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Clotrimazole nanoemulsion for malaria chemotherapy. Part I: Preformulation studies, formulation design and physicochemical evaluation

Vivek Borhade[a,1], Sulabha Pathak[b,2], Shobhona Sharma[b,2], Vandana Patravale[a,*]

[a] Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, N.P. Marg, Matunga, Mumbai 400019, Maharashtra, India
[b] Department of Biological Sciences, Tata Institute of Fundamental Research, Colaba, Mumbai 400005, Maharashtra, India

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ABSTRACT

Clotrimazole was formulated in nanoemulsion based system with the aim of improving its solubility and dissolution, which can further used for its preclinical evaluation. Clotrimazole nanoemulsion was prepared using spontaneous nanoemulsification method. Preformulation studies were preformed to evaluate drug-excipient compatibility, solution state pH stability and pH solubility profile. Solubility of clotrimazole in oils, surfactants and cosurfactants was determined to identify nanoemulsion components. Surfactants and cosurfactants were screened for their ability to emulsify selected oily phases. Phase diagrams were constructed to identify area of nanoemulsification. Influence of clotrimazole and pH of dilution medium on phase behavior were assessed. Drug-excipient chemical compatibility study facilitated to anticipate acid catalyzed degradation of clotrimazole. The pH of nanoemulsion was adjusted to 7.5, which could stabilize clotrimazole. Nanoemulsion composed of Capryol 90, Solutol HS 15 and Gelucire 44/14 enhanced solubility of clotrimazole up to 25 mg/ml. The optimized clotrimazole nanoemulsion could withstand the extensive dilution and did not show any phase separation or drug precipitation. The nanoemulsion exhibited mean globule size <25 nm, which was not affected by pH of dilution medium. Dissolution profile of clotrimazole nanoemulsion in various media showed 100% drug release within 15 min irrespective of pH of medium.

1. Introduction

Malaria is one of the oldest afflictions of man and even today approximately 40% of world's populations; primarily the most disadvantaged are at risk of this disease. Antimalarial drugs have played a mainstream role in management and control of malaria in human host. For decades, malaria chemotherapy has relied largely on comparatively small number of chemically related drugs with lack of structural diversity. These handful of drugs have their own limitations, of which the acquisition and spread of parasite multidrug resistance has been the most damaging. Because of which, the affordable drugs used in resource poor settings, such as chloroquine and sulfadoxine–pyrimethamine are of limited benefit across much of the world (Bangchang and Karbwang, 2009). Further, no new chemical class of antimalarials has been introduced into clinical practice since 1996 (Ekland and Fidock, 2008; Gamo et al., 2010). Recently, the last class of the newest and widely efficacious drugs, the artemisinins, is also being compromised by the rise of *Plasmodium falciparum* strains with reduced clinical response to artemisinin-containing drug combinations. Decline in efficacy of such therapies have been reported in South Asia, and there is persuasive concern that this would spread to other parts of world, notably to Africa (Andriantsoanirina et al., 2009; Bonnet et al., 2009; Carrara et al., 2009). The other problems associated with some existing drugs include unfavorable pharmacokinetics and adverse effects/toxicity (WHO, 2010). These factors underscore the continuing need for the development of new classes of antimalarial agents that are effective against multidrug resistant *Plasmodium* species. In this quest, one of the approaches to counterbalance the burgeoning quandary is the identification of antimalarial effects of older drug molecules that have already been evaluated for the treatment for other diseases, *i.e.* the piggyback approach. It represents a prospective strategy with potentially rapid clinical application (Bangchang and Karbwang, 2009; Gelb, 2007).

Many research groups have demonstrated a potent *in vitro* antimalarial activity of an antimycotic drug, clotrimazole on chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. The concentrations for 50% inhibition of parasitic growth (IC_{50}) were between 0.1 and 1.1 μM; and clotrimazole concentrations of 2 μM and above caused complete inhibition

* Corresponding author. Tel.: +91 22 3361 1111/2222/2217; fax: +91 22 3361 1020.
E-mail addresses: vivekborhade@gmail.com (V. Borhade), suepathak@tifr.res.in (S. Pathak), sharma@tifr.res.in (S. Sharma), vbp_muict@yahoo.co.in (V. Patravale).
[1] Tel.: +91 22 3361 1111/2222/2217; fax: +91 22 3361 1020.
[2] Tel.: +91 22 2278 2625/2865; fax: +91 22 2280 4610/4611.

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Table 1
Physiochemical properties of CLT.

Property	Value
Molecular weight	344.8 g/mol ($C_{22}H_{17}Cl\ N_2$)
log *P*	6.30
pK_a	4.70 and 6.02
Nature	Weak base
Melting range	141–145 °C
Solubility in water	0.49 μg/ml

of parasite replication within a single intraerythrocytic asexual cycle (Saliba and Kirk, 1998; Tiffert et al., 2000). A single 1 g oral dose of clotrimazole in humans could yield plasma levels up to 3.3 μM (Rifai et al., 1995). Pediatric and adult administrations of high doses of clotrimazole (100 mg/kg) for treatment of mycotic infections have been well tolerated (Holt and Newman, 1972).

Taken together, this information has led our research group to evaluate the potential of clotrimazole as antimalarial drug. However, it was reported earlier that clotrimazole has poor and erratic bioavailability with C_{max} attained after 6 h when administered orally and; also exhibits marked intra and inter-individual variations (Brugnara et al., 1995; Rifai et al., 1995). This is primarily attributed to its poor aqueous solubility and high lipophilicity (Table 1) (Balakrishnan et al., 2007). For such drugs, dissolution in gastrointestinal lumen is rate controlling step for absorption. Improved absorption can be achieved by use of delivery systems, which can enhance drug dissolution from its dosage form and maintain the drug in dissolved state in gastrointestinal fluids. Few attempts have been investigated to improve systemic delivery of clotrimazole and include complexation with cyclodextrins for oral delivery (Pedersen et al., 1998; Balakrishnan et al., 2007) and poloxamer–propylene glycol based suppository for rectal administration (Yong et al., 2006). However, these formulations were characterized by low drug loading and incomplete drug dissolution; and the physical stability of drug and cyclodextrin complexes was not evaluated. Moreover, our preformulation studies indicated that, though clotrimazole has good solubility in cosolvents such as ethanol and transcutol; these solutions became turbid immediately after dilution with water because of precipitation. Further, clotrimazole had improved solubility in acidic pH; the pH adjustment could not be used to improve its solubility due to its instability in acidic pH. Considering the limitations of these strategies; an approach which will increase its solubility and dissolution of clotrimazole is highly desirable. High lipophilicity of clotrimazole indicates good passive permeability through gastrointestinal membrane. It is also reported earlier that the absorption of clotrimazole was more efficient when given in oil solution than in tablets (Seo et al., 1977). These properties render clotrimazole a potential candidate for lipid based systems such as self-nanoemulsifying formulations and/or nanoemulsions.

Nanoemulsions are heterogeneous systems composed of oil droplets dispersed in aqueous media and stabilized by surfactant molecules. Moreover, they are kinetically stable without any apparent flocculation or coalescence during the long term storage due to their nanometer sized droplets (Nicolas and Vandamme, 2009, 2011). Recently, increasing attention has been focused on nanoemulsion based drug delivery system due to their ease of formulation with biocompatible excipients, and unique properties such as smaller droplet size (<200 nm), increasing solubility and dissolution rate, improving diffusion and mucosal permeability. The utility of nanoemulsions has been successfully established in optimizing the therapeutic performance of many lipophilic drugs (Date et al., 2010), which further justifies rationale of the study. In this context, this is the first part of the investigation aimed to formulate clotrimazole nanoemulsion for improving oral delivery; which can further used for its preclinical evaluation. The nanoemulsion was prepared by spontaneous emulsification method and optimized using phase diagrams. It was also evaluated for influence of drug and pH on phase behavior. A detailed preformulation study was also undertaken in order to select appropriate excipients and to stabilize the drug molecule.

