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In vitro characterization of self-emulsifying drug delivery system-based lipsticks loaded with ketoconazole

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Abstract

Background Self-emulsifying system-based lipstick may prove to have the potential of enhancing moisturizing characteristics and delivering hydrophobic drug antifungal drugs for the management of lip fungal infection, especially candidiasis. In this study, the self-emulsifying drug delivery system (SEDDs) of ketoconazole was obtained using IPM (Oil), Tween 80 (surfactant), and PEG 400 (co-surfactant). The medicinal lipstick was developed from the SEDDs, and the concentration of waxes (beeswax and carnauba wax) and cow ghee (penetration enhancer) was optimized using a Box–Behnken design. The lipstick formulations were assessed based on physicochemical features, such as pH, spreadability, softening point, breaking point, content uniformity, and in vitro drug permeation. Furthermore, the optimal lipstick formulation was tested for stability and antifungal activity.

Results The optimized formulation showed exceptional results in physicochemical analysis and ~ 87% release of the drug in 12 h. The formulations displayed adequate stability for 4 weeks at various temperature conditions such as room temperature, 40 °C, and 45 °C. The zone of inhibition produced by lipstick formulation was significantly higher as compared to the reference standard (ketoconazole in ethanol) which shows high antifungal activity.

Conclusion It was concluded from the outcomes that SEDDs-based lipstick formulation showed a lot of promise as a topical antifungal treatment option for Candidiasis.

Keywords Lipstick, Emulsions, Formulation, Stability, Box–behnken, Antifungal, Candidiasis

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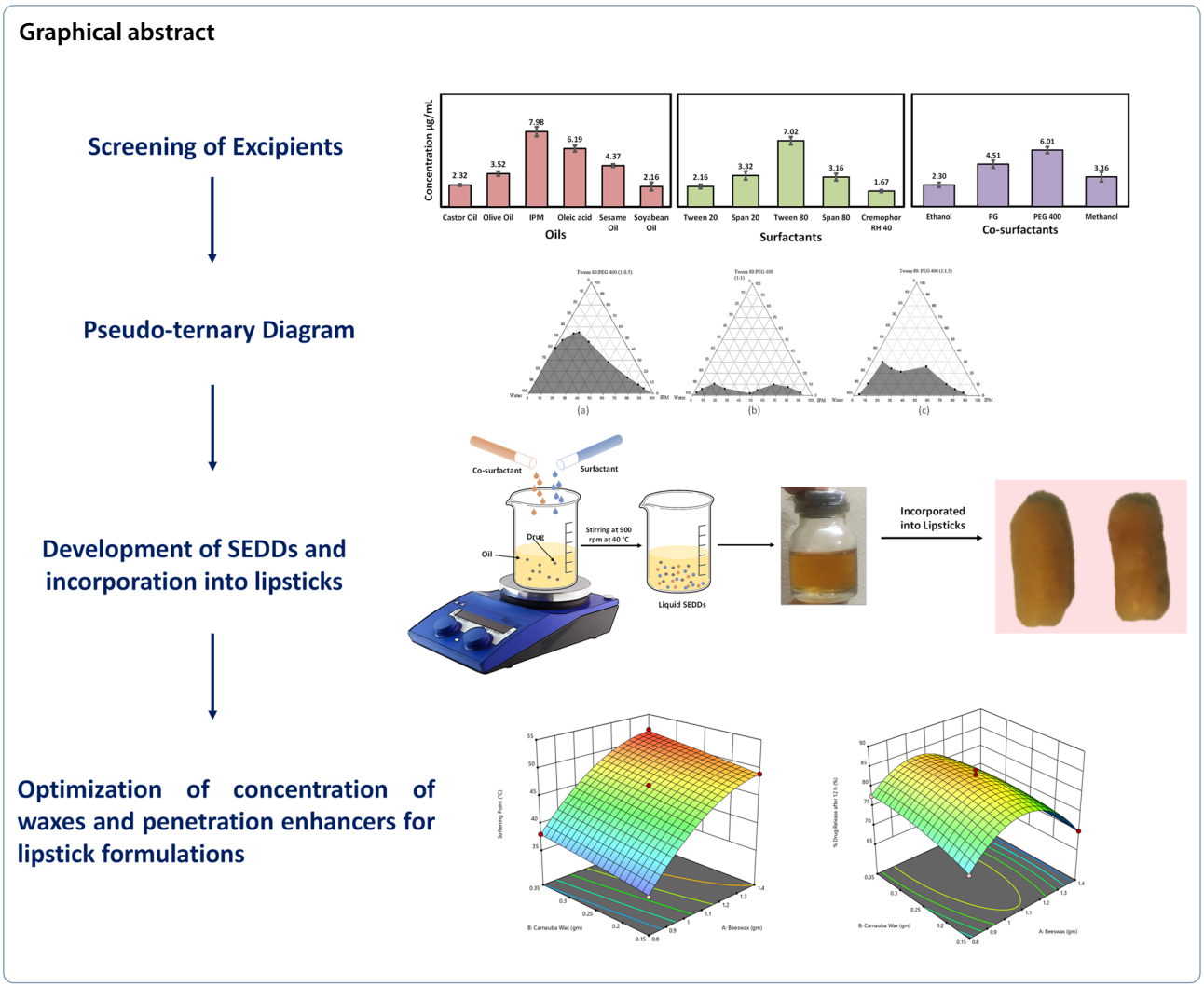
Graphical abstract



Background

Fungal infections are among the main health issues throughout the world. Fungal infection of the lips is a sort of opportunistic infection in which the weakening of host resistance permits the beginning and progression of pathogenic colonization [1, 2]. Generally, superficial infections manifest in the most abundant and prevalent types of mycoses [3]. Superficial infections such as lip fungal infections are mostly accompanied by pain, oral distress, paraguesia, and burning sensation [4]. However, these infections progress rarely into systemic infections, but their management tends to get complicated over time, especially in organ transplant recipients, AIDS patients, immunocompromised patients, etc. [5, 6]. Further, there are also chances of recurrence of the infections after some time of being treated with antifungal drugs [7].

