sample was placed in a beaker and was allowed to equilibrate for 5 min. The viscosity of the emulsion determined using spinning-rotating spindle at different rpm (0.5, 1, 2.5, and 5 rpm) by using spindle no 62 [29]. Placed the spindle in a gel formulation and noted the dial reading on the viscometer at each rpm of spindle or speed. The spindle speed was successively lowered and the equivalent dial reading was noted. The measurements were carried in triplicate ambient temperature. The viscosity in centipoises is finding out by the direct multiplication of the factors given in the Brookfield Viscometer catalogue with dial readings [30, 31].
In vitro permeability study

The in vitro permeability study of curcumin-loaded emulgel was conducted by Franz diffusion cell. The cellophane membrane and phosphate buffer solution of pH 5 was used for in vitro permeability study. The cellophane membrane was placed between donor and receptor compartments and it has a diffusion area of 1.77 cm² [19]. The 10 ml phosphate buffer (pH 5) was filled in the receptor compartment and this phosphate buffer in the receptor compartment of the Franz diffusion cell was kept under magnetic stirring at 500 rpm at 37±0.5 °C to avoid the stagnant layer formation [36, 38]. To 1 gm of curcumin-loaded emulgel was filled in the donor compartment and the temperature of the system was maintained at 37±0.5 °C. At the specific time interval, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h, 1 ml aliquots were withdrawn from the receptor compartment through a side tube and replaced with a fresh medium. The concentration or amount of curcumin permeated across vaginal mucosa was determined by suitable dilution using a UV-Visible spectrophotometer at 438 nm [34, 35].

Stability study

The prepared curcumin-loaded emulgel were placed in the airtight glass container in a clean and dry place for the stability study and this was stored at conditions such as 40 °C/75% relative humidity (RH), according to the guidelines of ICH for a period of 3 mo [29, 30]. After that, at particular time intervals (0, 30, 60, and 90 d), the formulations were withdrawn and checked for pH, viscosity, visual appearance, texture, and drug content [31, 33].

Antifungal activity

The Curcumin-loaded emulgel was tested by using an agar well diffusion method against Candida albicans strain. The potato dextrose agar medium was prepared by adding a sufficient amount of water into it by using an autoclave. The prepared potato dextrose agar medium was solidified, the fungal strain of Candida albicans was dispersed in the medium [36, 37]. After that, with the help of a sterile stainless steel borer 10 mm wells cut in the solidified medium. The prepared Curcumin-loaded emulgel and placebo were filled into each of the wells by using a sterile syringe. The prepared petri plate was kept for 48 h at room temperature (28-35 °C) for providing a suitable environment for the growth of Candida albicans. Thereafter, each of the wells was observed visually and the diameter of inhibition was calculated [38, 39].

RESULTS AND DISCUSSION

Solubility study

The solubility of curcumin in different oils is shown in fig. 1(A). The curcumin shows the highest solubility in isopropyl myristate and castor oil. However, Isopropyl myristate was reported to have increased skin absorption by acting as a skin penetration enhancer. Therefore, isopropyl myristate was chosen as the oil phase. The solubility of curcumin in various surfactants is shown in fig. 1(B). The Tween-80 exhibited the highest solubility for curcumin. Therefore, Tween-80 was selected as a surfactant. The solubility of curcumin in various cosurfactants as shown in fig. 1(C), the isopropyl alcohol shows the highest solubility of Curcumin therefore it was selected as cosurfactant. The selected isopropyl myristate (oil phase), Tween-80 (Surfactant), and co-surfactant (isopropyl alcohol) were further used for the construction of the pseudoternary phase diagram.

Solubility of curcumin in cosurfactant

Construction of pseudoternary phase diagram

The pseudoternary phase diagrams were constructed for the microemulsion system of Isopropyl myristate-Tween 80-Isopropyl Alcohol (IPM-Tween 80-IPA) by using a water titration method as shown in fig. 2. Each edge of the diagram represents each component of microemulsion i.e. Isopropyl myristate, Smix (Surfactant/Cosurfactant), and water. The gray shaded or highlighted region in the pseudoternary phase diagrams indicate the microemulsion region (clear, one phase, transparent, and absence of turbidity), and the remaining unshaded part indicates the turbid and phase separation region.

