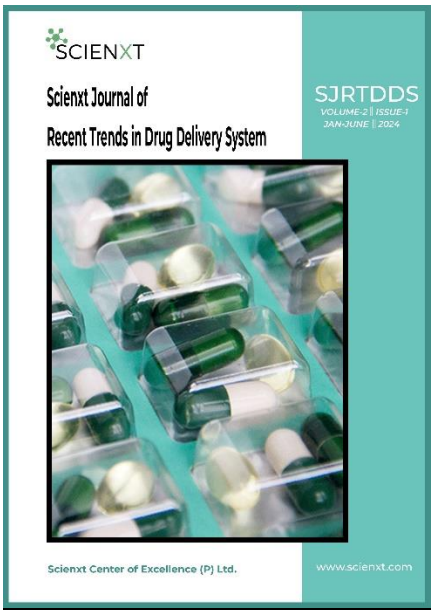

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Identifying the microemulsion region through pseudo-ternary phase diagrams, development and characterization of microemulsions to enhance percutaneous permeation of terbinafine hydrochloride

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Abstract:

Topical treatment of fungal infections of skin is often limited by the poor percutaneous permeation through human skin. For this reason, the development of topical formulations which are able to improve the percutaneous permeation of antifungal agents is of particular importance for skin conditions. A useful strategy for improving the percutaneous flux is to improve the concentration of drug and to choose the appropriate vehicle for the drug delivery across the skin. Various studies have proved that microemulsion can significantly increase topical and transdermal availability of poorly water soluble drug candidates. Terbinafine is a topically and orally active synthetic allylamine broad spectrum antifungal agent. Shorter courses of terbinafine are needed for the treatment of various fungal and yeast skin infections. Higher effectiveness of terbinafine has the advantage for using it in topical microemulsion. So present study is designed to develop the topical microemulsion of terbinafine hydrochloride.

Keywords:

Antifungal, Microemulsions, Phase Diagrams Percutaneous, Pseudo-ternary, Terbinafine hydrochloride.

1. Introduction:

The clinical efficacy of highly lipophilic drugs is being impeded by their low aqueous solubility resulting in poor absorption and penetration mainly when they are designed for topical administration. Among the different innovative approaches that have been suggested for enhancing the penetration of lipophilic drugs through topical administration, microemulsions have shown better results. Operationally microemulsion may be defined as dispersion of insoluble liquid in a second liquid that appears clear and homogenous to the naked eyes. A microemulsion, one of the pharmaceutical interests for new drug delivery is normally composed of oil, water surfactant and cosurfactant. The presence of surfactant and cosurfactant in the system makes the interfacial tension very low therefore the microemulsion is thermodynamically stable and forms spontaneously without any significant energy input in the form of shear or heat treatment. Microemulsions are readily distinguished from normal emulsions by their transparency, low viscosity and more fundamentally their thermodynamic stability. Microemulsion systems have extensive interfacial, aqueous and oily domains, so are quantities of oil soluble, water-soluble and amphiphilic materials. Microemulsion systems represent a promising prospect for the development of formulation suitable for the incorporation of poorly water- soluble drugs due to high solubilization capacity as well as the potential for enhanced absorption. In recent years microemulsion has been extensively studied for transdermal, parenteral and oral delivery of drugs. Recent increasing attention has focused on microemulsions for transdermal delivery of drugs. The transdermal delivery of ketoprofen, apomorphine, estradiol and lidocaine using microemulsions has been reported to show better drug availability over conventional formulations.

A topical treatment of several diseases is often limited by the poor percutaneous permeation through the human skin. For this reason, the realization of topical formulations which are able to improve the percutaneous permeation of antifungal drugs can be of particular importance for the success of topical therapeutic approaches. Terbinafine hydrochloride is a topically and orally effective synthetic allylamine antifungal agent. It is structurally and pharmacologically related to naftifine. Drug appears to act by preventing fungal ergosterol biosynthesis via specific and selective inhibition of fungal squale oxidase. In standard in-vitro susceptibility tests terbinafine has demonstrated activity against a wide range of dermatophyte filamentous, dimorphic fungi as well as yeast. High effectiveness of terbinafine hydrochloride than other

Topical antifungal drugs and lower drug requirement are the main advantages of this drug to be

used in topical microemulsion systems.

