International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 13, Issue 5, 2021

Original Article

FORMULATION AND DEVELOPMENT OF CURCUMIN BASED EMULGEL IN TREATMENT AND RECURRENCE OF VAGINAL *CANDIDIASIS*

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Received: 01 May 2021, Revised and Accepted: 05 Jul 2021

ABSTRACT

Objective: The main causative agent of vaginal candidiasis is *Candida albicans* and it develops resistance against several synthetic antifungal drugs and it has a high rate of infection in women. According to WHO report, around 75% of women are infected by *Candida albicans* and 50 % 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

DOI: https://dx.doi.org/10.22159/ijcpr.2021v13i5.1900 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

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* and5-8% 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, develop tolerance. As a result, there is an urgent need for a safe and efficient vaginal candidate 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, 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 exoenzymes 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 is 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 are 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 **s**olubility 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, Ethanol, 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 cosurfactant [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, add 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 pseudo ternary 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 microemulsions were 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 curcumin loaded microemulsion were measured by the Zetasizer (Nano ZS: Malvern Instruments. UK) [13]. The microemulsion sample was loaded into the cylindrical cuvettes and placed in a thermo stated 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 microemulsion 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 24h at 40 °C . The physical stability of 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].

$$\% \text{ Transmittance} = \text{Antilog } (2\text{-Absorbance})$$

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 cm^2. 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 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 1×10^3 CFU/ml–5 $\times10^3$ CFU/ml. To achieve final concentration 1×10^3CFU/ml–5 $\times10^3$ CFU/ml by further dilution 1 in 30 by using sterile distilled water [23, 24].

The stock solution of curcumin microemulsion and Fluconazole was prepared in dimethyl sulphoxide (DMSO) solution to achieve a 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 ml of RPMI-1640 medium is added to each well in the microplate. Starting with a final concentration of 0.050µg/ml, sample solutions (50µl) were serially diluted two-fold in the microplate with the RPMI-1640 medium. The prepared fungal strain inoculum was added to each well to achieve a final concentration of 0.5×10^3–2.5×10^3CFU/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 . At a wavelength of 492 nm, the amount of growth was quantified using a plate reader, [NCCLS document M27-A2] [25, 26].

Formulation and evaluation curcumin loaded emulgel

Formulation of curcumin-loaded emulgel

The Curcumin-loaded microemulsion was gelled by using Pluronic @F127 as a gelling agent. The emulgel was prepared by taken 2 gm of Pluronic@ F127 and dispersed in 6 ml cold distilled water and kept overnight in the freezer for soaking or to allow the Pluronic @F127 to swell completely. After that, remove the solution from the freezer and kept at room temperature for min [27]. Thereafter, the dispersed 20 ml of Curcumin-loaded microemulsion and 0.2% methylparaben and 0.1 % propyl paraben as preservative in above solution with continuous mixing at room temperature. The dispersion was neutralized by using 50% (w/w) triethanolamine or NaOH to obtain the emulgel and prepared emulgel kept in the freeze for 2 h to get homogenous 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 emulgel determined 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

Thespreadability of Curcumin-loaded emulgel was determined by using the following technique:-Weighed the 1 gm of gel formulation and placed it at the center of the glass slide or plate of standard dimensions (20×20 cm). After that, the second glass plate is placed over the first glass plate very carefully, on which emulgel formulation was placed. A weight of 0.5 kg was allowed to be placed on the center of the upper glass plate for 5 min, but avoid the sliding of the glass plate. The diameter of sample was measured in cm for triplicate to find the mean diameter [29].

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 in 100 ml volumetric flask and the resultant mixture stirred for 30 min at 25 °C for [19, 32]. 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 [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 cm^2 [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]. Te 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 poured into the sterile petri plate and thereafter this petri plate was allowed to cool at room temperature (25-28 °C). After the 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 gel 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