Among all the pseudoternary phase diagrams, the plot with Smix ratio 3:1 shows the highest microemulsion region (fig. 2C). Initially, the cosurfactant concentration kept constant and the concentration surfactant increased. As shown in fig. 2C, as the surfactant concentration was increased with concern to the cosurfactant (Smix ratio 3:1), the microemulsion area was increased as compared to the Smix ratio 1:1 and 3:1 in fig. 2A and fig. 2B respectively where surfactant concentration is lower. After that, the concentration of surfactant kept constant and cosurfactant concentration is increases (Smix ratio 1:2 and 1:3) fig. 2D and fig. 2E the microemulsion area was found to be decreased. Thus, to form a high microemulsion region required a high amount of surfactant and lower amount of cosurfactant.
Screening of smix (km value)
The pseudoternary phase diagram was constructed to determine the effect of the Smix ratio (km value) on the area of the microemulsion system. It has been reported that the stability of the microemulsion is dependent on the Km value. The different km values of Tween-80 and Isopropyl alcohol viz 1:1, 2:1, 3:1, 1:2, and 1:3 were taken for screening study. From the pseudoternary phase diagram, it was observed that the km value of 3:1, exhibited the highest microemulsion region of globule size 339.0 nm (fig. 2C). This will be found that the appropriate ratio of Tween 80, and isopropyl alcohol, which stabilizes the oil globule in microemulsion. Therefore the isopropyl myristate as oil phase, Tween 80, and isopropyl alcohol (km value 3:1) as surfactant and cosurfactant respectively were finalized for the formulation of microemulsion.

Formulation, optimization, and evaluation of microemulsion

Formulation of curcumin-loaded microemulsion
The optimized curcumin-loaded w/o microemulsion was prepared by loading 0.89 % of curcumin in the selected composition of isopropyl myristate, Tween-80, and isopropyl alcohol (S mix ratio 3:1). The microemulsion was evaluated for physical stability, accelerated stability study, globule size, zeta potential and polydispersity index, conductance, etc. The Curcumin-loaded w/o microemulsion was found to be a clear pale yellow-colored, homogenous, transparent system in appearance.

Effect of curcumin loading
The curcumin in different concentrations 0.6%, 0.89%, and 1.6% (w/v) was loaded into the optimized composition of oil, surfactant, and cosurfactants, obtained from pseudoternary phase diagram. It was found that the curcumin of concentration up to 0.89% can be effectively loaded in the optimized w/o microemulsion composition and it has physical stability (no phase separation and no drug precipitation) and expected globule size. Curcumin at 1.6% concentration becomes a turbid, shows phase separation and drug precipitation.

Evaluation of microemulsion
Measurement globule size, polydispersity index, and zeta potential
The average globule size of microemulsion was found to be 339.0 nm (fig. 3) with a 0.144 polydispersity index and zeta potential +44.47 mv
The globule size distribution graph was found to within an even size distribution range. The polydispersity index of microemulsion was found to be 0.144, which shows that the w/o microemulsion system had uniformity in globule size and higher stability.

![Graph](image)

**Fig. 3: Particle size distribution curve of microemulsion**

**Fig. 4: Zeta potential graph of curcumin-loaded microemulsion**

### Accelerated stability study

The globule size and polydispersity index of microemulsion were increased in microemulsion but they don't have any significant effect on the stability of the microemulsion system. There is a significant change in the zeta potential and conductivity of a w/o microemulsion. The zeta potential was decreased (39.76mV) and increased (52.86mV) after centrifugation and freeze-thaw cycle. There is no phase separation and no drug precipitation that occurs after the centrifugation and freeze-thaw cycle.

**Fig. 5: Scanning electron microscopy**

### Drug content and percent transmittance

The drug content of Curcumin-loaded microemulsion was found to be 96.50 % and it showed that the curcumin was uniformly distributed throughout w/o microemulsion.

The % transmittance of Curcumin-loaded microemulsion without dilution, after 10 times, and 100 times dilution with distilled water was found to 70.89%, 89.12% and 93.54% be and it represented the transparency and stability of microemulsion formulation.

