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RESEARCH ARTICLE

DEVELOPMENT AND EVALUATION OF MICROEMULSION FORMULATIONS OF BIFONAZOLE

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Abstract

Aim and objective: The purpose of this study was to develop microemulsion formulations for topical delivery of bifonazole with an objective to increase the solubility of the drug.
Methods: Total 5 microemulsion formulations were developed using water titration method. Capmul Pg-12 (oil), Tween-80**:** (surfactant) and Propylene glycol (co-surfactant) were used in different ratio based on the solubility and emulsification efficiency. Prepared formulations were evaluated on different parameters like transmittance (%), pH, refractive index, viscosity, drug content, solubility, and *in-vitro* release study Franz diffusion cell. Ternary phase diagram was prepared using oil (Capmul Pg-12), surfactant (Tween 80), co-surfactant (Propylene glycol) in different ratio.
Results: The % transmission was found to be in the range of 98.47±0.09 to 99.2±0.08%, the pH value was found to be in the range of 3.34±0.08 to 4.02±0.09, refractive index in the range of 1.3418±0.016 to 1.3818±0.004, drug content was found to be in the range of 98.47± 0.08 to 99.62±0.02%, viscosity was found to be in the range of 65.23±2.1 to 71.56±5.77%. It was seen that after 4 hours of diffusion, the drug released from the formulation ME5 faster and more than that of the other ratios i.e., 91.2±0.06%. It was observed that drug release was governed by the diffusion process.
Conclusion: On the basis of different evaluation parameters microemulsion formulations of batch ME5 were found to be the best. Present study concludes successful delivery of Bifonazole by the means of microemulsion formulations.
Keywords: Bifonazole, co-surfactant, microemulsion, oil, surfactant.

INTRODUCTION

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is that it has ability to deliver drugs more selectively to a specific site (local action). It provides utilization of drugs with short biological half-life, narrow therapeutic window to increase the duration of action[1,2].

Approximately 40% of new chemical entities exhibit poor aqueous solubility and presents major challenge to modern drug delivery systems which leads to poor absorption, poor bioavailability, and lack of dose proportionality. However, in many instances, oral administration is unsuitable when the drug undergoes significant degradation in the gastrointestinal tract or is metabolized to a high degree via the first pass effect in the liver. These disadvantages intensified the search for an alternative drug delivery in the form of microemulsion-based formulations for topical delivery.

A microemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension[3]. Because the droplet size is less than 25% of the wavelength of visible light, microemulsion are transparent.

ME are clear systems of with a droplets diameter>500 nm[4]. Due to small droplets size, they may serve an excellent carrier for drugs having poor water solubility. Aqueous phase consists of salt(s), and/or other ingredients, while oil consists of hydrocarbons and olefins[5]. There is need of high shear in the preparation of emulsions, but for ME, there is no need of high shear, they simply formulated by the mixing of different ingredients[6].

O/W microemulsion tends to increase solubility by changing in its dispersed phase and improve oral bioavailability by the means of increase in rate of absorption and its wettability[7,8].

Bifonazole is azole antifungal agent belonging to broad spectrum category; it is used for the treatment of skin infections like tinea, Athlete's foot, and ringworm of the body[9]. It is used topically in the form of gel for the treatment of Athlete's foot, but it has shown very low absorption (0.6% of an applied dose). Moreover the half life of bifonazole is only 1-2 h. So, for the sustain delivery of bifonazole, and to increase the duration of action, microemulsion-based formulations are developed in this study[10].

MATERIALS AND METHODS

Bifonazole was obtained from Genvio Pharma Ltd, Octanol, Castor Oil, Soyabean Oil, Linseed oil, Span 80, Tween 80 were obtained from Essential Drugs Company Ltd, Bangladesh. Other ingredients used were of analytical grade.