2. Materials and methods materials:

Terbinafine Hydrochloride was provided by Plethico Pharmaceuticals Ltd. Indore as a gift sample. Oleic acid Castor oil and tween 60 were purchased from Central Drug House (P) Ltd. Isopropyl alcohol was procured from Qualigens Fine Chemicals Mumbai. Methanol used was of analytical grade and was purchased from Ranbaxy Fine Chemicals Ltd.

2.1. Construction of pseudo-ternary phase diagrams:

In order to find out the construction range of components for the existing range of microemulsions pseudo-ternary phase diagrams were constructed using titration method at an ambient temperature.

The coarse emulsions were formed by mixing oil with water. These coarse emulsions were titrated with the combination of surfactant and co surfactant until the clear transparent microemulsions were formed and the volumes of surfactant/ co surfactant combination used were recorded.

2.2. Formulation of microemulsions:

Six (ME-1 ME-6) different ratios of surfactant and co-surfactant were selected from pseudo-ternary phase diagrams to prepare the emulsion. The measured quantities of surfactant- co-surfactant mix, oil and aqueous phase were stirred together at room temperature till the formation of transparent clear liquid formulation.

For drug loading the 1% w/v of Terbinafine hydrochloride was added to the microemulsion formulations, stirred for 4 hours, kept overnight for equilibration and stored at 25 ± 1^{0}C.

2.3. Characterization of microemulsions particle size determination:

The particle size of microemulsion formulations (ME-2, ME-3, and ME-4) was determined by using Quasi Dynamic Light Scattering Technique.

2.4. Determination of type of microemulsion:

Type of emulsion w/o or o/w was determined by electrical conductivity method. Solution of sodium chloride 1% w/v was used as the aqueous phase instead of the distilled water. Electrical conductivity in the solution was on the diffusion cell with the stratum corneum side facing towards the donor compartment. The area of the diffusion cell used for all in vitro permeation

studies was 0.786 cm^2 and the capacity of the receiver compartment was 61.5 ml. The skin was equilibrated for one hour with the receiver medium. A blank sample (4ml), was withdrawn from the receptor compartment and analyzed to ensure any residual absorbance. The receptor medium (20% v/v methanol in water) was replaced with the fresh medium. The receptor chamber was thermostated at 37+2°C and the magnetic stirrer stirred the solution in the receptor chamber continuously. The 1 ml. of microemulsion formulation containing 1% w/v Terbinafine hydrochloride was filled in the donor chamber. Samples (4 ml) were withdrawn from the receptor compartment for 24 hours at the interval of one hour and drug content was analyzed recorded as o/w while those that did not give conductivity were considered w/o type microemulsion systems.

2.5. Stability of microemulsion:

The physical stability of microemulsion was studied via clarity and phase separation. Microemulsions were stored in the dark at varying temperatures i.e., 5, 15, 25, 37 °C for three months. Then the clarity and phase separation were investigated to judge the optimal storage temperature.

2.6. Centrifugation studies:

In order to determine the metastable microemulsion system the selected microemulsion vehicles were centrifuged at 5000 rpm for 10 minutes.

2.7. In vitro skin permeation studies:

In vitro skin permeation across the rat abdomen skin was conducted using Franz diffusion cell. The excised skin was mounted using 20% v/v methanol in water as blank. The receptor volume was immediately replaced with an equal amount of receptor medium. Sampling port and donor chamber were covered by aluminum foil to prevent the evaporation of the receptor medium.