### In vitro release study

The in vitro drug diffusion study of curcumin-loaded microemulsion was performed by using the Franz Diffusion cell apparatus in phosphate buffer solution of pH 5. The drug release from microemulsion formulation exhibited 89.96% drug release in 10 h and the result depicted in fig. 6. This clearly indicates that the microemulsion has enhances the solubility of curcumin and it has the ability to permeate through the skin. It was observed that there was an increased permeation of curcumin across the membrane by using isopropyl myristate as oil phase in microemulsion formulation.

### Antifungal assay of curcumin-loaded microemulsion

The results showed that curcumin-loaded microemulsion and standard Fluconazole showed IC₅₀ value at 19.34 µg/ml and 38.12µg/ml respectively and which indicate that curcumin emulgel is most effective against Candida albicans than standard Fluconazole drug. The probable reason will be the incorporation of curcumin into the microemulsion based system and further fabricated into emulgel which increase the permeability rate of curcumin across the membrane. The result also revealed that the Candida albicans develop resistance against fluconazole and other antifungal drugs, thus curcumin is effective antifungal agent against Candida albicans as compare to the synthetic drugs.
Formulation of curcumin-loaded emulgel

The prepared emulgel of with 10% concentration of Pluronic®F127 is pale yellow-colored with a smooth texture, shear-thinning, and pseudoplastic behavior. Thus, 10% concentration of Pluronic®F127 is considered as the finalized concentration for the development of curcumin-loaded emulgel.

Evaluation of curcumin-loaded emulgel

Measurement globule size, polydispersity index, and zeta potential

The average globule size of microemulsion-based gel was found to be 286.3 nm with a 0.241 polydispersity index, and zeta potential+19.20. All these parameters are in the acceptable range of microemulsion-based gel and provide greater physical and chemical stability.
Physicochemical characterization of emulgel

The prepared emulgel is clear, pale yellow-colored, providing a lubricant feel to the skin, smooth, and soft in nature, and has a non-staining effect on the skin. The pH of the microemulsion-based gel (emulgel) was found to be 6.6±0.2 which is acceptable for vaginal application and does not cause irritation to the skin. The viscosity microemulsion-based gel (emulgel) at 5 rpm was found to be 11343±2cps. The formulated emulgel has a good consistency, hardness, spreadability (15.33 cm/min) and from the rheological study it is found that emulgel as shear thinning, pseudoplastic behavior, and which is easy to apply to the skin.

Drug content

The drug content in the emulgel was found to be 95.58%, which ensures that there was a very slight change in concentration or curcumin content in microemulsion as well as emulgel and this indicates that no degradation of the curcumin.

Texture profile analysis

The maximum force required to obtain a peak maximum or a positive peak which indicates good firmness. The area under the positive curve is measures the energy required to deform the formulation sample to define depth grade in order of its stability. Higher the firmness value indicates greater the hardness and thicker the gel and low spreadability. The negative force on the graph indicates the lesser adhesive force required by the formulation, the more negative value in the graph indicates the more sticky the formulation (greater adhesiveness). Adhesiveness is the area under the negative graph (energy required to break the sample-probe contact). The formulated gel was found to be medium firmness and not harder in nature, therefore it is easily spreadable and shows shear-thinning, pseudoplastic behavior (fig. 8.24). The formulated gel was found to be 0.33 ml and 0.39 respectively, which indicates that prepared gel not sticky and don’t show staining.

In vitro permeability study

The in vitro drug diffusion study of curcumin-loaded emulgel was performed by using Franz Diffusion cell apparatus in a phosphate buffer solution of pH 5. The drug release from the microemulsion based gel exhibited 82.35% drug release in 10 h and the result is depicted fig. 11. It was observed that the cumulative amount of drug permeated through the cellophane membrane was found to be 82.35% in 10 h. This clearly indicates that the microemulsion-based gel (emulgel) system has enhances the retention power of the formulation on the skin surface. The curcumin-loaded emulgel showed the slowed and complete permeation of the drug until 10 h. The slow permeation of curcumin across the membrane is due to the formulation of gel matrix with Pluronic®F127, which slows down the drug across the membrane and retained the formulation for a longer period of time on the application site.