Selection of the oil phase

Selection of the oil phase was based upon the maximum solubility of the drug. Different oils including castor oil, Capmul Pg-12, soyabean oil, Kollisolv GTA, MCT were taken for solubility studies. Based on the solubility Capmul Pg-12 was selected as the oil phase[10].

Selection of surfactants and co surfactant

Solubility of Bifonazole was checked in different surfactants and co surfactants. Emulsification efficiency of surfactants and co-surfactants to check their ability to emulsify selected oil.

To determine the emulsification ability, equal amount of surfactant was mixed with drug and after proper dilution, it was monitored for transmittance at 638 nm using UV-Vis spectrophotometer. The ease of formation of emulsion was monitored by the number inversions of volumetric flask required to produce uniform emulsion. Similarly co surfactant were selected based on their ability to form stable and clear microemulsion at a minimum concentration[11].

Solubility analysis

About 10 gm of oil was accurately weighed in 25 ml glass beaker and 100 mg of Bifonazole was added into it, followed by stirring on magnetic stirrer at moderate speed to dissolve the drug. When drug was dissolved completely another 10 mg Bifonazole of was added and stirring was continued. Addition of drug was continued until the saturated solution is obtained. Finally, the total amount of drug consumed was determined by using UV-spectrophotometer at 250 nm. In the similar way solubility of Bifonazole was checked in different surfactants and co-surfactants[12].

Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams were constructed using water titration method to determine the microemulsion area and to detect the possibility of making microemulsions with different possible compositions of oil, surfactant/co-surfactant and water respectively. The ratios of surfactant to co-surfactants were selected to be 1:1, 2:1 and 3:1 with fixed 5% oil amount. These mixtures (S/CoS) were mixed with the oil phase to give the weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. Water was added drop by drop and stirred using a magnetic stirrer at constant temperature. After each addition, the system was examined for the physical appearance. The end point of the titration was the point where the solution becomes transparent or translucent. The amount of the aqueous phase required to make the mixture turbid was noted. The percentages of the various incorporated pseudo phases were estimated, and the same procedure was followed for the other S/CoS ratios. All the ratios of S/Co gives dotted area in pseudo ternary phase diagram[13,14].

Preparation of drug loaded microemulsion

Formulations were developed using water titration method. Predetermined amounts of Bifonazole (100) mg was dissolved in the required quantity of Capmul Pg-12 (oil). Tween-80**:** (surfactant) and Propylene glycol (co-surfactant) were added to the above mixture in different ratio. Distilled water was added gradually with continuous stirring, which resulted in the formulation of a transparent and homogenous microemulsion[15,16].

Characterization of microemulsion formulations

Percentage Transmittance

Transparency of microemulsion formulations was determined by measuring percentage transmittance through UV Spectrophotometer at 250 nm with distilled water taken as blank and three replicates were performed for each sample[17,18].

Table 1: Solubility of Bifonazole.

Oils	Solubility	Surfactant	Solubility (mg/ml)	Cosurfactant	Solubility (mg/ml)
Castor Oil	1.23±0.35	Span 80	12.45±0.31	PEG 200	19.64±0.08
Soyabean Oil	0.46±0.04	Tween 80	14.43±0.87	PEG 400	8.65±0.41
Peanut oil	0.594±0.81	Labrasol	13.63±0.41	Propylene glycol	26.77±0.35
Capmul Pg-12	16.563±0.08	Tween-60	13.55±0.31	Iso propyl alcohol	0.86±0.54
Linseed oil	1.4453±0.0121				
Cottonseed oil	0.759±0.0048				

Table 2: Emulsification efficiency (selected oil and surfactant).

Surfactant	% Transmittance	HLB Value
Tween-80	88.147±0.0251	14
Labrasol	76.251±0.0228	13
Tween-60	85.167±0.0182	13.9

Table 3: Emulsification efficiency (selected surfactant and cosurfactant).

Co surfactant	% Transmittance	HLB Value
PEG 200	72.141±0.0148	5-6
PEG 400	73.132±0.0151	8-9
Propylene glycol	79.253±0.0241	11.6

Table 4: Composition of ternary phase diagrams (quantity in ml).