2.8. Determination of terbinafine hydrochloride:

Concentration of Terbinafine in receptor compartment of Franz's diffusion cell was determined by UV spectrophotometrically Analysis using Shimadzu-1700, Shimadzu Corporation Japan, UV visible double beam spectrophotometer with 1 cm quartz cuvettes. Calibration curve of Terbinafine hydrochloride was prepared by dissolving 100 mg of drug in 100 ml of 20% v/v methanol in water. 5 ml of this solution was pipette out and volume was made to 100 ml. in a volumetric flask to

prepare a stock solution of concentration 50 mg/ml. From this stock solution serial dilutions in concentration range 5-40 µg/ml were made and absorbance of these dilution were determined on UV microemulsion system not only reduces the interfacial tension between the oil phase and aqueous phase but also makes the lipophilic spectrophotometer at λmax 283 nm using 20% drug soluble in the system. According to the v/v methanol in water as a blank solution.

The absorbance values corresponding to each concentration were then statistically evaluated and plotted as a standard graph, between absorbance values on y-axis and concentrations on x-axis.

3. Results and discussion phase studies:

In general, a pseudo-ternary phase diagram was constructed to determine the composition of an aqueous phase, an oil phase, and a surfactant: co surfactant phase that will yield a microemulsion. For the present study phase diagrams for microemulsion systems were investigated using two different oils i.e., oleic acid and castor oil. The studied systems are composed of oil (oleic acid or castor oil), water Tween 60 and isopropyl alcohol. Tween 60 functions as surfactant with an HLB of 14.9 and isopropyl alcohol is a short chain alcohol functioning as co surfactant. For simplicity, the microemulsion is assumed to be a three-component system (oil, water, and the mixture of surfactant and co surfactant). Any combination of three components can be plotted as a percent on the pseudo-ternary phase diagram. Pseudo-ternary phase diagrams were constructed and the corresponding microemulsion regions were identified as shown in fig 1-5. The results indicate that the area of the microemulsion region increased in the system containing more amounts of isopropyl alcohol. The isopropyl alcohol incorporated into the pseudo-ternary phase diagrams with oleic acid at the surfactant/co surfactant ratio 1:3, microemulsion systems were obtained at oil concentrations ranging from 6.53-58.060% v/v and surfactant/co surfactant mixture was used ranging from 34.64-54.54% v/v. A maximum of 58.82 % v/v of water was solubilized in the microemulsion system. In the construction of phase diagram for microemulsion of oleic acid at the surfactant/co surfactant ratio 1:1, the maximum oil used was in the concentration 31.75% v/v and solubilizes water up to a maximum of 35.02% v/v. For the construction of phase diagram, the maximum amount of surfactant/co surfactant mixture used was 67.94%v/v.

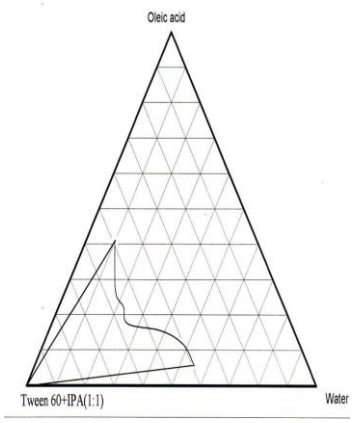

Figure. 1: Pseudoternary phase diagram for microemulsion using Oleic acid, tween60+IPA (1:3) & water

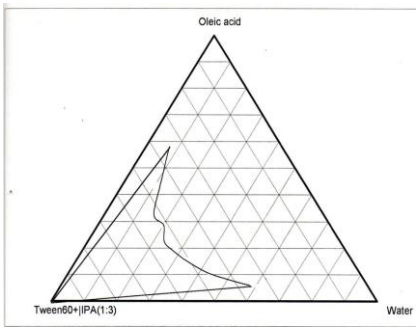

Figure. 2: Pseudoternary phase diagram for microemulsion using Oleic acid, tween60+IPA (1:1) & water