2. Materials and methods

2.1. Materials

Clotrimazole was obtained from Glenmark Generics Ltd., Mumbai, India. Capmul MCM C8, Captex 200 P, Captex 355 EP, Captex 1000, Captex 8000 (Abitec Corporation, Janesville, USA), Cremophor EL, Cremophor RH 40, Poloxamer 188, Poloxamer 407, Solutol HS 15 (BASF India Ltd., Mumbai, India), Capryol 90, Gelucire 44/14, Gelucire 50/13, Labrafac CC, Labrafac PG, Labrafil M 1944 CS, Labrafil M 2125 CS, Labrafil M 2130 CS, Labrasol, Lauroglycol 90, Maisine 35-1, Peceol, Plurol oleique CC 497, Transcutol HP (Gattefosse, St-Priest, France), Miglyol 810 (Sasol GmbH, Hamburg, Germany) were obtained as gift samples. Ethanol, polyethylene glycol 400, propylene glycol, soybean oil, tween 20, tween 80, HPLC grade acetonitrile, various buffer salts (S.D. Fine Chemicals, Mumbai, India) and Myrj 52 (Sigma Chemical Company, St. Louis, USA) were purchased. All chemicals and excipients were used as received. Freshly prepared double distilled water and buffers were filtered through 0.22 μm membrane filter (Pall India Pvt. Ltd., Ahmedabad, India) and used whenever required.

2.2. HPLC quantification of clotrimazole

A reversed phase HPLC method was developed for analysis of clotrimazole. The HPLC system consisted of Jasco PU-2080 Plus Intelligent HPLC pump (Jasco, Tokyo, Japan) equipped with UV-2075 Intelligent UV/VIS detector (Jasco, Tokyo, Japan), a Rheodyne 7725 injector (Rheodyne, Cotati, USA) and a Jasco ChromaPass Chromatography data system software (Version 1.8.6.1; Tokyo, Japan) was used. Chromatographic separation was performed on Hibar 250-4, 6, LiChrospher 60 RP-select B, 5 μm HPLC column (Merck KGaA, Darmstadt, Germany). The mobile phase consisted of acetonitrile:dibasic potassium phosphate buffer, pH 7.0 (75:25, v/v) was used. Freshly prepared mobile phase was filtered through 0.22 μm filter and degassed for 15 min before analysis. All samples were analyzed under isocratic elution at a flow rate of 1.0 ml/min, and effluent was monitored at 254 nm. A 100 μl of sample was injected onto the Rheodyne and analyzed at 25 °C. The retention time of ATQ was about 7.45 ± 0.31 min. The method was validated according to the ICH guidelines, Q2(R1). Assay was linear (r^2 = 0.9997) in the concentration range of 25–500 μg/ml. The method was found to be accurate, precise and robust as percent relative standard deviation was consistently <2%.

2.3. Preformulation studies

2.3.1. Drug-excipient chemical compatibility

Accurately weighed amounts of clotrimazole (100 mg) and each of selected excipients (500 mg) were placed in 5 ml glass vials and mixed thoroughly. Closed vials containing blends were stored in ovens at 60 °C and at 40 °C/75% RH for 14 days. A standard clotrimazole sample without mixing with excipients alone was also kept under similar conditions. The amount of drug substance in blends was determined on the basis of expected drug to excipient ratio in final formulation. Duplicate samples of drug-excipient blends were analyzed after 14 days by validated HPLC method.

Table 2
Visual assessment of emulsification efficiency.

Dispersability and appearance	Self-emulsification time (min)	Grade
Compositions spreads rapidly in water forming clear and transparent nanoemulsion	<1	+++ (very good)
Compositions droplets spread in water to form turbid emulsion	3–5	+ (good)
Compositions do not spread in water and form coalescence of oil droplets	Not emulsified	– (poor)

2.3.2. Solution state pH stability

A standard solution of clotrimazole, 1 mg/ml in acetonitrile was prepared. It was further diluted with USP buffers of pH 1.2, 4.5, 6.8 and 7.5, each to 10 ml. These solutions were incubated for 2, 4 and 24 h at 37 °C. In order to achieve adequate solubility levels; aqueous samples were prepared with acetonitrile as a cosolvent at an effective final concentration of 10% (v/v). The samples were assayed for drug content by validated HPLC method.

2.3.3. Solubility studies

The solubility of clotrimazole in oils, buffers and 10% (w/v) surfactant solutions was determined by shake flask method. Briefly, an excess amount of clotrimazole was added to each glass test tube containing selected vehicle and mixed using cyclomixer. The test tubes were incubated in reciprocating water bath shaker (Boekel Scientific, Pennsylvania, USA) at 37 °C and shaken for 48 h. Samples were centrifuged (Research Centrifuge, Eltek, TC 4100D, Elektrocrafts, Mumbai, India) at 5000 rpm for 10 min and subsequently filtered through 0.22 μm syringe driven membrane filter unit (Pall India Pvt. Ltd., Ahmedabad, India). The filtrates were assayed by HPLC to evaluate amount of drug dissolved. Experiments were conducted in triplicate.

2.3.4. Screening of surfactants and cosurfactants: assessment of dispersion properties

Initial evaluation of emulsifying properties was carried out by visual assessment. Selected oil and surfactant were mixed in 1:3 (w/w), heated at 40–50 °C and vortexed to form homogenous mixture. Oil–surfactant mixture, 500 mg dispersed into 10 ml of water with gentle stirring. Visual test was used to assess self-emulsification in terms of dispersability, ease of emulsification and final appearance using grading system (Table 2). Various cosurfactants were screened by mixing surfactants with selected cosurfactants in 2:1 (w/w) ratios. Oily phase was added to this mixture in 1:3 (w/w), heated and vortexed gently to form homogenous mixture. They were evaluated similar manner as explained earlier.

2.4. Clotrimazole nanoemulsion development and evaluation

2.4.1. Construction of phase diagrams

Phase diagrams of surfactant, cosurfactant and oil were plotted; each of them representing an apex of triangle (Kommuru et al., 2001). Ternary mixtures with varying compositions of surfactants (or cosurfactant) and oil were prepared. The surfactant concentration was varied from 15 to 70% (w/w) and oil concentration was varied from 15 to 45% (w/w). For any mixture, total of surfactants and oil concentrations always added to 100%. For example, first mixture consisted of 70% of surfactant (for example, Solutol HS 15), 15% of oily phase (Capryol 90) and 15% of second surfactant or cosurfactant (Gelucire 44/14 or Transcutol HP). In further experiments, second surfactant or cosurfactant was increased by 5% for each composition, oily phase concentration was kept constant and surfactant concentration was adjusted to make a total of 100%. Sixty-three such mixtures with varying surfactant and oil concentrations were prepared. The required amount of oil and surfactants were weighed accurately, gently heated at 45–50 °C and vortexed to form homogenous, isotropic mixtures. Compositions were evaluated for nanoemulsion formation by dispersing 500 mg of each mixture in 10 ml water. Visual observations were made immediately after dilution for dispersability, ease of emulsification and final appearance. The compositions that could self-emulsify under dilution and gentle agitation were identified from phase diagrams. Globule size of dispersions was determined immediately by dynamic light scattering (DLS). Dispersions, having globule size 100 nm or below were considered desirable. Nanoemulsions were observed for 24 h. Nanoemulsions which showed phase separation were rejected. The area of nanoemulsion formation (NE) was identified for respective system in which nanoemulsions with desired globule size and stability were obtained.

2.4.2. Effect of incorporation clotrimazole and pH of dispersion medium

Various compositions used for the study are presented in Table 3. Clotrimazole was dissolved in required amount of selected oil separately. Surfactants were weighed accurately and gently heated at 45–50 °C for 5 min. Both the phases were mixed to obtain homogenous isotropic mixtures. The influence of pH of dispersion media and addition of clotrimazole on the formation of nanoemulsion was investigated by dispersing 500 mg of each mixture with 10 ml of various USP buffers of pH 1.2, 4.5, 6.8 and 7.5; and water. Simultaneously, another set of similar compositions were prepared without clotrimazole to differentiate the effect of pH of dispersion medium and addition of clotrimazole. The mean globule size and polydispersity index (PI) of resulting dispersions were measured by DLS. Visual observations were made immediately after dispersion, for ease of dispersability, nanoemulsification efficiency and transparency. Nanoemulsions were observed for 24 h and compositions which exhibited phase separation and/or drug precipitation were rejected.