Antifungal activity of curcumin-loaded emulgel

In this study, the In vitro activity of Curcumin-loaded microemulsion-based gel against Candida albicans expressed as fungal inhibition zone diameter, are shown in fig. 13. By measuring the zone of inhibition after the incubation period, it was found that the curcumin-loaded emulgel had a remarkably greater zone of inhibition and superior in vitro antifungal activity against Candida albicans compared to the placebo gel. The value of zone of inhibition for curcumin-loaded emulgel and placebo gel was found to be 18 mm and 7 mm, respectively.

Fig. 10: Texture analysis graph of curcumin-loaded emulgel

Fig. 11: In vitro permeability study of curcumin-loaded emulgel
Stability study of emulgel
The data from the stability study suggests that the formulated curcumin-loaded emulgel showed good shelf stability, clear, pale yellow colored, physically and thermodynamically stable. There is a slight change in the pH and viscosity of microemulsion but not varied to a greater extent. The drug content of curcumin was found to be constant which indicate that emulgel maintained their potency and efficacy after the 3 mo stability study.

DISCUSSION
In the solubility study of curcumin in various oils, surfactants, and co-surfactants, it has been found that maximum solubility of curcumin was found to be in isopropyl myristate, Tween-80, and Isopropyl alcohol. Based on the resulted data of the solubility study, isopropyl myristate was selected as oil phase, and Tween-80 and Isopropyl alcohol selected as surfactant and cosurfactant, respectively for the construction of pseudoternary phase diagram and formulation of the microemulsion.

To find out the Smix (km value), the pseudoternary phase diagrams are plotted, using different Smix ratios (km value) (1:1, 1:2, 1:3, 2:1, and 3:1). From the results of pseudoternary phase diagrams, it was observed that Smix ratio 3:1 (km value=3), shows the maximum microemulsion area in the plot as shown in fig. 2(C). The increased concentration of Tween-80 is an optimized pseudoternary phase diagram and provides a greater w/o microemulsion region. The microemulsion region represents clear, transparent, homogenous, and thermodynamically stable microemulsion and doesn’t show phase separation when kept on standing. The curcumin-loaded w/o microemulsion was formulated by adding different concentrations [0.6%, 0.99%, and 1.6% (w/v)] of curcumin to the optimized composition of w/o microemulsion selected from the microemulsion region pseudoternary phase plot (Smix 3:1). The curcumin-loaded w/o microemulsion consists of curcumin concentration 0.89 % and was found to be a clear, isotatic, thermodynamically stable, and pale yellow colored microemulsion system. The prepared Curcumin-loaded w/o microemulsion was characterized for different physicochemical evaluation parameters.

The characterization of curcumin-loaded w/o microemulsion shows the formation of spherical globule with size 339.0 nm and polydispersity index 0.144. The polydispersity index value shows that the w/o microemulsion system had uniformity in globule size and higher stability because the higher the polydispersity index lower the uniformity of globule size in the microemulsion and formulation have polydispersity index ≤5 have greater stability [40]. The zeta potential of prepared w/o microemulsion was found to be 44.47 mv which indicate good dispersibility of microemulsion formulation and less aggregation with good stability. The accelerated stability study of w/o microemulsion by centrifugation and freeze-thaw cycle shows that, doesn’t show phase separation and drug precipitation. But there is a slight change in globule size, polydispersity index, and zeta potential.

The drug content of Curcumin-loaded microemulsion was found to be 96.50 % and it showed that the curcumin was uniformly distributed throughout w/o microemulsion. The percentage transmittance of plane w/o microemulsion, after 10 times dilution and after 100 times dilution was found to be 70.89%, 89.12%, and 93.54%, respectively and it represents the transparency and stability of the w/o microemulsion. The incorporation of curcumin into a w/o microemulsion system indicates the improvement in solubility profile and with the complete release in 10 h and it indicates that increased permeation of curcumin across the membrane by using isopropyl myristate as oil phase and acts as a penetration enhancer. Antifungal assay of Curcumin-loaded microemulsion by microdilution technique shows that, the IC50 value of Curcumin-loaded microemulsion and Fluconazole was found to be 19.34 µg/ml and 38.12 µg/ml respectively and which indicates that curcumin are most effective against Candida albicans than Fluconazole. The data from the stability study suggests that the formulated w/o microemulsion is clear, homogenous, transparent, and thermodynamically stable and doesn’t show the phase separation.