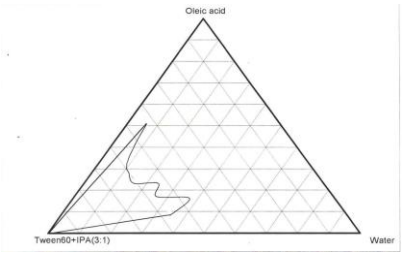

Figure. 3: Pseudoternary phase diagram for microemulsion using oleic acid, tween 60+IPA (3:1) & water

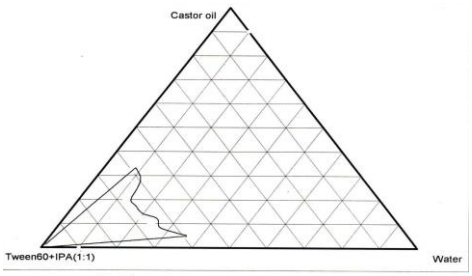

Figure. 4: Pseudoternary phase diagram for microemulsion system, using castor oil, Tween 60+IPA (1:1) & water

3.1. Preparation of microemulsion formulations:

Six (ME-1 ME-6) different ratios of surfactant and co-surfactant were selected from pseudo-ternary phase diagrams to prepare the microemulsions. The composition of all six microemulsions is given in the table below:

Table. 1: composition of microemulsion formulations

Ingredient (In ml)	***ME-1***	***ME-2***	***ME-3***	***ME-4***	***ME-5***	***ME-6***
Oleic acid	43.00	15	40.8	16.2	23.53	-
Castor oil	-	-	-	-	-	27.5
TWEEN 60	11.56	12.5	24.5	23.12	39.07	49.6
IPA	24.68	37.5	24.5	23.12	13.24	17.6
Distilled water	10.75	25	10.2	37.64	23.53	10.5

3.2. Standard curve of terbinafine hydrochloride:

The standard curve obtained for Terbinafine hydrochloride in 20% solution of methanol in water at 283 nm wavelength obeyed the Beer-Lambert's law in the selected concentration range and R^2=0.9999.

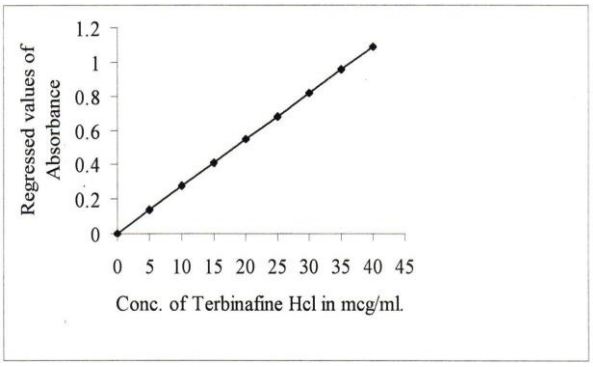

Figure. 6: Calibration curve of Terbinafine hydrochloride in 20% solution of methanol in water at 283 nm

3.3. Characterization of microemulsions:

Type of microemulsion whether o/w or w/o was determined by electrical conductivity method and the results showed that ME-1, ME-3 and ME-6 were w/o while ME-2, ME-4 and ME-5 were o/w type microemulsions.

3.4. Light scattering study:

Quasi-light scattering studies were performed to determine the mean particle size of

microemulsion formulations, ME-2, ME-3, and ME-4. Laser scattering studies of microemulsion formulations indicate that these are within the range of microemulsion formulation i.e., 171.66, 100, and 266.72 nm in diameter, which imparts thermodynamic stability.

3.5. Stability study:

All microemulsion formulations were stable at 37^0C. No change in physical appearance was determined in stability studies during three months. The turbidity and phase separation were observed in the microemulsion formulation stored at 5^oC. The coagulation of the oily phase might lead to this instability, but these microemulsions were easily recovered by keeping at room temperature.