Ketoconazole is an imidazole antifungal class of drug which has been used for the treatment of fungal infections [8]. It has broad spectrum activity against most candida infections, along with fungicidal activity and fungistatic activity against dermatophytes, eumycetes, coccidioides, dimorphous fungi, histoplasma, etc. [9, 10]. It is associated with serious complications upon systemic administration, while topical administration induces significantly lesser side effects [11]. Furthermore, it is tough to develop a solubilized form of ketoconazole owing to its poor water solubility [12]. Therefore, it is required to evade the formulation issues, reduce side effects, and improve the antifungal action of the drug [13–15].

Self-emulsifying systems are a mixture of oils, surfactants, and co-surfactants that can be used for the delivery of drugs. SEDDs are known to be highly useful as the delivery system for enhancing the solubility of poorly water-soluble drugs [16–18]. The combination

of surfactants (surfactant and co-surfactant) is added to reduce the interfacial tension and rapid emulsification of the system in the body fluid [19, 20]. SEDDs have emerged as an important approach to enhance the bioavailability of low water-soluble drugs [21]. Moreover, they do not contain any aqueous phase, therefore making them adequate for scale-up and preparation simpler than other nanoparticle-based drug delivery systems, such as polymeric nanoparticles, liposomes, niosomes, dendrimers, and carbon nanotubes [22]. The self-emulsification tendency of SEDDs is affected by the nature, concentration, and specific ratio of the surfactant mixture; along with physiological parameters, like temperature and pH [23].

The novel strategy of the research work was to utilize the SEEDs-based lipstick to improve the dissolution, stability, and antifungal activity of ketoconazole (KTZ). It was expected that the incorporation of the drug in SEDDs will increase the solubility and stability; therefore, firstly SEDDs of KTZ were prepared. Secondly, the lipstick was fabricated by loading the SEDDs into the lipstick base comprising of waxes and cow ghee was used as a penetration enhancer. The lipstick containing SEDDs was evaluated for melting point, breaking load test, spreadability test, surface anomalies, drug content, and softening point. Further, the researchers studied the in vitro permeation, stability study, and antifungal activity of the lipsticks.

Methods

Materials

KTZ was obtained as a gift sample from Modex Pharma Ltd. (Gujarat, India). Beeswax, Carnauba wax, and lanolin anhydrous (LR) were purchased from S.D Fine Chemicals Ltd. (Maharasthra, India). Tween 80, PEG-400, butylated hydroxyanisole (BHA), and transcutol P were purchased from Finar Ltd. (Gujarat, India). Titanium dioxide was purchased from Qualigens Fine Chemicals Pvt. Ltd. (Maharasthra, India). Iso-propylmyristate (IPM) and methanol were obtained from Chemdyes Corporation (Gujarat, India) and Central Drug House Ltd. (New Delhi, India). Cow ghee and cold press castor oil were obtained from the Ashtamangal Oil mill (Maharasthra, India). These materials were food grade, and all the other chemical reagents were of analytical grade.

Screening of Excipients for the development of SEDDs

The selection of excipients was done based on the solubility of KTZ in the various oils, surfactants, co-surfactants, and co-solvents. The solubility study was carried out by incorporating the excess quantity of KTZ in different excipients and mixing vigorously with the help of a magnetic stirrer. These mixtures are then stored at room temperature for 24 h for equilibration, followed by centrifugation at 5,500 to 6,000 rpm for 15 min. The supernatant was filtered, diluted, and analyzed using a UV-1800 UV–visible spectrophotometer (Shimadzu, Kyoto, Japan) at 220 nm. λ max of KTZ for determining the solubility [24].

Construction of pseudo-ternary phase diagram

The pseudo-ternary phase diagram was plotted for finding out the formulation which is suitable for generating the oil-in-water emulsions. Based on solubility studies, IPM, Tween 80, and PEG 400 were chosen as oil phase, surfactant, and co-surfactant. The phase diagram was constructed by employing the water titration method wherein the S_{mix} (Tween 80: PEG 400) was taken in 1:0.5, 1:1, 1:1.5 ratios. The different S_{mix} ratios were mixed with oil in specific combinations of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 using a magnetic stirrer set to 900 RPM and 40 °C. This is followed by dilution of 0.2 mL of formulation up to 100 times with distilled water under continuous stirring. The phase diagrams were plotted using Sigma Plot 12.0 (Systat Software Inc., USA) [25, 26].

Preparation of KTZ-SEDDs

KTZ-SEDDs were developed using a one-step emulsification method (Fig. 1). In this method, KTZ (0.2 g) was mixed with IPM (1.85 g) and heated at 40 °C with continuous stirring at 900 rpm for preparing the oil phase. To the above phase, Tween 80 and PEG 400 were added followed by heating the mixtures at 40 °C and stirring until a clear homogeneous solution is produced. The SEDDs were allowed to cool down to room temperature and emulsification was tested by diluting 0.1 g of the sample up to 200 times with deionized water. Oil-in-water emulsion could be spontaneously generated in the aqueous solution which confirms the self-emulsification tendency of the optimized combination [25, 26].