2.4.3. Effect of increasing clotrimazole loading

Effect of increasing clotrimazole loading on physical stability of nanoemulsions was studied using optimized composition *i.e.* formulation F3. Accordingly, a series of oil–surfactants mixture was prepared with varying amount of clotrimazole (0–10%, w/w) and

Table 3
Compositions of oil-surfactants used to study effect of incorporation of CLT and pH of dispersion medium. All quantities expressed in mg.

Ingredients	Formulations											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Capryol 90	125	125	125	150	150	150	175	175	175	200	200	200
Solutol HS 15	108	162	216	100	150	200	92	137	183	83	125	167
Gelucire 44/14	216	162	108	200	150	100	183	137	92	167	125	83
Clotrimazole	25	25	25	25	25	25	25	25	25	25	25	25

500 mg of it was dispersed in 10 ml of different USP buffers and the globule size and PI were determined immediately by DLS.

2.4.4. Preparation of nanoemulsion formulations

Clotrimazole nanoemulsions were prepared by dissolving drug into oily phase separately. Surfactants were weighed accurately and gently heated at 45–50 °C for 5 min. Both phases were mixed to form homogenous isotropic mixtures. Required amount of phosphate buffer, pH 7.5 was added to mixture to obtain nanoemulsion. The nanoemulsions were stored at room temperature until used.

2.4.5. Robustness to dilution: impact of dispersion media

The robustness of nanoemulsions to dilution was studied by diluting 500 mg of clotrimazole incorporated oil–surfactants mixture with 250 ml of various buffers. Visual observations were made immediately after dilution for self-nanoemulsification efficiency and transparency. Resulting nanoemulsions were kept at room temperature for 24 h to assess the phase separation and drug precipitation, if any. Globule size and PI was determined immediately and after 24 h.

2.4.6. Dynamic light scattering (DLS) measurements

The average globule size and its distribution (polydispersity index, PI) of nanoemulsions were measured in triplicate at 25 °C by DLS using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The instrument utilizes a 4 mW He–Ne red laser at 633 nm. The light scattering is detected at 173° by noninvasive backscatter (NIBS) technology with a measuring range from approximately 0.6 nm to 6 μm. Disposable polystyrene cuvettes, 1 ml were used for measurements. Water or buffers (filtered through a 0.22 μm membrane filter) were used to dilute the formulations. The DLS measurement yields *z*-average mean hydrodynamic diameter of the sample, which is an intensity weighted mean diameter of the bulk population. Whereas, the PI value obtained is a measure for the width of size distribution and ranges from 0 to 1. The values near to zero indicate monodispersed particle population where as values >0.5 signifies a very broad size distribution.

2.4.7. In vitro dissolution profile: dispersion and precipitation assessment

Dissolution studies were performed using USP Apparatus II; paddle (USP Dissolution Tester TDT-06T, Electrolab, Mumbai, India) at 37 ± 0.5 °C. Accurately weighed samples (clotrimazole as plain drug and nanoemulsion) containing 300 mg equivalent of clotrimazole were placed in 500 ml of dissolution medium. USP buffers of pH 1.2, 4.5 and 6.8 were used as dissolution media. The paddle revolution speed was set to 50 rpm. At predefined time intervals (15, 30, 45, 60, and 120 min); 5 ml aliquots were withdrawn from each vessel and replaced with a similar volume of fresh media. Aliquots were centrifuged at 5000 rpm for 10 min and subsequently filtered through 0.22 μm syringe driven membrane filter unit. The filtrates were assayed for drug concentration.

3. Results and discussion

3.1. Preformulation studies

3.1.1. Drug-excipient chemical compatibility

The total number of drug-excipient blends in the study may be very high; therefore excipients rank ordered with their solubility for clotrimazole were selected for primary screening. For example, oils such as Capryol 90, Lauroglycol 90, and Capmul MCM C8 exhibiting higher solubility for clotrimazole were selected. As summarized in Table 4, every excipient clotrimazole had degraded approximately 5–15% in 14 days at both storage conditions. The rate of degradation increased with an increase in temperature. Similar degradation peak of clotrimazole was evident in chromatograms of all samples. The representative chromatogram of sample stored at 60 °C for 14 days is shown in Fig. 1A, which shows well resolved degradation product of clotrimazole. Interestingly, chromatogram is similar to that obtained after forced degradation of clotrimazole in 1 N HCl, indicating its possible degradation mechanism. In solution state, stability of clotrimazole is pH dependent. Clotrimazole hydrolyzes in acidic medium to (o-chlorophenyl) diphenylmethanol and imidazole (Hoogerheide and Wyka, 1982). With the knowledge that clotrimazole is predominately stable under neutral to basic pH, degradation products formed by acid catalyzed degradation are shown in Fig. 1B. This clearly indicated that degradation of clotrimazole was catalyzed by acidic nature of excipients, attributed to presence of free fatty acids. For example, Capryol 90 and Lauroglycol 90 contain trace amounts of caprylic acid and lauric acid, respectively. Another example is Solutol HS 15 (Macrogol 15 hydroxystearate), which is a mixture of mainly monoesters and diesters of 12-hydroxystearic acid and macrogols obtained by ethoxylation of 12-hydroxystearic acid. The main fatty acid component is 12-hydroxystearic acid with stearic acid and palmitic acid also present in noticeable amounts. Virtually, all lipidic excipients including modified oils and surfactants are synthesized by esterification or ethoxylation with various fatty acids and hence contain detectable amount of free fatty acids. Clotrimazole in solid state is stable and unaffected by heat (up to 70 °C) and light (Hoogerheide and Wyka, 1982) and therefore the control sample, *i.e.* clotrimazole alone kept at above mentioned storage conditions, remained stable. Excipients used in lipid based formulations are usually thought to be inert. However they may initiate, propagate or participate in chemical or physical interactions with drug, which may compromise the quality and effectiveness of overall medication. The stability aspects of drug molecules in these formulations have not been reported in the most of investigations. Paradoxically, all these excipients are routinely used in lipid based formulations. The observation of degradation of clotrimazole in present study clearly indicated the need to assess the stability of drug in individual components before proceeding to formulation development. Further, the understanding of chemical and physical nature of excipients, impurities or residues associated with them and how they may interact with each other or drug compound is critical; which can forewarns the possibilities of undesirable developments. The study also demonstrated that it is possible to reduce or avoid the occurrence of such unwanted scenarios by allying knowledge of the propensity of a drug to undergo degradation

Table 4
Percent amount of drug remained after a storage period of 14 days at 60 °C and 40 °C/75% RH.

Excipients	60 °C (14 days)	40 °C/75% RH (14 days)
	% drug remained (mean ± SD, *n* = 3)	
CLT alone	99.52 ± 0.12	99.47 ± 0.54
Oily phases		
Capmul MCM C8	89.07 ± 3.63	93.51 ± 1.06
Capryol 90	87.58 ± 1.33	92.73 ± 2.03
Labrafac PG	86.96 ± 2.96	93.07 ± 1.65
Lauroglycol 90	89.35 ± 1.83	94.18 ± 1.32
Surfactants		
Cremophor EL	89.05 ± 1.25	90.52 ± 2.20
Cremophor RH 40	88.75 ± 2.20	91.87 ± 1.25
Gelucire 44/14	87.95 ± 2.90	92.87 ± 2.52
Labrasol	86.72 ± 1.36	93.36 ± 1.99
Solutol HS 15	91.92 ± 1.92	94.72 ± 1.27
Tween 20	90.46 ± 1.24	93.15 ± 2.42
Cosurfactants		
Labrafil M 1944 CS	88.48 ± 0.92	91.39 ± 1.86
Transcutol HP	93.34 ± 1.05	93.25 ± 1.36