Centrifugation studies of microemulsion systems were carried out at 5000 rpm for 10 minutes. Centrifuge tests showed that microemulsion formulations ME-2, ME-4, ME-5, and ME-6 had good physical stability. The formulations ME-1 and ME-3 showed phase separation and turbidity after centrifuge tests. But these were recovered by keeping at room temperature.

3.6. Interaction study:

No significant shifting in characteristic peaks of Terbinafine hydrochloride was observed in the FTIR | spectra of microemulsions of castor oil and oleic acid. So Terbinafine hydrochloride can be expected to be compatible with the excipients used.

3.7. In vitro permeation studies:

To evaluate the ability of topical microemulsions formulated to deliver Terbinafine hydrochloride through the skin, in vitro permeation experiments through rat abdomen skin were carried out. The permeation profiles of various microemulsion formulations are presented in the figs.7 and 8. The microemulsion formulation ME-3 is characterized by the highest drug permeation while the marketed cream showed the lowest permeation profile. The significant difference in the in vitro skin permeation of Terbinafine hydrochloride between conventional formulations and the microemulsions was principally due to mean size diameter of internal phase, which are noticeably smaller in case of microemulsions. Besides the improved colloidal properties of microemulsion dispersed phase, other factors which may contribute to increased drug permeation can be high drug

solubilizing capacity of microemulsions and the permeation enhancing effect mediated by isopropyl alcohol and oleic acid. Microemulsions solubilize the poorly water-soluble drug and deliver it to the membranes where the drug molecules are released from the microemulsion systems to the skin surface thus increasing the drug skin permeation. In addition, improvement in the permeation of drugs can also be elicited by the increased skin hydration of stratum corneum by microemulsion formulations. The results of in vitro skin permeation studies of microemulsion formulations and that of marketed cream are reported in the table below:

Table. 2: In-vitro cumulative drugs permeation from microemulsions

Time in hrs.	***Cumulative amount Permeated (μg/cm²)***					
	ME-1	***ME-2***	***ME-3***	***ME-4***	***ME-5***	***ME-6***
1	87.43±1.03	94.63±1.72	101.91±1.09	40.07±3.18	32.08±2.31	38.62±1.51
2	167. 57±1.76	182.11±3.64	191.08±2.71	73.02±4.29	88.42±3.46	64.10±2.61
3	222.68±2.54	267.65±2.73	280.25±4.58	112.11±8.21	122.20±7.21	95.78±5.12
4	269.37±4.83	280.43±6.212	388.71±7.43	146.78±7.23	148.76±5.83	140.29±7.43
5	301.83±3.27	319.96±6.34	481.19±3.14	153.27±11.91	150.32±8.31	165.61±6.13
6	346.2±6.84	347.70±8.55	495.74±6.70	166.31±12.72	153.18±4.29	192.22±9.00
7	369.6±87.6	376.72±10.01	714.64±11.22	172.13±11.25	153.97±2.09	216.77±7.03
8	382.48±10.01	395.84±5.38	801.27±9.87	181.29±13.66	154.34±9.52	243.13±11.46
9	407.73±8.53	410.98±7.46	907.00±13.24	191.37±17.24	154.89±11.43	268.63±9.11
10	412.35±10.68	414.35±8.93	1031.84±10.08	205.50±9.08	155.20±15.61	319.32±10.34
11	419.54±13.22	421.55±13.86	1110.82±13.51	219.11±11.72	155.93±8.32	332.36±13.61
12	424.04±12.5	427.54±15.6	1236.94±15.7	232.25±19.3	156.35±14.0	356.85±13.5

	3	5	0	4	7	7
15	436.27±14.19	440.39±14.00	1338.35±12.05	259.38±21.21	157.77±17.13	395.50±9.34
18	447.38±13.87	452.50±17.57	1486.62±7.83	267.92±18.67	160.59±13.29	446.86±15.57
21	452.5±14.44	459.60±11.28	1557.14±15.20	308.30±23.65	162.02±11.73	497.59±14.38
24	476.91±14.92	480.89±16.47	1676.43±14.56	363.31±17.50	164.66±14.68	574.10±13.66