Development of lipstick from KTZ-SEDDs using experimental design

The combined effect of three independent variables, i.e., amount of beeswax (X_1), carnauba wax (X_2), and cow ghee (X_3), was evaluated using a Box–Behnken design. The concentrations of the variables were taken in three levels, i.e., upper (+1), middle (0), and lower (-1) limits (Table 1). The randomized run presented 15 different formulation batches (F1 to F15) is represented in Table 2, wherein F7, F10, and F13 are triplicates of central values. The quantity of independent variables given in Table 1 is selected based on a literature review and preliminary trials. The dependent variables or responses chosen for the experiment were softening point (Y_1) and percentage drug release after 12 h (Y_2). The models were validated

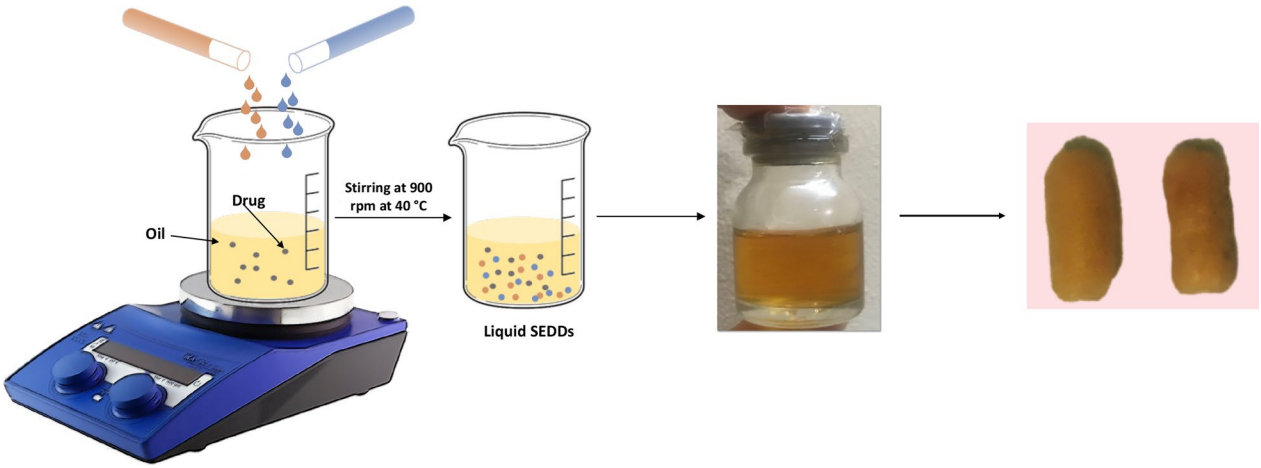


Fig. 1 Preparation of the KTZ-SEDDs and transformation of SEDDs into lipstick

Table 1 Variables in the experimental design

Independent factors	Levels		
	Low (− 1)	Medium (0)	High (+1)
X_1: Beeswax (gm)	0.8	1.1	1.4
X_2: Carnauba wax (gm)	0.15	0.25	0.35
X_3: Cow ghee (mL)	0.5	0.75	1

by lack of fit, ANOVA, and multiple correlation coefficient tests. The experimental design was conducted using Design-Expert® 13 (version 13.0.5.0, Stat-Ease Inc., Minneapolis, USA).

The prepared SEDDs were transformed into lipstick formulations for topical delivery of KTZ. For this purpose, the weighed quantities of lanolin, beeswax, and carnauba wax were melted in a porcelain dish in the water bath to prepare the melted wax mixture (mixture 1). Similarly, the cow ghee was melted in a separate porcelain dish, and castor oil was added to it (mixture 2). A measured quantity of SEDDs was incorporated into mixture 1, followed by the addition of mixture 2 along with vigorous stirring until a soft emulsion is generated. The quantity of components used in the preparation of 15 formulations is given in Table 2. The molding of the emulsion into lipstick was done by adding it to

Table 2 Composition of formulations as per randomized runs in Box–Behnken design

Formulation code	Castor oil (ml)	SEDDS (ml)	Beeswax (gm)	Carnauba Wax (gm)	Lanolin (gm)	Ghee (ml)	BHA (gm)	TiO_2 (gm)
F1	2.3	1	***1.1***	***0.35***	0.3	***1***	0.002	0.02
F2	2.3	1	***1.1***	***0.15***	0.3	***0.5***	0.002	0.02
F3	2.3	1	***1.4***	***0.15***	0.3	***0.75***	0.002	0.02
F4	2.3	1	***0.8***	***0.25***	0.3	***1***	0.002	0.02
F5	2.3	1	***1.4***	***0.35***	0.3	***0.75***	0.002	0.02
F6	2.3	1	***0.8***	***0.35***	0.3	***0.75***	0.002	0.02
F7	2.3	1	***0.8***	***0.25***	0.3	***0.5***	0.002	0.02
F8	2.3	1	***1.4***	***0.25***	0.3	***1***	0.002	0.02
F9	2.3	1	***1.4***	***0.25***	0.3	***0.5***	0.002	0.02
F10	*2.3*	*1*	***1.1***	***0.25***	*0.3*	***0.75***	*0.002*	*0.02*
F11	2.3	1	***1.1***	***0.35***	0.3	***0.5***	0.002	0.02
F12	2.3	1	***0.8***	***0.15***	0.3	***0.75***	0.002	0.02
F13	*2.3*	*1*	***1.1***	***0.25***	*0.3*	***0.75***	*0.002*	*0.02*
F14	*2.3*	*1*	***1.1***	***0.25***	*0.3*	***0.75***	*0.002*	*0.02*
F15	2.3	1	***1.1***	***0.15***	0.3	***1***	0.002	0.02