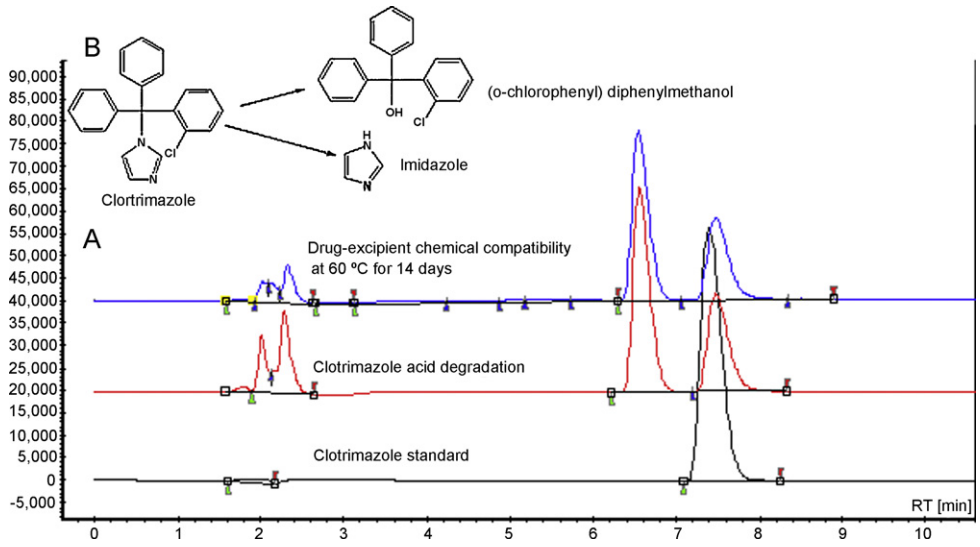

Fig. 1. (A) Chromatograms of clotrimazole stored at 60 °C for 14 days for drug-excipient chemical compatibility study and (B) acid degradation products of clotrimazole.

reactions. In conclusion, drug-excipient chemical compatibility study in present investigation facilitated to anticipate the undesirable interaction and was found to be a prerequisite for development of dosage forms that are stable and of good quality.

3.1.2. Solution state pH stability

Information on stability of drug in solution is required to understand its characteristics under physiological conditions. The pH-dependent stability was studied covering pH range in the gastrointestinal tract using USP buffers from pH 1.2 to 7.5. It is obvious that drug should not degrade significantly before being absorbed. Compounds with more than 5% degradation at physiological pH within the investigated period are expected to raise issues in this respect (Balbach and Korn, 2004). As seen in Table 5, clotrimazole did not degrade at any of pH condition in the investigation period. Even at pH 1.2, it was stable when assayed after 4 h incubation. It could be because of the fact that, clotrimazole to degrade in acidic environment requires either higher temperature or long incubation periods. The time periods investigated in present study were sufficient, considering the gastrointestinal tract physiology and time required for absorption of drug from immediate release product.

3.1.3. Solubility studies

Solubility of clotrimazole in various buffers is shown in Fig. 2. Clotrimazole is a weak base and is reported to be practically insoluble in water (Hoogerheide and Wyka, 1982). It can be clearly seen that clotrimazole has pH dependant solubility. The solubility of clotrimazole increased with decreasing pH. It is due to presence of ionizable imidazole group present in its structure. The nitrogen atoms are protonated in acidic pH and impart polar nature to clotrimazole, making it more soluble in lower pH buffers. The solubility of clotrimazole in water and in buffers pH 6.8 and 7.5 could not be estimated, as it was below the limit of quantification of developed method. However, it must be less than the reported solubility in water (0.49 μg/ml) and can be classified as practically insoluble.

Further solubility studies were mainly carried out for identifying suitable excipients for development nanoemulsion for clotrimazole. Identifying suitable oil, surfactant/cosurfactant having maximal solubilizing potential for drug under investigation is very important to achieve optimum drug loading. Solubility studies help mainly in selection of oily phases. The selected oily phases should be able to solubilize maximum amount of drug. This is important to achieve successful emulsification and to avoid precipitation of drug. Unmodified edible oils are the logical and preferred lipid excipients; however, they exhibit relatively poor emulsification efficiency (Pouton and Porter, 2008). Clotrimazole exhibited comparatively lower solubility in unmodified edible oils such as soybean oil and medium chain triglycerides (Fig. 3). However, clotrimazole was found to have good solubility in semisynthetic, modified oils. Among these, Capryol 90 was able to solubilize

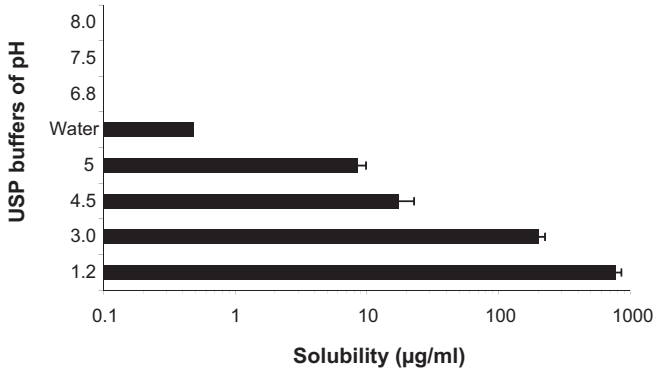

Fig. 2. pH solubility profile of clotrimazole. Data expressed as mean ± SD, $n = 3$.

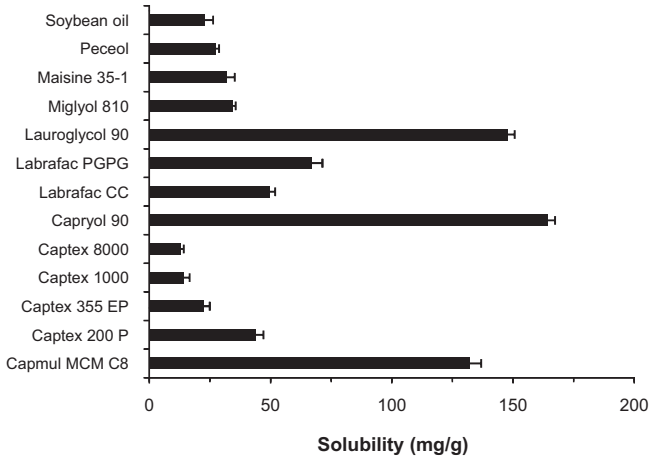

Fig. 3. Solubility of clotrimazole in various oily phases. Data expressed as mean ± SD, $n = 3$.

Table 5
Percent amount of drug remained in solution state pH stability study.

Time	pH 1.2	pH 3.5	pH 4.5	pH 6.8	pH 8.0
	% drug remained (mean ± SD, $n = 3$)				
Initial	98.94 ± 1.55	99.11 ± 0.45	98.86 ± 1.02	99.58 ± 1.72	99.39 ± 1.56
2 h	98.25 ± 1.66	99.19 ± 0.94	98.25 ± 1.21	99.35 ± 1.54	99.21 ± 1.21
4 h	99.14 ± 0.39	99.73 ± 1.42	98.21 ± 2.74	98.88 ± 1.71	98.97 ± 1.03
24 h	–	–	98.75 ± 1.64	99.26 ± 1.43	98.33 ± 1.35

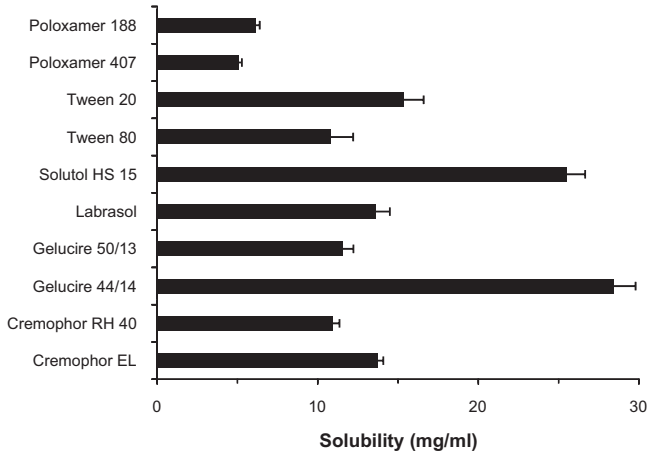

Fig. 4. Solubility of clotrimazole in various surfactant solutions (10%, w/v). Data expressed as mean ± SD, $n = 3$.

clotrimazole to the maximum extent. However, Lauroglycol 90 also showed comparable solubilizing potential. Hence, Capryol 90 and Lauroglycol 90 were selected as oily phases and were assessed further for their ease of emulsification in order to arrive at final oily phase to be used in nanoemulsion.