The curcumin-loaded microemulsion-based system has poor retention on the vaginal mucosa because of its liquid state and exhibits poor and short-term efficacy. Thus, to solve a problem and improve the retention, the curcumin-loaded w/o microemulsion was formulated into an emulgel dosage form by using a Pluronic® F127 as a gelling agent (10%). The prepared emulgel was subjected to various physical evaluation parameters. The Globule size, polydispersity index, and zeta potential of Curcumin-loaded emulgel were found to be 286.3 nm, 0.241, and +19.20, respectively. All these parameters are in the acceptable range of emulgel and provide greater physical and chemical stability.

The prepared curcumin-loaded emulgel gel was clear, pale yellow colored, providing a lubricant feels to the skin and the pH of the emulgel was found to be 6.6±0.3, which acceptable for vaginal application. The viscosity of curcumin-loaded emulgel at 5 rpm was found to be 11.34±2.329. Viscosity is the rheological parameter concerned with the physical and mechanical properties of the gel as such as hardness, spreadability, consistency. From the viscosity study, we found that the formulated emulgel has a good consistency, hardness, soft and smooth texture, and which is easy to apply to the skin. From the texture profile analysis, it was found that formulated emulgel was found to be medium firmness and not harder in nature, therefore it is easily spreadable and shows shear-thinning, pseudoplastic behavior. The adhesiveness and cohesiveness of the prepared emulgel was found to be 0.33 mJ and 0.39 respectively, which indicates that prepared emulgel not sticky and don’t show staining. The spreadability of microemulsion-based gel was found to be 15.33 cm/5 min.

The drug content in the curcumin-loaded emulgel was found to be 95.58%, which ensures that their no degradation of the curcumin in microemulsion as well as in emulgel gel. The curcumin-loaded emulgel showed the slowed and complete permeation of the drug until 10 h. The slow permeation of curcumin across the membrane is due to the formulation of gel matrix with Phrronic® F127, which slows down the drug across the membrane and retained the formulation for a longer period of time on the application site.
The value of zone of inhibition for curcumin-loaded emulgel and placebo gel was found to be 18 mm and 7 mm (fig 12), respectively and curcumin-loaded emulgel had a remarkably greater zone of inhibition and superior in vitro antifungal activity against Candida albicans compared to the placebo gel. From the stability study, we found that there is no change in the appearance, pH, viscosity, spreadability, and drug content of Curcumin-loaded emulgel, and maintained their efficacy and potency.

CONCLUSION

The curcumin loaded emulgel was successfully developed for the delivery of curcumin in the vagina for the treatment of vaginal candidiasis. The conversion of microemulsion into emulgel makes it a dual control release system and overcomes the problems associated with microemulsion such as phase separation, creaming are resolved. The drug release from microemulsion formulation exhibited 89.96% drug release in 10 h and this clearly indicates that the microemulsion has enhances the solubility of curcumin and penetration across the vaginal mucosa. The physicochemical characteristics of emulgel has better mechanical characteristics and has a soft and smooth texture. Based on in vitro permeability study shows that the prepared emulgel has slowed and complete permeation of the curcumin and it is due to the formulation of gel matrix with Pluronic®F127, which retained the formulation for a longer period of time on the application site and releases curcumin in a sustained manner. The developed emulgel showed promising antifungal activity against Candida albicans. The curcumin-loaded emulgel was a potential alternative as compared to the conventional dosage for the treatment of vaginal candidiasis.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA

Not applicable

ACKNOWLEDGEMENT

Authors are highly thankful to Rajiv Gandhi Science and Technology Commission, Mumbai (Maharashtra) for financial support and DST-FIST supported Laboratory, Sanjivani college of Pharmaceutical Education and Research Kopargaon Maharashtra for necessary instrumentation and facilities to carry out the Research. The author is very thankful to the Principal and all supporting members of the institutions for providing all the facilities to conduct this research work. Finally, the author is thankful to everyone for providing direct or indirect support to conduct this study.

FUNDING

Not applicable

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declare no conflict of interest, financial or otherwise. The author alone is responsible for the content and writing of the article.