Oil: SA/CoSA	Capmul Pg-12 (Oil)	Tween-80:Propylene glycol (Surfactant:Cosurfactant)					
		1:1		2:1		3:1	
1:9	1	4.5	4.5	6.0	3.0	6.7	2.3
2:8	2	4.0	4.0	5.3	2.7	6.0	2.0
3:7	3	3.5	3.5	4.6	2.3	5.3	1.7
4:6	4	3.0	3.0	4.0	2.0	4.5	1.5
5:5	5	2.5	2.5	3.3	1.7	3.8	1.2
6:4	6	2.0	2.0	2.6	1.3	3.0	1.0
7:3	7	1.5	1.5	2.0	1.0	2.3	0.7
8:2	8	1.0	1.0	1.3	0.7	1.5	0.5
9:1	9	0.5	0.5	0.7	0.3	0.7	0.3

(Bifonazole=100 mg)

Table 5: Composition of batches for Bifonazole microemulsion.

Code	S_{mix} ratio	% w/w composition		
		% Oil	S_{mix}	% Water
ME1	1:1	35	65	5
ME2	1:2	60	35	5
ME3	1:3	35	60	10
ME4	2:1	50	40	10
ME5	3:1	40	55	5

pH determination

The apparent pH of all microemulsion formulations was determined at 25°C by immersing the electrode directly into the microemulsion formulations using a digital pH meter[19].

Refractive index

Refractive indices of the prepared microemulsion formulations were determined at 25°C by Abbe's refractometer by placing one drop of microemulsion on the slide[20].

Viscosity measurement

Microemulsion are generally low viscosity systems. The viscosity of the prepared microemulsion was measured at 25°C at 60 rpm by LV spindle no. 63 using a Brookfield viscometer[21].

Determination of Drug Content in the Bifonazole microemulsion formulations

The drug content of the microemulsion formulations was determined by dissolving 1 ml (equivalent to 10 mg drug) of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer keeping blank microemulsion as control at wavelength 250 nm and three replicates were performed for each sample[22].

Drug solubility study

Bifonazole was added in excess to the optimized microemulsion formulation as well as each individual ingredient of the formulation. After continuous stirring for 4 hours at room temperature, samples were withdrawn and centrifuged for 10 minutes. The amount of drug soluble in optimized formulation as well as each individual ingredient of the formulation was calculated by subtracting the drug in the sediment from the total amount of drug added. The solubility of drug in microemulsion was compared with respect to its individual ingredients[23].

***In-vitro* drug release**

The diffusion study was carried out on a modified Franz diffusion cell of volume 20ml. The receptor compartment was filled with 20 ml of Phosphate buffer (pH 7.4). The donor compartment was fixed with cellophane membrane (Cut Off weight=1000 Da) contains Bifonazole microemulsion formulation (equivalent to 5 mg of drug) and plain drug solution separately. At predetermined time intervals samples were withdrawn from receptor compartment and analyzed for drug content by UV Spectrophotometer at 250 nm[24].

Drug release kinetic data analysis

Release data was evaluated through PCP disso software for the kinetic models. First, and Peppas and Korsmeyer model were studied[25].

Stability study

Based on different evaluation parameters micro-emulsion formulations of batch ME5 was used for stability testing. The formulations were air tight packed and kept for three months on 40°C and 75% RH. The samples were observed by UV spectrophotometer at 250 nm for the absorbance[25].

Statistical analysis

The data obtained for different formulations was analyzed by one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Solubility of Bifonazole was checked in different oil to select the oil for the preparation of microemulsion formulation. On the basis of solubility Capmul Pg-12 was selected as the oil and on the basis of emulsification efficiency and solubility Tween 80 was selected as the surfactant and Propylene glycol as the co-surfactant.