The solubility of clotrimazole in various surfactants and cosurfactants is shown in Figs. 4 and 5, respectively. Clotrimazole exhibited solubility in the order, Gelucire 44/14 > Solutol HS 15 > Tween 20 > Cremophor EL. The nonionic surfactants used in solubility studies are known to be less irritant and cytotoxic than anionic and cationic surfactants. Moreover, they are less affected by pH and changes in ionic strength that are likely to occur in the gastrointestinal tract. These characteristics would be important for successful development of clotrimazole nanoemulsion in order to achieve pH independent solubility and release profile of clotrimazole. Among the cosurfactants, Transcutol HP showed the highest solubility of clotrimazole. Selection of surfactants and cosurfactants was primarily governed by their emulsification efficiency for selected oily phases and their solubility potential for clotrimazole is considered as an added advantage.

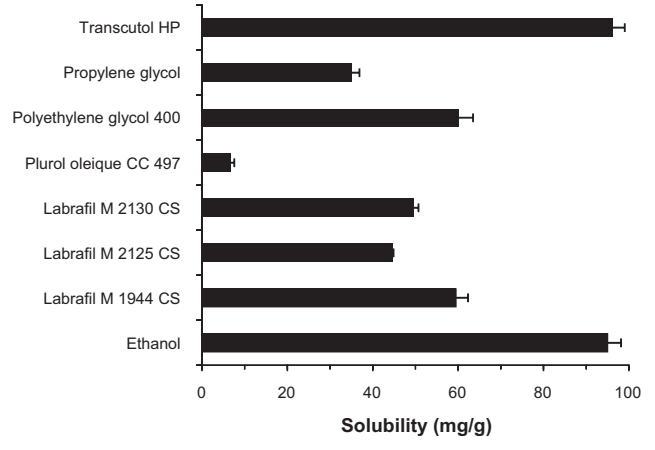

Fig. 5. Solubility of clotrimazole in various cosurfactants. Data expressed as mean ± SD, $n = 3$.

3.1.4. Screening of surfactants and cosurfactants: assessment of dispersion properties

The study was undertaken to identify suitable surfactants which can emulsify the selected oily phase. Ratio of oil to surfactant was decided on the basis of requirements stated by Pouton for spontaneously emulsifying systems (Pouton, 2006). Capryol 90 and Lauroglycol 90 were selected as oily phases based on their solubility for clotrimazole and screened against various surfactants. Grading of surfactants for relative emulsification is shown in Table 6 which clearly distinguished ability of surfactants to emulsify selected oily phases. The study indicated that Cremophor EL, Cremophor RH 40, Gelucire 44/14, Solutol HS 15 and Tween 20 had very good ability to emulsify Capryol 90 whereas Gelucire 55/13, Labrasol, Poloxamer 188, Poloxamer 407 and Tween 80 appeared to be poor emulsifiers. None of the surfactant could emulsify Lauroglycol 90 effectively. Although, HLB values of surfactants used in study were >10, there were considerable differences in their ability to emulsify oils. Results obtained indicated that apart from HLB value, other factors such as structure and relative length of hydrophobic chains of surfactants had influence on nanoemulsification. These results are in conformation with results reported in literature (Borhade et al., 2008). Gelucire 44/14 and Solutol HS 15 rendered effective nanoemulsification with added advantage of good solubilization potential for clotrimazole over other surfactants, and therefore were selected for further study.

Among the oils investigated, Lauroglycol 90 was difficult to emulsify whereas Capryol 90 was emulsified easily. This is explained by fact that ease of emulsification of oil and its amount incorporated in nanoemulsion are affected by molecular volume of oil. As number and length of hydrophobic alkyl chains increases, molecular volume increases and the oil becomes difficult to emulsify. Chemically Lauroglycol 90 and Capryol 90 are propylene glycol monolaurate and propylene glycol monocaprylate *i.e.*, monoesters of lauric acid and caprylic acid, respectively. Lauric acid has longer chain length (C12) than caprylic acid (C8) which

Table 6
Emulsification efficiency of various surfactants.

Surfactant	Oily phases	
	Capryol 90	Lauroglycol 90
Cremophor EL	+++	+
Cremophor RH 40	+++	+
Gelucire 44/14	+++	+
Gelucire 50/13	–	–
Myrj 52	+	–
Labrasol	–	–
Solutol HS 15	+++	+
Tween 20	+++	–
Tween 80	–	–
Poloxamer 188	–	–
Poloxamer 407	–	–

Table 7
Emulsification efficiencies of surfactant–cosurfactant combinations.

Cosurfactants	Gelucire 44/14	Solutol HS 15
Labrafil M 1944 CS	–	–
Labrafil M 2125 CS	–	–
Labrafil M 2130 CS	–	–
Plurol oleique CC 497	–	–
Polyethylene glycol 400	+	+
Propylene glycol	+	+
Transcutol HP	+++	+++

limits emulsification of Lauroglycol 90 compared to Capryol 90. The observations are in line with studies reported by Malcolmson et al. (1998) and Warisnoicharoen et al. (2000). Therefore, Capryol 90 was selected as oily phase for further study due to its relative ease of emulsification.

Table 7 shows relative efficacy of cosurfactants to improve emulsification of surfactants. As ratio of surfactant to cosurfactant is constant, the study clearly distinguished the ability of cosurfactants both hydrophilic and lipophilic, to improve emulsification of selected surfactants. Transcutol HP, propylene glycol and polyethylene glycol 400 which are hydrophilic cosurfactants increased spontaneity of nanoemulsion formation. However lipophilic cosurfactants in general were less effective as they could not improve emulsification of selected surfactants. Furthermore, as cosurfactants improve emulsification of surfactants by penetrating interfacial surfactant monolayer, their performance is affected by their structure and chain length (Malcolmson et al., 1998; Warisnoicharoen et al., 2000). Labrafils and Plurol olique CC 497 have oleate/linoleate and oleate backbones, respectively which increased their molecular volume and affect penetration at interface. In contrast, hydrophilic cosurfactants used were short chain amphiphiles and could penetrate interface effectively. Although other hydrophilic cosurfactants investigated were could improve emulsification of selected surfactants; Transcutol HP due to its superior solubilizing potential for clotrimazole was selected. Overall, the study gave an insight into relative emulsification properties of various components and formed the basis of their selection.