Table. 3: In-vitro flux of Terbinafine Hydrochloride

S.No.	***Formulation***	***Mean flux(µg/cm²/hr)***
1	ME-1	49.18±20.68
2	ME-2	51.98±23.77
3	ME-3	94.36±10.04
4	ME-4	24.20±8.56
5	ME-5	21.56±11.73
6	ME-6	30.29±3.89
7	Marketed cream	14.43±5.35

In microemulsion formulation (ME-1) high cumulative drug permeation was principally due to the great potential of microemulsion concentration gradient across the membrane due lower drug content in the continuous phase. The mean flux across the skin was itself. In this formulation a lower amount of 24.20 µg/cm^2/hr. It is also assumed that the surfactant is used. The mean flux from the role of isopropyl alcohol remains insignificant formulation is 49.18 µg/cm^2/hr. Also, the due to poor solubility of the drug in the effect of IPA in this formulation cannot be neglected. The cumulative amount of Terbinafine hydrochloride permeated in 24 hours was 476µg/cm^2. The better flux from this formulation may be due to better hydration of skin as the formulation contains a considerable amount of water. The release seems of non-zero order. The

mean flux for ME-2 was 51.98 µg/cm^2/hr. and the external phase.

The equal amounts of oil and water used in the formulation suggest the bicontinuous structure of microemulsion formulation ME-5. The highest amount of surfactant used in this formulation, may be the cause of poor permeation of drug as drug might not be released easily from the interfacial film and hence poor mean flux, 21.56 µg/cm^2/hour was cumulative amount of Terbinafine hydrochloride permeated in 24 hours was found to be almost equal to that of ME-1 i.e., 480.89 µg/cm^2.

The flux from formulation ME-3 is highest (94.36µg/cm^2/hr.). The release is of zero order. The better permeation may be due to the increased solubility of the drug in the internal phase of microemulsion formulation. The cumulative amount permeated from the formulation in 24 hours was 1676.43 µg/cm^2. This formulation has the least amount of surfactant and has the highest concentration gradient. Flux across the skin is highest among all the formulations. Formulation has equal amounts of surfactant and co surfactant, which decrease the interfacial tension and thus the reduced mean diameter of dispersed phase can be responsible for increased drug permeation through the skin.

The cumulative amount of drug released from the formulation ME-4 was 363.31 µg/cm. in 24 hours. The structure of microemulsion formulation was o/w type and has poor observed. The lamellar structure of microemulsion formulation itself has poor permeating effect. The cumulative amount of drug permeated in 24 hours was 164.66 µ g/cm^2. In order to explore the effect of formulation components on the permeation of drug, castor oil was used as the oil phase. Castor oil and isopropyl alcohol is a good candidate for topical formulation. The permeation followed zero order release with castor oil microemulsion (ME-6). Its poor flux indicates poor concentration gradient across the membrane though it has maximum amount of isopropyl alcohol among all the formulations. The cumulative amount of drug permeated was nearly average (574.10µ g/cm^2). It is assumed that the drug is concentrated on the film formed around the droplet of water. The mean flux of drug permeated from the microemulsion formulation ME-6 was 30.29 µg/cm^2/hr. The rate of permeation obeys zero order rate equation.