*Rows highlighted in italic represent the central values of experimental design, while columns indicated by bolditalic background are independent factors

previously lubricated and chilled molds. The solidified lipsticks were removed from the mold by using a scrapper and reshaped from the edges (Fig. 1).

Characterization of KTZ-SEDDs lipstick formulation

Melting point

The lipstick sample (about 2 g) was taken in a glass capillary tube. The tube was immersed in the beaker-filled water, which was placed over the water bath for heating. The melting point was estimated as the temperature at which the material starts to melt and forms a liquid drop [27].

Breaking load test

The strength of the lipstick formulation was assessed by the breaking load test. In this, the lipsticks were held horizontally into an opening close to the edge of the support, and the weights attached to it were gradually increased (10 g) at a specific interval of 30 s. The weight at which the lipstick breaks is recorded as the breaking point weight [28].

Spreadability test

The spreadability of lipstick was assessed by spreading it over a transparent glass slide at an angle of 45°. The surface of the slide was observed visually against the dark background for its uniformity in forming a consistent layer, fragmentation, or deformation upon application. The following criteria laid down for this test are the symbol 'G' indicates a Good and uniform layer without fragments and deformation, the symbol 'M' indicates moderate uniformity with few fragments, but no deformation, and 'P' indicates poor uniformity with high fragmentation and clear deformation [29–31].

Surface anomalies

The quality of lipstick may be indicated by estimating the surface anomalies. The samples were examined visually to check for anomalies that may occur on the surface of the lipstick. In this study, the lipsticks were checked for marks (representing scratches or dents on the surface), heterogeneity (color or texture differences), pollutants (dust or other undesirable particles), and distortion (irregular line, deformation, etc.). The surface of the product should be completely devoid of crystal formation with no signs of contamination from the molds and fungi [32, 33].

Percentage drug content

For the determination of drug content, a weighed quantity of lipstick (100 mg) sample was transferred into a 100-mL volumetric flask and dissolved into appropriate amount of methanol. The absorbance was measured using a UV–Vis spectrophotometer at 220 nm after suitable dilution [34, 35].

Softening point

The softening point test was performed by inserting the lipstick sample into an aluminum ring, and extra mass above and below was removed. The ring containing the sample was placed in the refrigerator at 6 °C for 10 min. After removal, the ring was fastened into a stand and dipped into a beaker filled with water. The whole assembly was heated with continuous stirring and the softening point was recorded as the temperature at which the lipstick mass loosened and fell into the beaker [36, 37].

Permeability study

The lipstick formulations were tested for permeability using vertical glass Franz diffusion cells (Electrolab Private Limited, India). The transparent jacketed cell with a flat bottom and a 5-mL receptor was used for the experiment. The release of KTZ was studied by employing a cellophane membrane (Sigma-Aldrich, 0.22 µm pore size). The mixture of deionized water and methanol was selected as receptor media which was filled in the receptor compartment and magnetically stirred at 600 rpm. The experiment was conducted at 32 ± 0.5 °C for 12 h involving withdrawing samples at 1-h interval and analyzed using a UV spectrophotometer at 220 nm [38, 39].

Stability study

The stability of lipstick was estimated at room temperature (25 °C), 40 °C, and 45 °C in the stability chamber for 4 weeks. Samples of the selected formulation were examined for their melting point, spreadability, breaking point, surface anomalies, drug content, and percentage drug release after 12 h after completion of the 1st, 2nd, and 4th week [40].

In vitro antifungal activity

The antifungal activity of the formulation was studied using the cup-plate method. The dextrose agar media was dissolved in 100 mL of distilled water with the help of continuous stirring and heating. The solution was boiled for some time for effective dissolution, followed by maintaining pH at 5.5 and autoclaving at a temperature of 121 °C for 15 min. The media was poured into two sterile Petri plates in an aseptic area and the plates were set aside for solidification. After solidification, the *Candida albicans* were inoculated on the surface of media in both plates and small holes were created. In one of the plates, 1% w/v KTZ solution in ethanol was placed in the holes as a reference. The formulation was subjected to the holes of agar media in another plate and both plates

were incubated for 24 h at a temperature of 25 °C. The plates were removed after 24 h, and the zone of inhibition was estimated in millimeters by utilizing a vernier caliper. The diameter of the zone of inhibition was recorded, and each measurement was performed in triplicate [41, 42].

Statistical analysis

Statistical analysis was performed using Design-Expert® 13, and one-way ANOVA was performed for the statistical comparisons. The analysis was done using statistical parameters such as multiple correlation coefficient (r^2), adjusted multiple correlation coefficient (adjusted r^2), coefficient of variation (C.V.), and lack of fit proven by Design-Expert software.