3.2. Clotrimazole nanoemulsion development and evaluation

3.2.1. Construction of phase diagrams

Phase diagrams were constructed in the absence of clotrimazole to identify self-nanoemulsifying regions and would help to optimize the concentration of oil and surfactants in nanoemulsion. The phase diagrams were studied for following three combinations namely, Capryol 90–Solutol HS 15–Transcutol HP, Capryol 90–Gelucire 44/14–Transcutol HP and Capryol 90–Solutol HS 15–Gelucire 44/14 and are shown in Fig. 6A–C. These combinations were selected based on solubility and emulsification studies. The percentage of surfactant, cosurfactant and oil used herein was decided on the basis of requirements stated by Pouton for spontaneously emulsifying systems (Pouton, 2006). The area occupied by square dots indicates the area explored for locating emulsification region whereas the region outlined by blue line indicates the area in which nanoemulsions of desired size and stability were obtained. The size of nanoemulsion region was compared; larger the size, greater is the self-emulsification efficiency. From Fig. 6A–C, it can be interpreted that the Capryol 90–Solutol HS 15–Gelucire 44/14 system has larger nanoemulsification region as compared to Capryol 90–Solutol HS 15–Transcutol HP and Capryol 90–Gelucire 44/14–Transcutol HP based compositions. Capryol 90–Solutol HS 15–Gelucire 44/14 could form nanoemulsion for the compositions that had oily phase concentration as high as 45% (w/w). Nanoemulsion formation area was increased with an increase in concentration of Solutol HS 15 compared to Gelucire 44/14 indicating its superior emulsification ability. Transcutol HP as cosurfactant was comparatively less effective, as it could not improve emulsification of both of surfactants resulting in smaller nanoemulsion area with low oil incorporation. The combined use of surfactants (Solutol HS 15–Gelucire 44/14) showed apparent advantages over the use of surfactant and cosurfactant combination. The nanoemulsion region was greatly increased in the phase diagram. The oil compositions were also broadened so that high drug loading became possible. Surfactants used in the study, Solutol HS 15 and Gelucire 44/14; both are hydrophilic having HLB values of 14–16 and 14, respectively; which render them highly suitable for generation of self-emulsifying compositions. Their combined use might have provided better hydrophilic–lipophilic balance. As a result, it enhanced the flexibility of surfactant layer that was formed at interface; it also improved their ability to partition in greater extent into oil–water interface; both of which resulted in stabilization of formed nanoemulsion. Similar observations were reported in literature where combined used of surfactants had increased the emulsification area with greater incorporation of oily phase (Moreno et al., 2003; Li et al., 2005). Therefore in view of current investigation, for improved physical stability and high clotrimazole solubilization; Capryol 90–Solutol HS 15–Gelucire 44/14 system was selected.

3.2.2. Selection of excipients

Apart from obvious formulation considerations, it is essential to use the excipients with defined regulatory status (Chen, 2008). The excipients which were screened earlier and selected for final formulation are generally regarded as safe (GRAS) listed. Capryol 90, an oily liquid chemically is propylene glycol monocaprylate, monoester of a medium chain fatty acid. It is listed in USP NF and widely used in oral formulations as oily solubilizer for bioavailability improvement. Gelucire 44/14 is a nonionic semisolid surfactant, included in USP NF and EP monographs as "Lauroyl polyoxylglycerides" and is a well defined mixture of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol. It is also included in FDA's inactive ingredients guide (IIG) for its use in oral tablets and capsules (1464 mg/day). Solutol HS 15 is polyethylene glycol 660 hydroxystearate, a nonionic solubilizer widely used in oral and parenteral formulations in various countries. It is available as, well defined chemical composition of polyglycol ester of 12-hydroxystearic acid (70%) and polyethylene glycol (30%), the main fatty acid component is 12-hydroxystearic acid. Its LD50 in rats by oral route is >20 g/kg. It is official in BP and PhEur monographs as Macrogol hydroxystearate 15.

3.2.3. Selection of formulation strategy for clotrimazole

As per initial proof of concept, clotrimazole was thought to be formulated as self-nanoemulsifying drug delivery system (SNEDDS). SNEDDS, an anhydrous form of nanoemulsion are isotropic mixtures of oil, surfactant(s) and cosurfactant(s), which when introduced into aqueous medium under conditions of gentle agitation, spontaneously form fine oil-in-water nanoemulsions. However in view of free fatty acid catalyzed degradation of clotrimazole as revealed in "drug-excipient chemical compatibility", it was essential to identify a suitable strategy to stabilize it. A simple approach would be pH adjustment. It was observed in our forced degradation studies that; clotrimazole is stable in neutral to basic conditions. Further, it was reported earlier that clotrimazole is very stable from pH 6.0 onwards, when heated at 95 °C for a period of week (Hoogerheide and Wyka, 1982). However it was difficult to adjust the pH of SNEDDS because of their anhydrous nature. So it was decided to formulate clotrimazole as a nanoemulsion based drug delivery system, which allowed the desired pH adjustment due to its water content. The pH of clotrimazole nanoemulsion was adjusted to 7.5 with phosphate buffer. Based on this

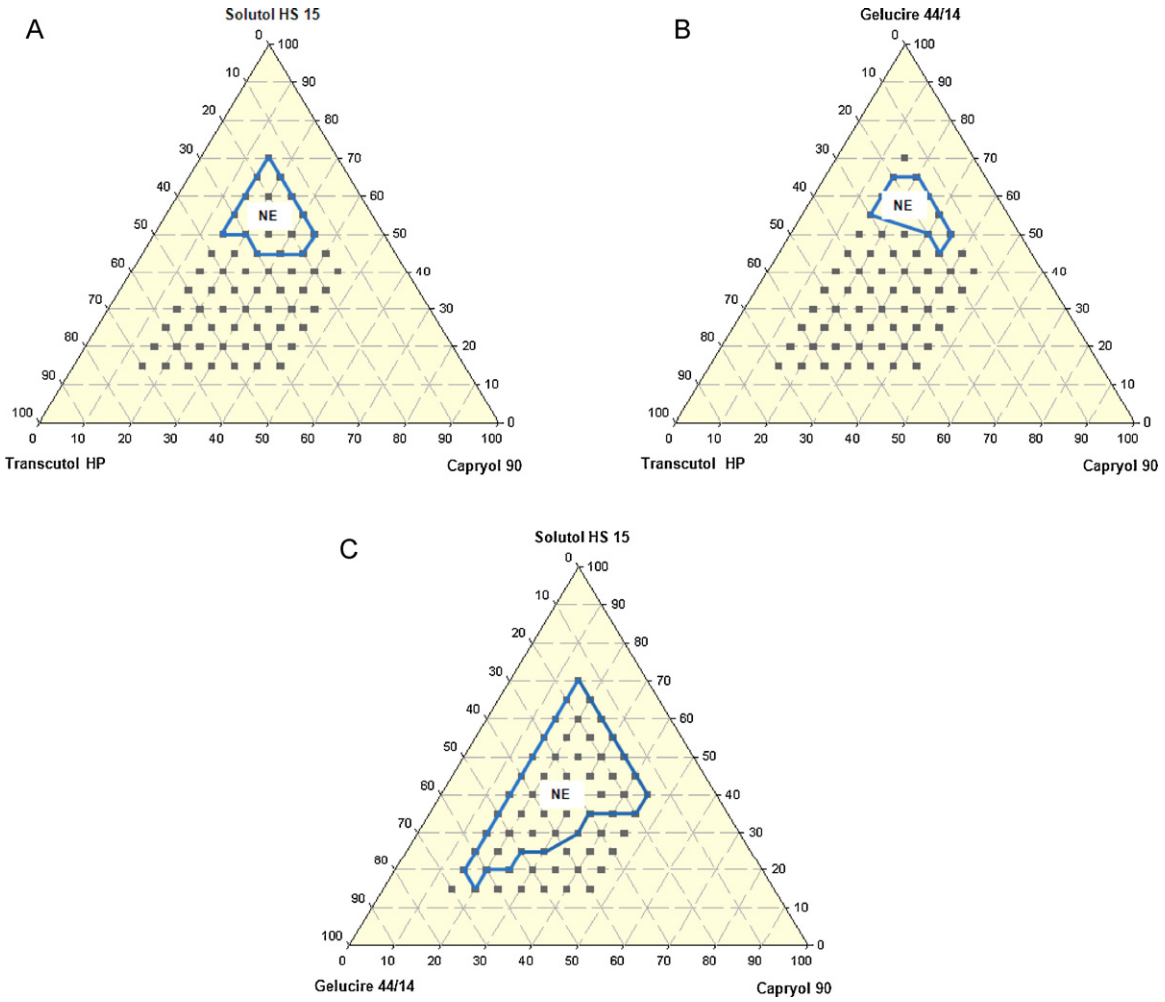

Fig. 6. Phase diagrams of (A) Capryol 90–Solutol HS 15–Transcutol HP, (B) Capryol 90–Gelucire 44/14–Transcutol HP and (C) Capryol 90–Solutol HS 15–Gelucire 44/14.

assumption, prototype stability evaluation of pH adjusted clotrimazole nanoemulsion was performed at 40 °C/75% RH for 6 months. This approach successfully stabilized clotrimazole nanoemulsion, without any decrease in drug content (data not shown). This was also supported by the fact that the commercial formulation of clotrimazole (Candid-V gel) has pH value of 7.0 and it claims the stability up to 2 years (Bachhav and Patravale1, 2009). This also corroborated the assumption drawn about the acid catalyzed degradation of clotrimazole and its stabilization at neutral to basic pH.