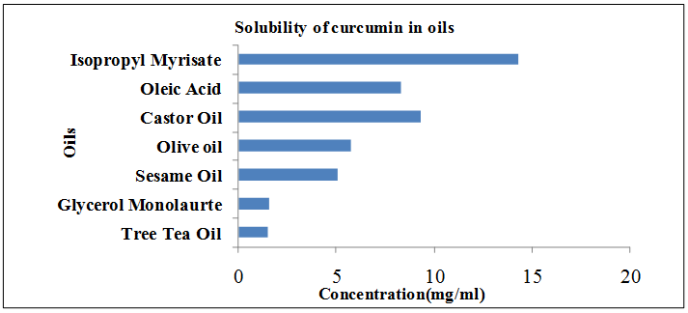
The solubility of curcumin in different oils is shown in fig. 1(A). The curcumin shows the highest solubility in isopropyl myristate and castor oil. However, Isopropyl myristate was reported to have increased skin absorption by acting as a skin penetration enhancer. Therefore, isopropyl myristate was 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

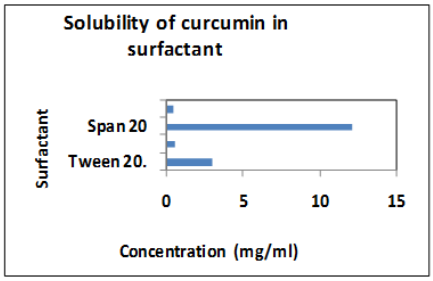
Construction of pseudoternary phase diagram

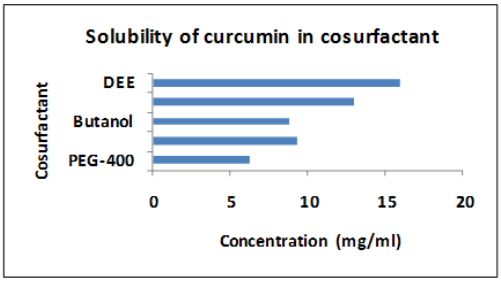
The pseudoternary phase diagrams were constructed for the microemulsion system of Isopropyl myristate-Tween 80-Isopropyl Alcohol (IPM-Tween 80-IPA) by using a water titration method as shown in fig. 2. Each edge of the diagram represents each component of microemulsion i.e. Isopropyl myristate, Smix (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 1:1and 2:1 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.



(A)





(B) **(C)**

Fig. 1A: Solubility of curcumin in oils B: Solubility of curcumin in surfactant C)