REFERENCES

1. Nagashima M, Yamagishi Y, Mikamo H. Antifungal susceptibilities of Candida species isolated from the patients with vaginal candidiasis. J Infect Chemother. 2016;22(2):124-6. doi: 10.1016/j.jiac.2015.08.008, PMID 26262736.
2. Palmeira-de-Oliveira R, Palmeira-de-Oliveira A, Martinez-de-Oliveira J. New strategies for local treatment of vaginal infections. Adv Drug Deliv Rev. 2015;92:105-22. doi: 10.1016/j.addr.2015.06.008, PMID 26144995.
3. Sobel JD, Faro S, Force RW, Fousan B, Ledger WJ, Nyirjesy PR, Reed BD, Summers PR. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol. 1998;178(2):203-11. doi: 10.1016/s0002-9378(98)80001-x, PMID 9500475.
4. Alasamydai A, Jabar N. Pharmacological aspects of curcumin: review article. Int J Pharmaceut. 2018;5(6):313-26.
5. Andrade JT, Fantini de Figureiredo G, Cruz LF, Eliza de Moraes S, Souza CDF, Pinto FCH, Ferreira IMS, Araújo MGM. Efficacy of curcumin in the treatment of experimental vulvovaginal candidiasis. Rev Iberoam Micol. 2019;36(4):192-9. doi: 10.1016/j.riamol.2019.01.003, PMID 31757595.
6. Talegaonkar S, Azeem A, Ahmad FJ, Khan RK, Pathan SA, Khan ZI. Microemulsions: A novel approach to enhanced drug delivery. Recent Pat Drug Deliv Formul. 2008;2(3):238-57. doi: 10.2174/187221087686241679, PMID 19075911.
7. Sreevidya VS. An overview on emulgel. Int J Pharm Phytopharm Res. 2019;9(3):92-7.
8. Thakkar V, Shah A. Optimization of self-microemulsifying drug delivery system containing curcumin and artemisinin using D-optimal mixture design. Saudi J Pharm Sci 2017;3(5):388-98.
9. Hu L, Jia Y, Niu F, Jia Z, Yang X, Jiao K. Preparation and enhancement of oral bioavailability of curcumin using microemulsion vehicles. J Agric Food Chem. 2012;60(29):13737-41. doi: 10.1021/jf204078r, PMID 22588756.
10. Hao Y, Ding W, Wei J. Preparation of a bis-demethoxy curcumin microemulsion based on pseudo-ternary phase diagrams and an orthogonal test analysis. J Pest Sci. 2011;36(2):248-51. doi: 10.1594/jpestics.G10-79, PMID 22341488.
11. Zhang H, Shen Y, Bao Y, He Y, Feng F, Zheng X. Characterization and synergistic antimicrobial activities of food-grade dilution-stable microemulsions against Bacillus subtilis. Food Res Int. 2008;41(5):495-9. doi: 10.1016/j.jfoodres.2008.02.006.
12. Zhang H, Shen Y, Wang P, Zhao G, Feng F, Zheng X. Antimicrobial activity of a food-grade fully dilutable microemulsion against Escherichia coli and Staphylococcus aureus. Int J Food Microbiol. 2009;135(3):211-5. doi: 10.1016/j.jfoodmicro.2009.08.015, PMID 19717202.
13. Mirani A, Kundaikar H, Velhal S, Patel V, Bandivdekar A, Degani M, Patrawale V. Tetrahydrocurcumin-loaded vaginal nanomicrobicide for prophylaxis of HIV/AIDS: in silico study, formulation development, and in vitro evaluation. Drug Deliv Transl Res. 2019;9(4):828-47. doi: 10.1007/s13346-019-00633-2, PMID 30990133.
14. Lin GC, Lin HY, Chi MH, Shen CM, Chen HW, Yang WJ, Lee MH. Preparation of curcumin microemulsions with food-grade xanthan gum/lecithin and their cytotoxicity on the HepG2 cell line. Food Chem. 2014;154:282-90. doi: 10.1016/j.jfoodchem.2014.01.012, PMID 24518344.
15. Chen YC, Chen BH. Preparation of curcuminoid microemulsions from Curcuma longa L. to enhance inhibition effects on growth of colon cancer cells HT-29. RSC Adv. 2016;8(5):2323-37. doi: 10.1039/C5RA22977G.
16. Das S, Lee SH, Chia VD, Chow PS, Mabeath C, Liu Y, Shlouout G. Development of microemulsion based topical ivermectin formulations: pre-formulation and formulation studies. Colloids Surf B Biointerfaces. 2020;189:110823. doi: 10.1016/j.colsurfb.2020.110823.
17. Khokhra S, Diwan A. Microemulsion based transdermal Drug delivery of tea tree oil. Int J Drug Dev Res. 2011;3(1):191-8.
18. Zhang L, Zhu W, Yang C, Guo H, Yu A, JI, Gao Y, Sun M, Zhai G. A novel folate-modified self-microemulsifying drug delivery system of curcumin for colon targeting. Int J Nanomedicine. 2019;7:151-62. doi: 10.2147/INJN.S27639, PMID 22275831.
19. Sharma S, Ganju E, Upmanyu N, Jain P. Therapeutic microemulsion of curcumin for the management of osteoarthritis. J Drug Delivery Ther. 2018;8(5-6):341-7. doi: 10.22720/jddt.v8i5-s.1395, PMID 30390122.
20. Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS, Pottos PH. Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC Adv. 2019;9(35):20192-206. doi: 10.1039/C9RA03102B.
21. Solanki SS, Sarkar B, Dhanwani RK. Microemulsion drug delivery systems for bioavailability enhancement of amlopiniol. ISRN Pharm. 2012;2012:108164. doi: 10.5105/2012/108164, PMID 22830055.
22. Kuang J, Gao J, Xie S, Lei Q, Fang W, Xie H, Lu X. Phase behaviors and curcumin encapsulation performance of Gemini surfactant microemulsion. J Mol Liq. 2020;315. doi: 10.1016/j.molliq.2020.113786, PMID 3113786.
23. ERDAL MS, GÜRBÜZ A, Birtekşöz Tan S, GÜNGÖR S, ÖZSOY Y. In vitro skin permeation and antifungal activity of natifline microemulsions. Turk J Pharm Sci. 2020;17(1):43-8. doi: 10.4247/ijps.galenos.2018.87699, PMID 32454759.