3.2.4. *Effect of incorporation clotrimazole and pH of dispersion medium*

The drug and pH of dispersion medium may have considerable influence on the behavior of nanoemulsifying formulations. Additionally keeping in view that the high concentration of

Table 8
Globule size and polydispersity index of various nanoemulsifying formulations without incorporation of CLT diluted with various buffers as dispersion medium. Data expressed as mean, $n = 3$, where relative standard deviation was < 10%.

Formulations	Hydrochloric acid buffer pH 1.2		Acetate buffer pH 4.5		Water		Phosphate buffer pH 6.8		Phosphate buffer pH 7.5	
	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI
F1	25.52	0.12	17.33	0.04	23.83	0.26	18.21	0.09	15.23	0.06
F2	17.26	0.04	16.13	0.07	21.37	0.22	21.43	0.08	16.08	0.06
F3	23.69	0.18	16.69	0.18	26.54	0.25	17.08	0.12	18.42	0.07
F4	20.23	0.12	18.17	0.18	19.04	0.05	19.84	0.10	17.27	0.05
F5	27.72	0.21	29.09	0.20	19.24	0.09	24.03	0.14	26.08	0.22
F6	19.31	0.01	19.24	0.10	22.79	0.18	21.74	0.15	22.42	0.19
F7	121.51	0.29	166.12	0.50	227.94	0.50	162.24	0.42	180.51	0.44
F8	22.12	0.01	22.93	0.04	21.26	0.09	24.53	0.05	22.20	0.05
F9	23.15	0.05	22.51	0.17	22.32	0.08	24.48	0.19	30.09	0.19
F10	148.34	0.34	168.9	0.37	136.94	0.52	162.73	0.37	173.78	0.35
F11	88.77	0.47	191.7	0.66	200.63	0.79	185.47	0.34	183.13	0.33
F12	23.41	0.12	26.23	0.09	24.49	0.07	30.14	0.21	28.48	0.18

Table 9
Globule size and polydispersity index of various nanoemulsifying formulations incorporated with CLT diluted with various buffers as dispersion medium. Data expressed as mean, $n = 3$, where relative standard deviation was <10%. Values in parentheses represent polydispersity index, $n = 3$.

Formulations	Hydrochloric acid buffer pH 1.2		Acetate buffer pH 4.5		Water		Phosphate buffer pH 6.8		Phosphate buffer pH 7.5	
	Globule size (nm)	Precipitation	Globule size (nm)	Precipitation	Globule size (nm)	Precipitation	Globule size (nm)	Precipitation	Globule size (nm)	Precipitation
F1	16.43 (0.08)	No	170.60 (0.46)	Yes	92.45 (0.34)	Yes	186.57 (0.43)	Yes	198.40 (0.45)	Yes
F2[a]	18.03 (0.13)	No	20.41 (0.08)	No	19.12 (0.16)	No	19.16 (0.11)	No	19.56 (0.07)	No
F3	17.97 (0.08)	No	19.38 (0.10)	No	18.55 (0.08)	No	18.07 (0.09)	No	20.02 (0.06)	No
F4	19.01 (0.05)	No	183.90 (0.44)	Yes	98.42 (0.19)	Yes	237.86 (0.30)	Yes	248.80 (0.31)	Yes
F5	20.25 (0.07)	No	145.40 (0.34)	Yes	119.10 (0.41)	Yes	117.16 (0.37)	Yes	192.25 (0.40)	Yes
F6 [a]	23.52 (0.10)	No	22.63 (0.09)	No	19.91 (0.13)	No	21.43 (0.12)	No	22.88 (0.09)	No
F7	113.59 (0.15)	No	159.40 (0.33)	Yes	187.70 (0.23)	Yes	223.49 (0.24)	Yes	273.80 (0.18)	Yes
F8	22.66 (0.06)	No	160.40 (0.35)	Yes	144.50 (0.24)	Yes	189.32 (0.29)	Yes	184.26 (0.16)	Yes
F9[a]	31.47 (0.14)	No	32.10 (0.24)	No	26.26 (0.12)	No	24.17 (0.15)	No	29.39 (0.14)	No
F10	145.82 (0.21)	No	168.40 (0.21)	Yes	142.60 (0.19)	Yes	188.95 (0.29)	Yes	252.40 (0.16)	Yes
F11	84.29 (0.29)	No	204.40 (0.42)	Yes	211.60 (0.24)	Yes	243.08 (0.32)	Yes	294.50 (0.47)	Yes
F12	32.71 (0.11)	No	178.40 (0.37)	Yes	147.30 (0.28)	Yes	198.43 (0.37)	Yes	231.14 (0.28)	Yes

[a] Formulations exhibited a tendency of drug precipitation at pH 7.5 when observed after 24 h.

clotrimazole to be incorporated and its pH dependent solubility, studies on effect of clotrimazole and pH of dispersion medium on the behavior of selected compositions were investigated. The compositions were selected based on phase diagrams obtained earlier and contain increasing concentration of oily phase with varying relative proportion of Solutol HS 15 and Gelucire 44/14. The effect of pH of dispersion medium on nanoemulsifying formulations without incorporation of clotrimazole, *i.e.* placebo formulations is shown in Table 8 and that with clotrimazole is shown in Table 9. As seen in Table 8, the placebo compositions were not affected by pH of dispersion medium. It was expected because the compositions contained nonionic surfactants. However, few of the compositions with higher oil content and relatively higher proportion of Gelucire 44/14 (F7, F10 and F11), exhibited higher particle size at pH 4.5 and above. This is in line with the conclusion drawn from the phase diagrams, where smaller nanoemulsion regions were obtained with relatively higher proportion of Gelucire 44/14.

Further, it was expected that incorporation clotrimazole would influence the behavior nanoemulsifying compositions used in the study. Table 9 clearly indicated that there was remarkable effect of clotrimazole along with pH of dispersion medium on particle size and stability of formed nanoemulsions. Incorporation of clotrimazole in nanoemulsifying compositions led to considerable increase in the globule size of nanoemulsions at pH 4.5 and above, except formulations F2, F3, F6 and F9 when compared to compositions without incorporation of clotrimazole. Due to low aqueous solubility, clotrimazole is likely to influence the nanoemulsion formation by orienting partly at interface. The increased globule size of nanoemulsions could be due to altered interaction of surfactants with oil at interface in the presence of clotrimazole molecules. Interestingly, the compositions whose globule size increased at pH 4.5 and above, exhibited very low globule size (similar to that without incorporation of clotrimazole) at pH 1.2 and were found to be stable. This behavior supports aforementioned hypothesis about the orientation of clotrimazole. As clotrimazole is more soluble in pH 1.2 than in pH 4.5 and above; it likely to migrate more in the external phase leading to reduction in its amount present at interface. This may increase the effective concentrations of surfactants available for nanoemulsion formation, which may be responsible for their low globule size and stability at pH 1.2. The globule size of formed nanoemulsion increased further for buffer pH 6.8 and 7.5. Formulations F2, F3, F6 and F9 remained unaffected by incorporation of clotrimazole and pH of dispersion medium, when globule size measured immediately after nanoemulsion formation. However, formulations F2, F6 and F9 when observed after 24 h exhibited tendency of drug precipitation at pH 7.5. Hence, further studies of drug loading were performed with formulation F3. Assessment of *in vitro* precipitation at this stage was very critical in order to eliminate the potentially precipitating compositions at early development stage. Dispersion medium volume used was 10 ml at this point with pH value ranging from 1.2 to 7.5. Therefore, the compositions which exhibited precipitation at this dilution level will definitely precipitate the drug at extensive dilution and pH changes encountered in gastrointestinal tract. Although precipitation was evaluated visually, a quantitative assessment of precipitation at larger dilution levels is preformed in following sections. Additionally, it should be bear in mind that the pH of nanoemulsion is required to be adjusted to 7.5; in order to prevent the hydrolysis of clotrimazole molecules and therefore the selected nanoemulsion should physically be stable at pH 7.5.