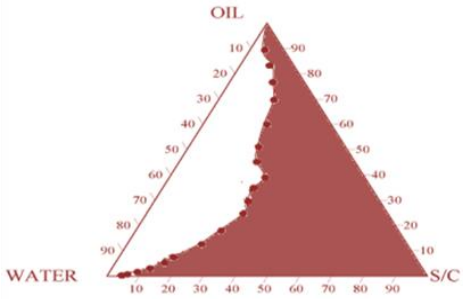

Figure 1: Pseudo ternary phase diagram for 1:1.

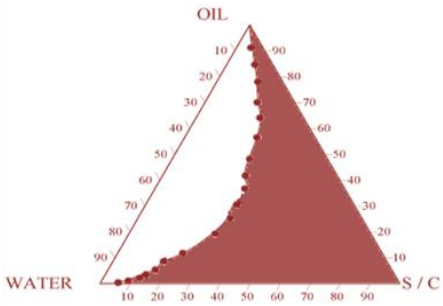

Figure 2: Pseudo ternary phase diagram for 2:1.

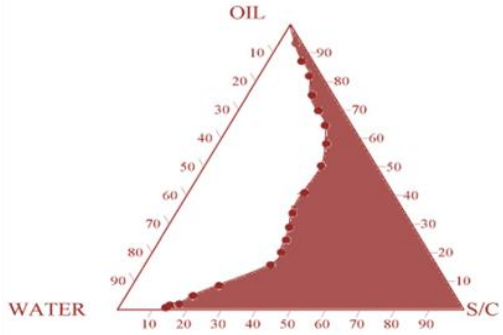

Figure 3: Pseudo ternary phase diagram for 3:1.

Ternary phase diagram was prepared using oil (Capmul Pg-12), surfactant (Tween 80), co-surfactant (Propylene glycol) in different ratio to identify the microemulsion existing zone from which appropriate concentration ranges of components of microemulsion can be obtained. The ternary phase diagrams of all the ratios are shown in Figure 1 to Figure 3. Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behaviour investigations of this system demonstrated the suitable approach to determine the water phase, oil phase, surfactant concentration, and co surfactant concentration with which the transparent, one-phase, low-viscous micro-emulsion system was formed. Total five formulations were developed to enhance the solubility of the Bifonazole. Prepared formulations were further studied for different parameters including percent transmittance, drug content, pH determination, refractive index, viscosity, drug release. The percent transmission carried out on UV spectrophotometer at 638 nm was found to be in the range of 98.47±0.09 to 99.2±0.08% for all which confirms good transparent nature of formulations. For the microemulsion formulations, the pH value was found to be in the range of 3.34±0.08 to 4.02±0.09.

Table 6: Evaluation parameters of prepared ME Bifonazole formulations.

Batch	Transmittance (%)	pH	Refractive index	Viscosity (cp)	Drug content (%)	Solubility mg/ml
ME1	99.4±0.12	3.73±0.08	1.3648±0.007	64.23±0.12	98.37±0.08	26.47±0.07
ME2	99.37±0.31	3.75±0.12	1.3420±0.008	66.46±0.14	99.52±0.04	27.77±0.18
ME3	99.42±0.23	3.82±0.07	1.3718±0.004	70.56±0.77	99.61±0.03	28.67±0.09
ME4	98.57±0.09	3.44±0.08	1.3720±0.008	68.43±0.34	99.32±0.06	25.87±0.09
ME5	99.63±0.03	4.12±0.09	1.3518±0.016	66.36±0.74	98.23±0.08	31.87±0.07

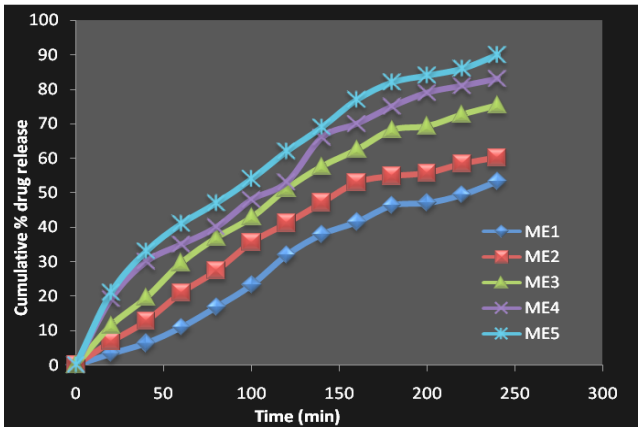

Figure 4: *In vitro* study of prepared Bifonazole microemulsion formulations.