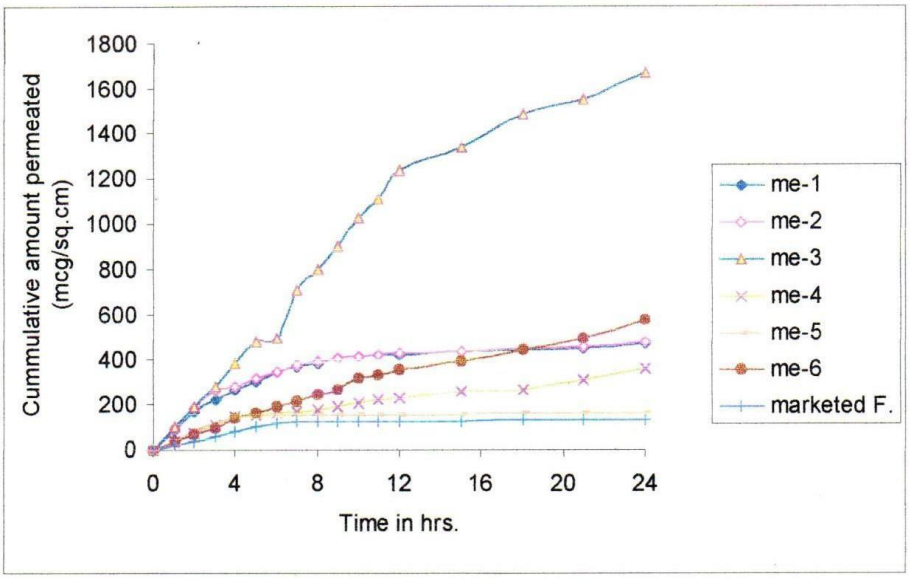

Figure. 7: Cumulative drug release from microemulsions and marketed cream

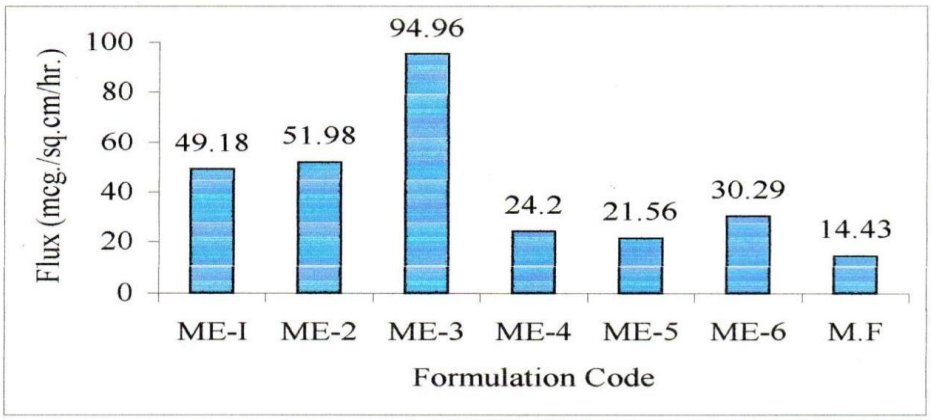

Figure. 8: In-vitro flux of terbinafine from microemulsions and marketed cream through rat abdomen skin

Permeation of drug from the marketed formulation shows poor flux (14.43 µ g/cm^2/hour) and less cumulative amount permeated (i30.07 µg/cm^2). The poor permeation across the skin in comparison to microemulsions may be due to large mean diameter of dispersed phase and high viscosity of formulation.

From the in vitro permeation studies across the present rat abdomen skin, it is asserted that microemulsion as topical delivery vehicle enhances the topical bioavailability of the Terbinafine hydrochloride. Laser Scattering studies of microemulsions indicate that these are within the range of micro emulsion formulation which imparts thermodynamic stability.

4. Conclusion:

The present study was carried out to investigate the possibility of topical microemulsion of Terbinafine hydrochloride with increased permeation through skin.

In order to determine the effect of formulation components, castor oil and oleic acid were chosen as oil phase. To investigate the microemulsion existence region pseudo-ternary phase diagrams were constructed using oleic acid or castor oil as oil phase, water as aqueous phase and the combination of tween 60 and isopropyl alcohol as surfactant-cosurfactant mixture in varying ratios. The phase studies showed a larger microemulsion region with oleic acid in comparison to castor oil, also the microemulsion region increased with increase in IPA content. Study shows that topical microemulsions of terbinafine hydrochloride can be successfully formulated having a good drug release profile.

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