3.2.5. Effect of increasing clotrimazole loading

Effect of increasing clotrimazole loading on mean globule size of nanoemulsions is shown in Table 10. Drug incorporation can have significant influence on mean globule size and needs to be investigated. Because of hydrophobic nature and pH dependent solubility of clotrimazole, it may affect globule size of nanoemulsions or may precipitate upon dilution. Globule size experiments showed that

Table 10
Effect of increasing CLT loading on globule size and polydispersity index of nanoemulsions (composition F3). Data expressed as mean, $n = 3$, where relative standard deviation was <10%.

(% drug loaded)	Hydrochloric acid buffer pH 1.2		Acetate buffer pH 4.5		Water		Phosphate buffer pH 6.8		Phosphate buffer pH 7.5	
	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI
0	19.14	0.11	16.69	0.12	18.54	0.09	17.08	0.12	18.47	0.07
2.5	21.17	0.07	18.46	0.08	18.75	0.12	19.47	0.11	21.22	0.12
5	19.97	0.14	19.38	0.04	19.55	0.08	18.07	0.09	20.08	0.06
7.5	20.60	0.18	32.53	0.12	34.88	0.13	38.04	0.11	41.83	0.12
10	21.87	0.17	82.70	0.45	81.28	0.27	87.17	0.27	94.03	0.38

Table 11
Globule size and polydispersity index of CLT nanoemulsions (composition F3) diluted with 250 ml of USP buffers of different pH. Data expressed as mean, $n = 3$, where relative standard deviation was <10%.

	Hydrochloric acid buffer pH 1.2		Acetate buffer pH 4.5		Bi-distilled water		Phosphate buffer pH 6.8		Phosphate buffer pH 7.5	
	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI	Globule size (nm)	PI
F3										
Initial	19.22	0.08	20.92	0.06	19.95	0.06	20.07	0.12	19.10	0.09
After 24 h	21.54	0.13	18.61	0.12	20.07	0.11	17.84	0.08	20.81	0.11

incorporation of clotrimazole does not have any impact on globule size when its concentration was up to 5% (w/w) and did not precipitate. This behavior was independent of pH of the dilution medium. However, mean globule size increased significantly when clotrimazole concentration was 7.5 and 10% (w/w), and drug precipitated out from nanoemulsions after 2 h at pH 4.5 and above. However at these drug loadings nanoemulsion remained stable at pH 1.2. These observations are in line with the results discussed in above section.

3.2.6. Selection of optimum composition

Optimized composition was selected based on, larger nanoemulsification area with high amount of oil incorporation and drug loading efficiency. The selected composition should also exhibit minimum effect of pH of dispersion medium and dilution on mean globule size and have the desired physicochemical stability without precipitation of drug. Accordingly, formulation F3 was selected for further experiments.

3.2.7. Robustness to dilution: impact of dispersion media

Physical integrity of nanoemulsion and its drug solubilization capacity after dilution must be assessed and ensured as it gives an idea about its performance *in vivo*. In view of this, composition F3 was dispersed in 250 ml of various USP buffers and results are depicted in Table 11. Clotrimazole nanoemulsion dispersed effectively without any precipitation of drug. It was also observed that there were no significant differences in nanoemulsions obtained either with water or buffers in the pH range of 1.2–7.5 used as dispersion medium. Nanoemulsion exhibited globule size <25 nm with narrow distribution irrespective of pH of dispersion medium. It was robust to dilution as it did not show any phase separation, increase in globule size and drug precipitation even after 24 h storage at room temperature.

3.2.8. In vitro dissolution profile: dispersion and precipitation assessment

To investigate the effect of pH on dissolution of clotrimazole, dissolution studies were performed in USP buffers of pH 1.2, 4.5 and 6.8. The comparative dissolution profiles of clotrimazole as plain drug powder and clotrimazole nanoemulsion in various media are shown in Fig. 7. The dissolution of drug was strongly affected by pH, with significantly greater dissolution observed at pH 1.2 than at 6.8. Clotrimazole is a weak base with two ionizable nitrogen atoms. The pK_a value of 4.7 may result in protonation of nitrogens below pH 3. This explains the drop in solubility above pH 3 and why the changes in pH profoundly influenced both solubility and dissolution. Clotrimazole was characterized by less than 30 and 10% dissolution at the end of 2 h in pH 4.5 and 6.8, respectively. Further, another important aspect that should be consider is that, weakly basic drugs such as Clotrimazole might exhibit good dissolution behavior under acidic conditions in stomach (as observed in present study), but are likely to precipitate further in gastrointestinal tract because of a sharp increase in pH under the conditions found in the intestine. Thus, such drugs may precipitate *in vivo* before their absorption in the intestine and may significantly affect the bioavailability. As a result, avoidance of drug precipitation is one of the most important considerations for oral formulation screening and development. Such kind of precipitation is reported in literature for weakly basic drugs (Kostewicz et al., 2004). Therefore, it is essential to prevent precipitation and maintain high drug concentrations in solubilized state in the intestine to improve *in vivo* performance. A 100% release of clotrimazole was obtained from nanoemulsion in 15 min in all dissolution media and was unaffected by the pH of dissolution medium. Dramatic increase in rate of release of clotrimazole from nanoemulsion compared to clotrimazole as plain drug is attributed to its quick dispersability and ability to keep drug in solubilized state. The dissolution studies conducted for 2 h to observe the occurrence of precipitation over a time. The amount of drug dissolved at the end of 2 h was close to 100%. Visual observations also indicated no sign of drug precipitation.

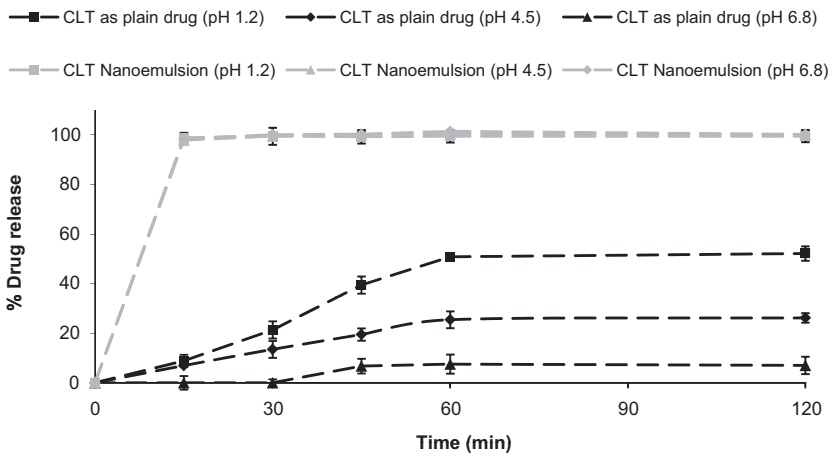

Fig. 7. Dissolution profiles of clotrimazole as pure drug and clotrimazole nanoemulsion in USP buffers of pH 1.2, 4.5 and 6.8 using USP paddle apparatus at 37 °C. Data expressed as mean ± SD, $n = 3$.

4. Conclusion

Clotrimazole nanoemulsion was prepared using spontaneous nanoemulsification method for improving its solubility and dissolution. Drug-excipient chemical compatibility facilitated to anticipate the potential degradation of clotrimazole in various excipients and was helpful to design a suitable strategy for its stabilization in proposed formulation. Screening of surfactants and cosurfactants studies helped to identify the most suitable excipients, whereas the phase diagrams gave a good idea about the concentrations of the nanoemulsion components that should be employed to achieve self-nanoemulsifying formulations. The pH and drug incorporation studies could differentiate the stability of different compositions and facilitated the selection of most stable formulation. The chemical stability of clotrimazole was preserved in the investigated nanoemulsion. The optimized clotrimazole nanoemulsion could withstand the extensive dilution and exhibited 100% drug release in 15 min irrespective of pH of medium. It would be used further for preclinical evaluation of antimalarial activity and the toxicity.

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