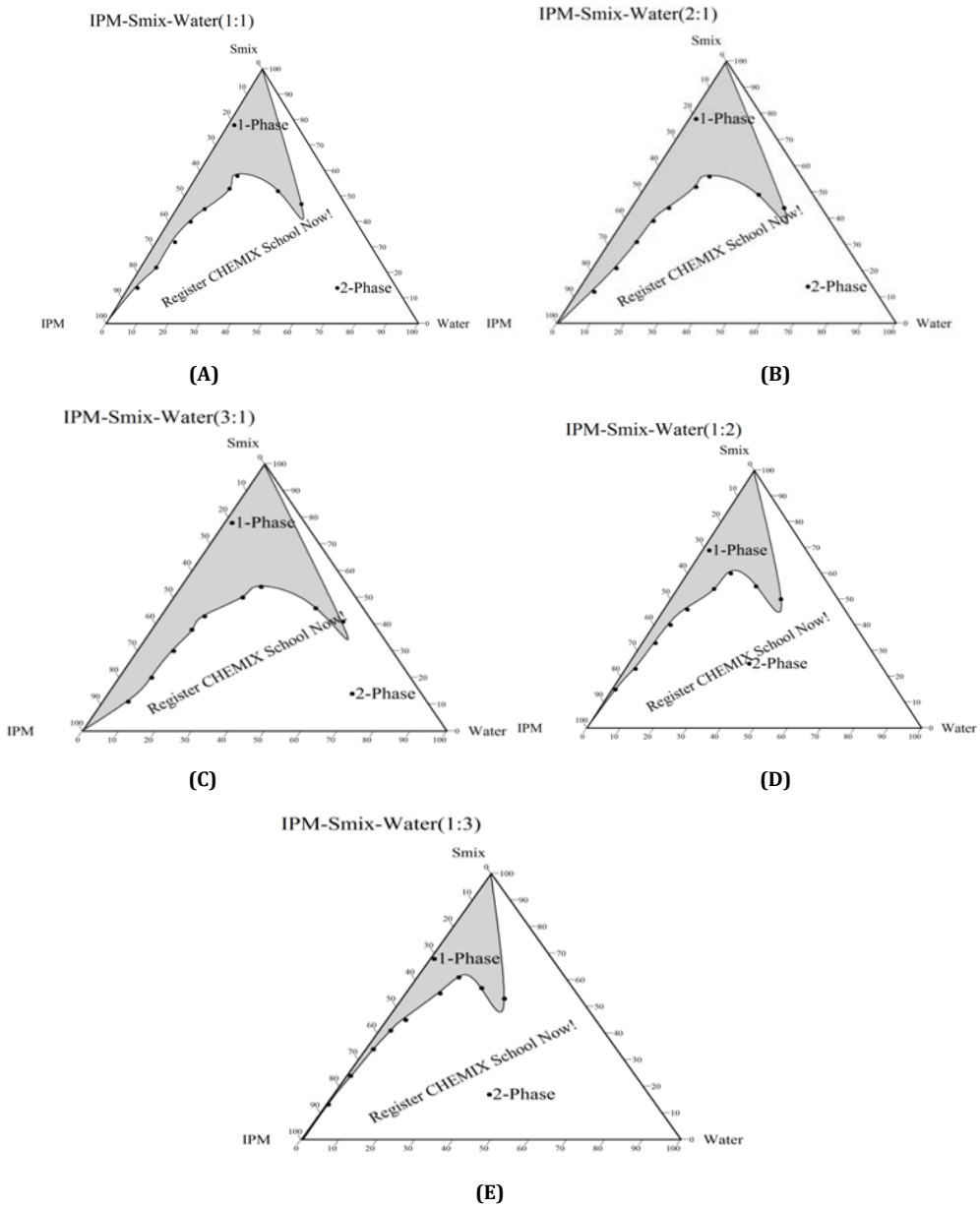


Fig. 2: Pseudoternary phase diagram with surfactant: cosurfactant ratio A (1:1) B (2:1), C (3:1), D (1:2) and E (1:3)

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 size339.0 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

(fig. 4). 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.

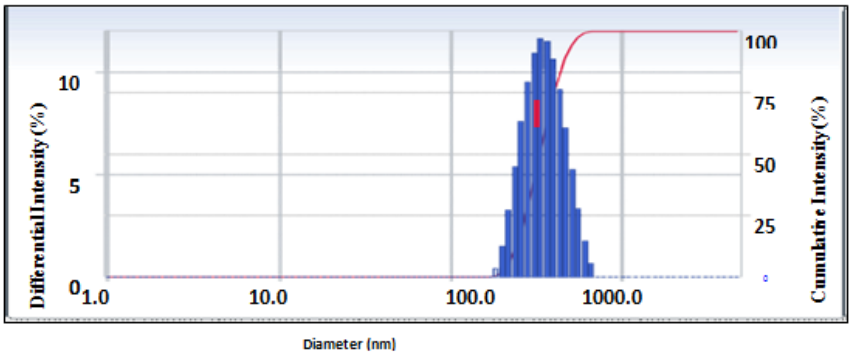


Fig. 3: Particle size distribution curve of microemulsion

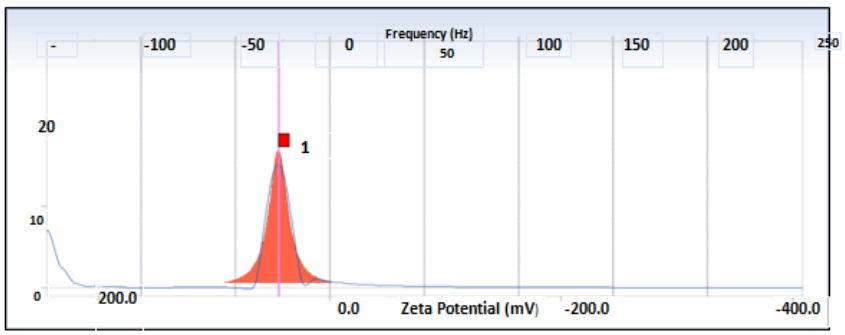


Fig. 4: Zeta potential graph of curcumin-loaded microemulsion

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.