Results

Screening of excipients for the development of SEDDs

The solubility study of KTZ in various excipients was estimated to verify that optimum drug loading takes place. The solubility of KTZ in oils, surfactants, and co-surfactants is reported in Fig. 2. Among different oils, the solubility of the drug was observed to be higher in IPM than in oleic acid, sesame oil, olive oil, castor oil, and soybean oil. Similarly, the Tween 80 and PEG 400 showed the highest solubility among different surfactants and co-surfactants, respectively. The excipient selected based on solubility was further used in the study for the development of SEDDs.

Construction of pseudo-ternary phase diagram

The pseudo-ternary diagram was constructed to estimate the combination of surfactants yielding the highest region of self-emulsification. The pseudo-ternary phase diagram of the KTZ-SEDDs was designed by using IPM as the oil phase and Tween 80 (surfactant) and PEG 400 (co-surfactant) were taken in ratios of 1:0.5, 1:1, and 1:1.5 as shown in Fig. 3. The self-emulsification region indicated in the figure represents that formulae selected from these regions have the capability of self-emulsification to form oil-in-water emulsions. It can be observed from the plots that a 1:1 combination of Tween 80 and PEG 400 produced the largest self-emulsification region, followed by 1:1.5 and then 1:0.5 (Fig. 3).

Development of lipstick from KTZ-SEDDs using experimental design

The study is focused on effectively correlating the variables and outcomes of the experiment. The composition of the KTZ-SEDDs lipstick was optimized using a Box–Behnken design for estimating the most suitable

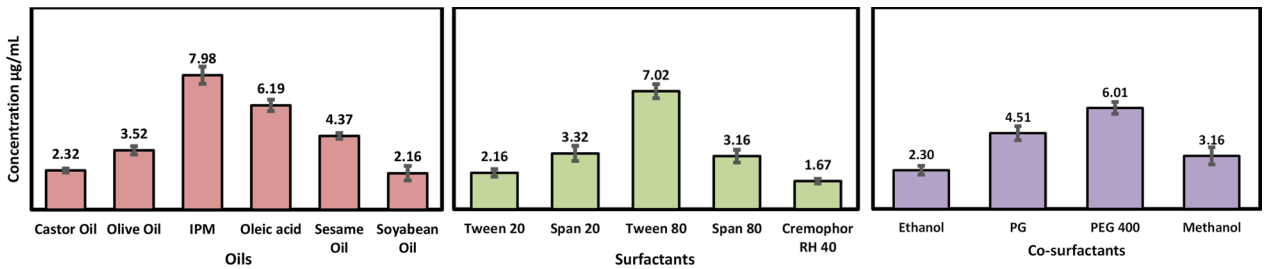


Fig. 2 Solubility of KTZ in different excipients

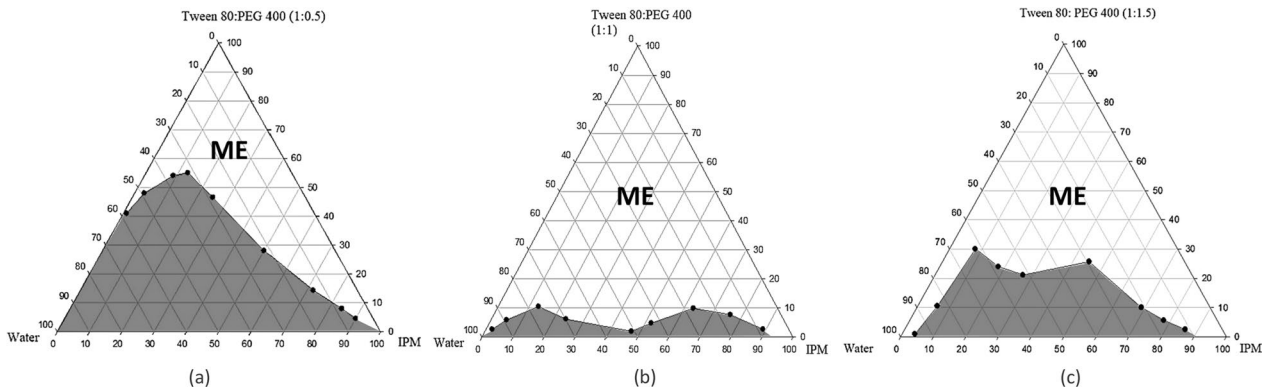


Fig. 3 Pseudo-ternary phase diagrams of SEDDs where Tween 80 and PEG 400 were used in the ratio: **a** 1:0.5, **b** 1:1, and **c** 1:1.5. The non-shaded region in the ternary phase diagram indicates the microemulsion region represented by 'ME'

composition of formulation variables which affects the quality of the product. In this study, the best-fitting models were produced for the softening point and percentage drug release after 12 h as response variables of the lipstick formulation (Table 3).

Table 3 Observed responses for softening point (Y_1) and percentage drug release after 12 h (Y_2) from randomized runs in the BBD

Formulation	Independent variables			Response variables	
	X_1	X_2	X_3	Y_1 (°C)	Y_2 (%)
F1	0	1	1	48	78.97
F2	0	− 1	− 1	46	79.83
F3	1	− 1	0	49	70.03
F4	− 1	0	1	37	75.63
F5	1	1	0	52	69.12
F6	− 1	1	0	38	77.68
F7	− 1	0	− 1	37	77.93
F8	1	0	1	51	80.38
F9	1	0	− 1	50	79.44
F10	0	0	0	47	81.76
F11	0	1	− 1	48	86.94
F12	− 1	− 1	0	35	72.68
F13	0	0	0	46	83.66
F14	0	0	0	47	84.91
F15	0	− 1	1	45	82.1

Characterization of KTZ-SEDDs lipstick formulation

The lipstick formulation was characterized by melting point, breaking point, spreadability, surface anomalies, and drug content after the fabrication of lipsticks. The results are indicated in Table 4. The melting point of the lipsticks was found to vary from 50 to 64 °C. The melting point is expected to be high for evading the chances of deterioration of lipsticks during their development and application. Studies in the past have stated that the melting point of lipsticks should be in the range of 40 to 56 °C [43], or even slightly higher up to 64 °C is acceptable [44].