The refractive index for the microemulsion formulations was found to be in the range of 1.3418±0.016 to 1.3818±0.004. The drug content was found to be in the range of 98.47±0.08 to 99.62±0.02% in the microemulsion formulations. The Viscosity was found to be in the range of 65.23±2.1 to 71.56±5.77% in the microemulsion formulations. The viscosity of the microemulsion increased with increasing concentration of the surfactant. It was seen that after 4 hours of diffusion, the drug released from the formulation ME5 faster and more than that of the other ratios i.e., 91.2±0.06%. In present study PCP disso Version 2 software was used for the estimation of release pattern. Models for the release kinetic profile are shown in Table 7.

Table 7: Different release models for Bifonazole microemulsion formulations.

Batch	Kinetic model	Parameters
ME1	Peppas and Korsmeyer	R = 0.955, K1 = 4.334, n = 0.780
ME2	Peppas and Korsmeyer	R = 0.984, K1 = 3.247, n = 0.864
ME3	First order	R = 0.972, K1 = 5.81, n = 0.770
ME4	Peppas and Korsmeyer	R = 0.954, K1= -0.070
ME5	Peppas and Korsmeyer	R = 0.983, K1 = 6.712, n = 0.762

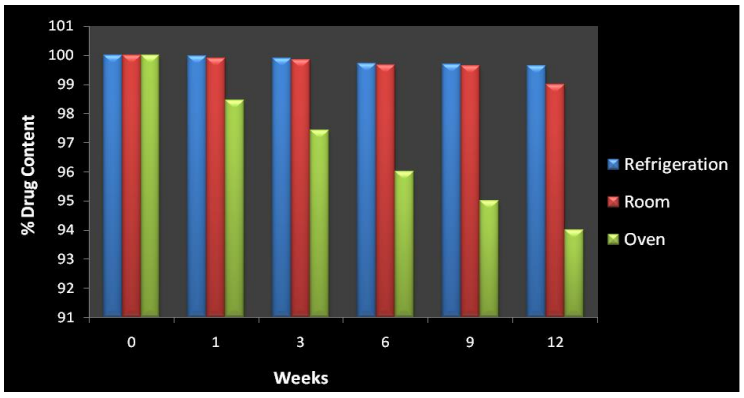

Figure 5: Stability studies of microemulsion formulations of batch ME5 at different temperature.

In-vitro release data were plotted in 2 different models i.e. first, and Korsemeyer peppas. It was observed that release was governed by the diffusion process. Stability studies indicated that the preparation was stable at room temperature over the period of 3 months (Figure 5).

Limitation of the study: There is need of *in-vivo* study for the estimation of the effectiveness of the prepared formulations.

CONCLUSIONS

ME are clear systems of with a droplets diameter>500 nm[1]. Due to small droplets size, they may serve an excellent carrier for drugs having poor water solubility. Aqueous phase consists of salt(s), and/or other ingredients, while oil consists of hydrocarbons and olefins. There is need of high shear in the preparation of emulsions, but for ME, there is no need of high shear, they simply formulated by the mixing of different ingredients. Present study concludes successful delivery of Bifonazole by the means of microemulsion formulations.

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AUTHOR'S CONTRIBUTIONS

Islam MS: writing, gathered and analyzed data. **Mojumder TJ:** data analysis, report drafting. **Nawrin F:** editing, review. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

No conflict of interested is associated with this work.

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