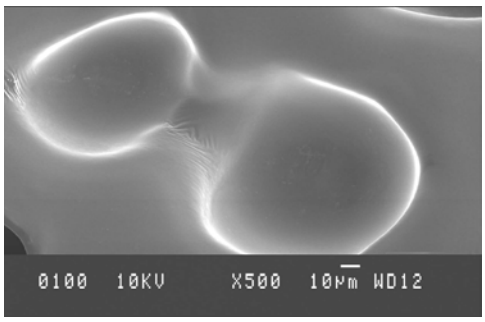


Fig. 5: Scanning electron microscopy

Scanning electron microscopy analysis

The SEM analysis of Curcumin-loaded w/o microemulsion formulation is shown in fig. 5. The size obtained from the scanning electron microscope analysis was found to be in concurrence with the globule size obtained from the Zetasizer.

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 IC_{50} 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.

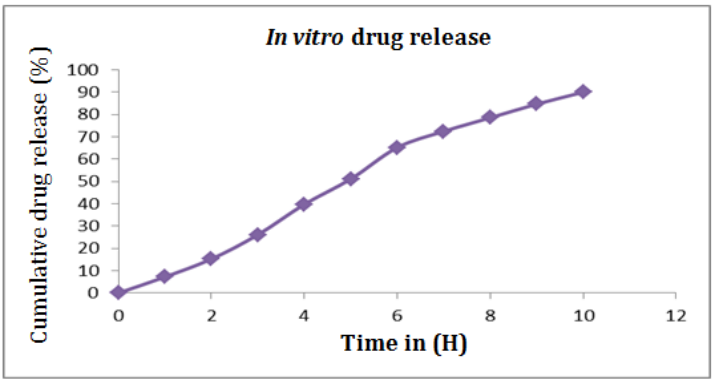


Fig. 6: *In vitro* release study of curcumin-loaded microemulsion

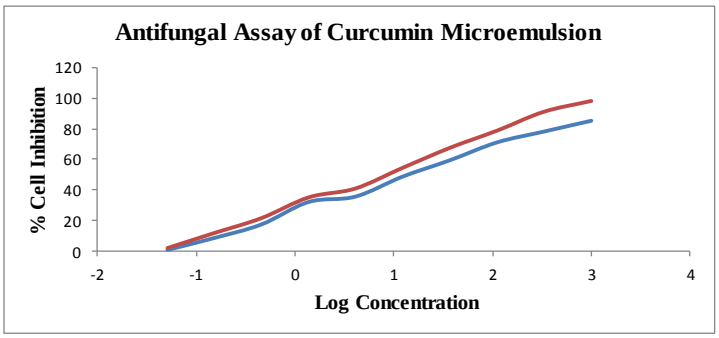


Fig. 7: Dose response curves of test compounds against candida albicans and fluconazole

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.

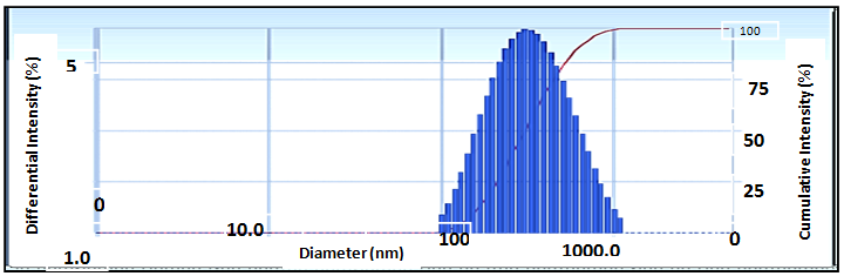


Fig. 8: Particle size distribution curve of curcumin-loaded emulgel

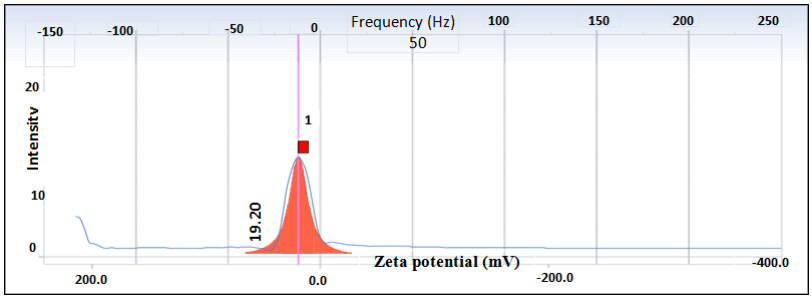


Fig. 9: Zeta potential graph of curcumin-loaded emulgel

Physicochemical characterization of emulgel

The prepared emulgel is clear, pale yellow-colored, providing a lubricant feels 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 adhesiveness and cohesiveness of the prepared gel was found to be 0.33 ml and 0.39 respectively, which indicates that prepared gel not sticky and don't show staining.