The breaking point of the lipsticks varied between 127 to 134 gm, and there was no significant difference between the results of the breaking point for all the formulations. The spreadability of the formulations was assessed visually for the uniformity and smoothness of the protective layer formed by the lipstick. F2, F6, F7, and F9 showed fragmentation, non-uniformity, and deformation upon application, while other formulations showed moderate to good spreadability. Apart from this, slight or moderate cracking was observed in the F2, F4, F6, and F7 indicating defective lipsticks. There were no major signs of defect observed visually in all the other formulations. The surface anomalies may be produced due to contamination or excessive heating during the preparation of lipsticks. The drug content of all the formulations was higher than 90%, while F5, F8, F9, F11 to F15 formulations showed a high percentage drug content in comparison with the other formulations.

Table 4 Physicochemical Characterization of lipstick formulations data

Formulation	Melting Point (°C)	Breaking Point (gm)	Spreadability test	Surface anomalies	% Drug content
F1	56 ± 1.02	129 ± 1.71	G	No defects	95.44 ± 1.91
F2	57 ± 0.92	126 ± 1.65	P	Slight cracking	94.13 ± 2.38
F3	59 ± 0.56	130 ± 2.22	G	No defects	93.42 ± 1.77
F4	51 ± 1.34	127 ± 2.61	M	Slight cracking	94.28 ± 2.49
F5	64 ± 0.97	134 ± 1.73	M	No defects	98.22 ± 2.38
F6	52 ± 0.88	128 ± 2.17	P	Cracks	90.63 ± 1.42
F7	53 ± 0.99	127 ± 2.29	P	Slight cracking	93.95 ± 1.56
F8	61 ± 1.41	131 ± 2.15	G	No defects	98.07 ± 1.49
F9	61 ± 0.59	131 ± 1.91	M	Slight cracking	97.26 ± 2.07
F10	55 ± 1.15	130 ± 2.63	G	No defects	94.49 ± 2.26
F11	58 ± 2.1	129 ± 1.02	G	No defects	99.35 ± 1.64
F12	50 ± 0.94	125 ± 1.43	G	No defects	98.91 ± 2.42
F13	56 ± 1.67	128 ± 1.76	M	No defects	99.05 ± 2.2
F14	55 ± 0.87	127 ± 2.12	M	No defects	99.84 ± 1.25
F15	53 ± 0.61	126 ± 2.67	G	No defects	97.64 ± 1.52

All the data are results of three analyses (n = 3), indicated as mean ± SD

Letters 'G', 'M', and 'P' indicates the good, moderate, and poor spreadability of the formulations

Optimization of lipstick formulation by response analysis

Model fitting the outcomes of the experiments and the ANOVA of the model are given in Table 5. In the development of lipstick of KTZ from SEDDs, the amount of beeswax and carnauba wax was found to be considerably effective ($p < 0.0001$). The formulation of lipstick was carried out with about 20–30% of beeswax and 5–10% carnauba wax. The quadratic model was used for the optimization, and binary interactions are also included in the equation for Y_1. The surface and contour plots of the independent variables showing a significant impact on the responses are depicted in Fig. 4a, b. The percentage drug release after 12 h (Y_2) of the systems depicting the movement of the drug from the formulation into the receptor media is shown in Table 5. The surface and contour plots of the independent variables which are statistically significant for the percentage drug release after 12 h are depicted in Fig. 4c and d. The correlation plots of the softening point and percentage drug release after 12 h between predicted and experimental data are generated from the mathematical model as indicated in Fig. 4e and f, respectively. The data estimated from the outcomes of softening point and percentage drug release data models were comparable and significant. The lipstick formulation F11 was chosen for the stability testing due to its relatively high melting point, good spreadability, no surface anomalies, and high softening point along with reasonably high drug content, and better drug release after 12 h.

Stability study

The stability study involved the estimation of melting point, spreadability, breaking point, surface anomalies, drug content, and percentage drug release after 12 h of the optimized formulation at different temperature conditions. The lipstick exhibited a bit softer consistency at 45 °C without impairing the spreadability and deformation in the lipstick.

In vitro antifungal activity

The microbiological study revealed that the KTZ-SEDDs-lipsticks significantly inhibited the growth of *C. albicans* as compared to plain KTZ solution (1% w/v). The mean diameter of zones of inhibition was found to be 27.36 ± 1.55 mm for KTZ-SEDDs-lipsticks and 19.84 ± 2.02 mm for KTZ solution (Fig. 5).