24. Chryssanthou E, Cuenca-Estrella M. Comparison of the antifungal susceptibility testing subcommittee of the European committee on antibiotic susceptibility testing proposed standard and the E-test with the NCCLS broth microdilution method for voriconazole and caspofungin susceptibility testing of yeast species. J Clin Microbiol. 2002;40(10):3841-4. doi: 10.1128/JCM.40.10.3841-3844.2002, PMID 12354895.

25. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163-75. doi: 10.1038/nprot.2007.521, PMID 18274517.

26. Rahdar A, Hajinezhad MR, Sargazi S, Zaboli M, Barani M, Bano M, Bilal M, Sanchoooli E. Biochemical, ameliorative and cytotoxic effects of newly synthesized curcumin microemulsions: evidence from in vitro and in vivo Studies. Nanomaterials (Basel). 2021;11(3):317. doi: 10.3390/nano11030317, PMID 33806829.

27. El-Kamel A.H. In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. Int J Pharm. 2002;241(1):47-55. doi: 10.1016/s0378-5173(02)00234-x, PMID 12086720.

28. Jaceczak M, Wach R, Maras P, Dudek M, Kozicki M. Substituting gelatine with P1 pluronic F-127 matrix in 3D polymer gel dosimeters can improve nuclear magnetic resonance, thermal and optical properties. Phys Med Biol.. 2018;63(17):175010:175010. doi: 10.1088/1361-6560/aad9d5.

29. Patel MR, Patel RB, Patel RG. Novel microemulsion-based gel formulation of tazarotene for therapy of acne. Pharm Dev Technol.. 2018;23(2):642-47. doi: 10.3390/pharmaceutics8040007, PMID 29927972.

30. Al-Khordagui LK. Itraconazole liquid crystalline systems for controlled drug release and improved Treatment of Vulvovaginal Candidiasis. Mol Pharm.. 2018;15(10):4491-4504. doi: 10.1021/acs.molpharmaceut.8b00507, PMID 30184431.

31. Hussain A, Samad A, Singh SK, Haque MW, Faruk A, Ahmed FJ. Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. Drug Deliv. 2016;23(2):642-47. doi: 10.3390/pharmaceutics8040007, PMID 29927972.