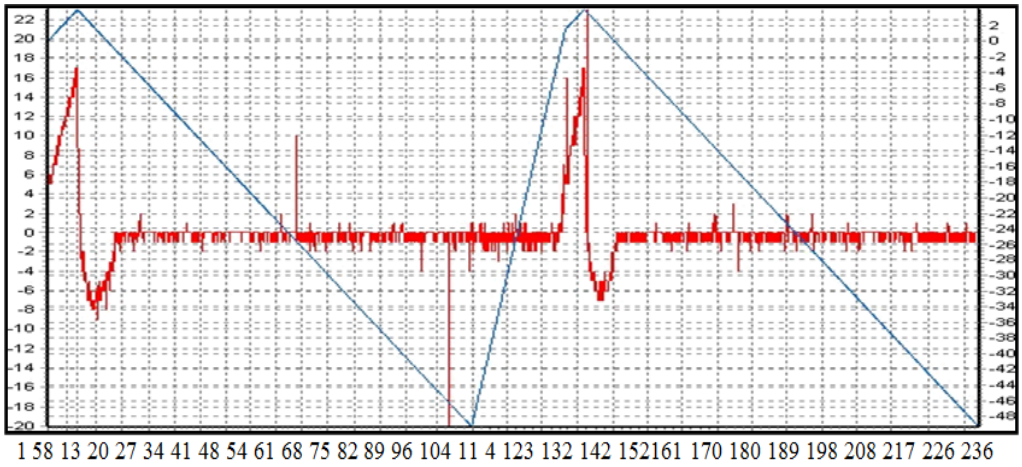


Fig. 10: Texture analysis graph of curcumin-loaded emulgel

***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.

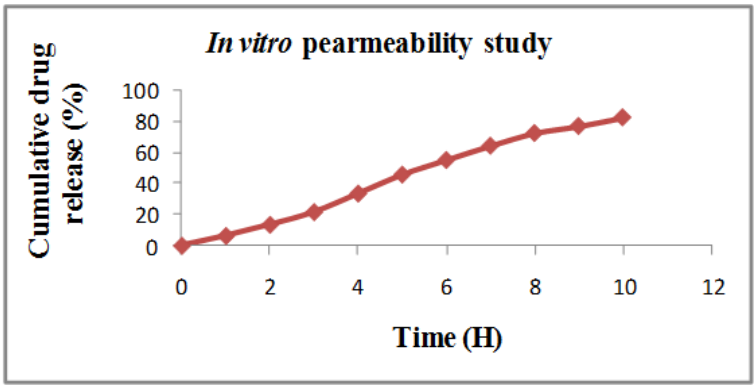


Fig. 11: *In vitro* permeability study of curcumin-loaded emulgel

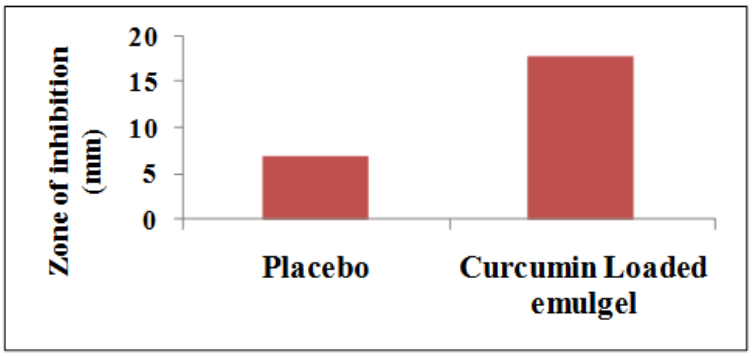


Fig. 13: Antifungal activity (Zone of inhibition comparison)

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, by using different Smix ratios (km value) (1:1, 1:2, 1:3, 1:2, and 1:3). 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%, 0.89%, 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, isocratic, thermodynamically stable, and pale yellow coloured 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 dispersibity 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 IC_{50} value of Curcumin-loaded microemulsion and Fluconazole was found to be 19.34 µg/ml and 38.12µg/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 a 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 11343±2cps. 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 Pluronic@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 instrumentations 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 articl

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