Discussion

The solubility of the drug was observed to be higher in IPM, Tween 80 and PEG 400 as compared to other oils, surfactants and co-surfactants, respectively, therefore selected for development of SEDDs. The emulsification region indicated better self-emulsification of 1:1 combination of Tween 80 and PEG 400 as compared to other ratios. The high amount of PEG 400 in the 1:1.5 combination led to a reduction in the self-emulsification area, possibly due to the hydrophilic nature of PEG 400. However, the increase in PEG 400 concentration allowed more dissolution of KTZ but the microemulsion region was considerably reduced. The outcomes of the run showed that the correlation coefficients (R^2) of the equations estimated using experimental results were well-fitted to the data. The R^2 values are indicated in Table 5, and the *P*-value should be smaller along with a high *F*-value for depicting the more relevant influence over response variables in the models. It was evident from the outcomes that the physicochemical properties of the lipsticks were affected by the different components used for the formulation. The amount of cow ghee used in the formulation displayed an insignificant effect on the melting point of the lipstick. However, the melting

Table 5 ANOVA of the fitted equation for softening point and percentage release after 12 h from lipstick formulation

	Softening point				% Drug release after 12 h			
	Sum of Squares	df	Mean Square	*F*-value	Sum of Squares	df	Mean Square	*F*-value
Model	429.52	9	47.72	168.44	475.64	9	52.85	39.32
A-Beeswax	378.12	1	378.12	1334.56	71.70	1	71.70	53.35
B-Carnauba Wax	15.12	1	15.12	53.38	8.14	1	8.14	6.06
C-Cow Ghee	0.20	1	0.20	0.24	8.12	1	8.12	6.04
Residual	1.42	5	0.2833		6.72	5	1.34	
Lack of Fit	0.7500	3	0.2500	0.7500	0.99	3	0.5629	0.2237
Pure Error	0.6667	2	0.3333		1.03	2	0.52	
Std. Dev	0.5323				1.16			
Adjusted R^2	0.9908				0.9610			
Predicted R^2	0.9687				0.9205			
R^2	0.9967				0.9861			

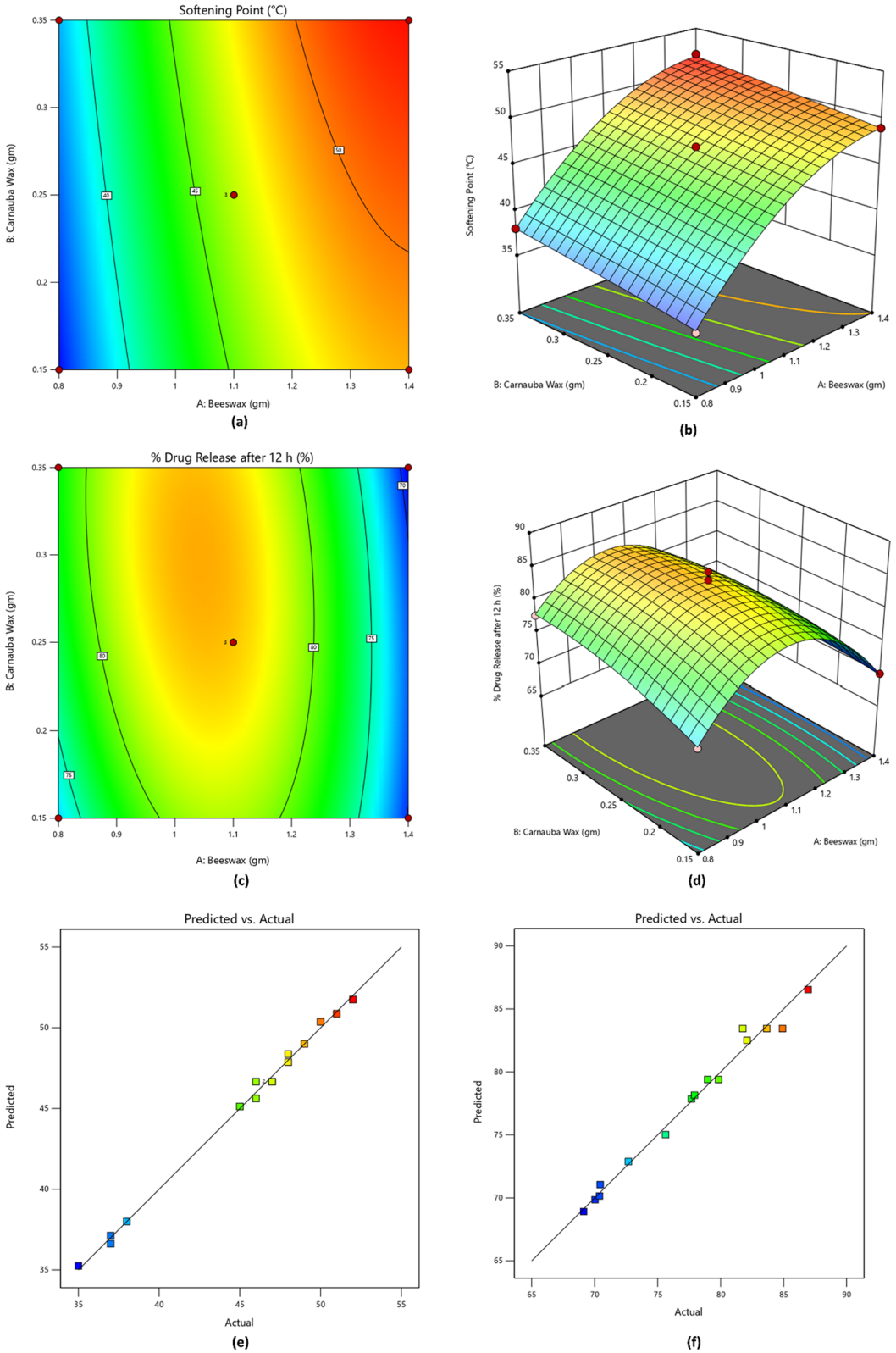


Fig. 4 Illustration of influence of the amount of beeswax and carnauba wax interaction: **a** Contour plot, **b** 3D surface plot for softening point and **c** Contour plot, and **d** 3D surface plot for percent drug release after 12 h. A comparison of the predicted and actual experimental data of the lipstick formulation is given in (**e**) softening point, and (**f**) percent drug release after 12 h

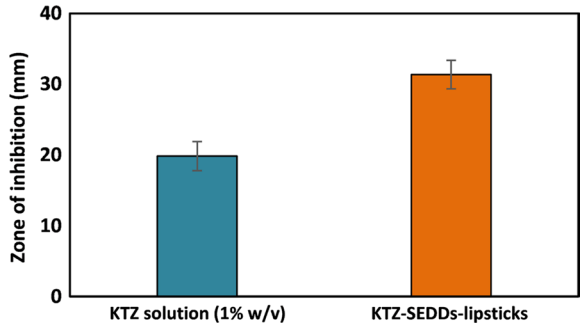


Fig. 5 In vitro antifungal activity of KTZ solution (1% w/v) and KTZ-SEDDs-lipsticks against *C. albicans*

point increased with the increase in the concentration of beeswax and carnauba wax. It suggests that lipstick with the desired melting point can be produced by optimizing the concentration of waxes. It was clear that lipsticks with a higher quantity of waxes showed relatively high breaking points. However, the outcomes of the breaking point test do not indicate the superiority of any specific formulations. There were no defective indications, as well as significantly high percentage drug content for optimized lipstick formulation. A high *F*-value indicated that the model is significant in assessing the impact of the independent variables over responses. The change in the quantity of cow ghee which was used as a penetration enhancer and moisturizer in the system was not statistically significant; therefore, it was taken as constant in the response plots. It was observed that as softening point of the lipstick increased with the increase in the amount of beeswax and carnauba wax used. When the responses were evaluated for the lipstick formulation, the amount of beeswax and carnauba wax used was observed to have a significant impact on the quadratic model. When the amount of beeswax increased up to 1.1 g, the percentage release of KTZ from the formulation also increased but the drug release decreases upon the subsequent increase in beeswax up to 1.4 g. There was no significant difference in the melting point and breaking point of the lipstick throughout the study. They showed no defects at different temperature conditions, and the surface of the lipstick was intact for 4 weeks. The formulation showed good spreadability when being stored at room temperature, 40 °C, and 45 °C allowing uniform application without getting fragmented or deformed throughout the entire stability study. The lipstick formulations showed considerably high inhibition of growth C. albicans in comparison to 1% w/v drug solution. The results indicated enhanced antifungal activity which could also be due to the synergistic effect of KTZ along with castor oil and lanolin. Therefore, the product will be useful in the management of Candidiasis, caused by Candida albicans which is one of the most common type of lip fungal infections.

Conclusion

The research was focused on loading the KTZ in SEDDs before incorporating it into waxes to develop lipsticks. IPM, Tween 80, and PEG 400 were selected as oil phase, surfactant, and co-surfactant, respectively, by estimating the solubility of the drug. Smix ratio (Tween80: PEG 400) of 1:1 showed the highest microemulsion ratio, therefore chosen for further loading into lipsticks. The concentrations of waxes (beeswax and carnauba wax) and cow ghee were optimized using a Box–Behnken design. The optimal SEDDs were loaded into different lipstick formulations, and the concentration of waxes and cow ghee was generated from the software while taking a suitable concentration of other excipients as reported from previous literature [45, 46]. The physicochemical characterization of the lipsticks, such as melting point, spreadability, surface anomalies, breaking point, and drug content, was evaluated and most of the lipsticks fall within the acceptable range. However, the characteristics of some of the lipsticks were superior owing to physicochemical features, optimum softening point, and relatively better release of drug after 12 h. The optimum lipstick formulation (F11) examined for stability at different temperature conditions (room temperature, 40 °C, and 45 °C) showed no signs of instabilities throughout the study period. Finally, the microbiological assay of the KTZ-SEDDs-lipsticks showed high antifungal activity as compared to plain KTZ solution. Therefore, it was deduced that the self-emulsifying systems loaded lipsticks may be suitable for attaining better drug delivery and enhancing the therapeutic potential of the drug in candidiasis.

Abbreviations

SEDDs	Self-emulsifying drug delivery systems
Tween 80	Polysorbate 80, Polyoxyethylene sorbitan monooleate
PEG 400	Polyethylene glycol 400
AIDS	Acquired immune deficiency syndrome
KTZ	Ketoconazole
LR	Lanolin anhydrous
BHA	Butylated hydroxyanisole
IPM	Iso-propylmyristate
ANOVA	Analysis of variance
r^2	Multiple correlation coefficient
adjusted r^2	Adjusted multiple correlation coefficient
C.V.	Coefficient of variation
UV–Vis	Ultraviolet–visible
df	Degree of freedom

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Author contributions

Dr. BG.P was responsible for the conceptualization and planning of the research work. Dr. HP and Mr. PA.S developed and evaluated the

KTZ-SEDDs-based lipstick formulation under the guidance of Dr. BG.P. Dr. HP has compiled and prepared the manuscript and all authors have read and approved the final manuscript.

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