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Formulation development and characterization of microemulsion for topical delivery of Glipizide

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Abstract

In the present work glipizide based microemulsion was developed and its usefulness as topical drug carrier system for the non-insulin dependent diabetes mellitus (NIDDM) was investigated. Microemulsion was prepared by water titration method using oleic acid as oil phase, tween-80 as surfactant and propylene glycol as co-surfactant. Menthol as a permeation enhancer was added to a final drug-loaded microemulsion formulation at the level of 1% w/w. Microemulsions were characterized for pseudo-ternary phase diagrams, pH, viscosity, droplet size, in vitro release profile, ex-vivo diffusion study, irritancy tests, stability and in vivo evaluation. The optimized microemulsion formulation was found to be o/w type emulsion by pseudo ternary phase diagram and having mean particle size of 138±4.5 nm. The results indicated that the developed microemulsion systems, especially ME-3, may be promising vehicles for the transdermal delivery of glipizide. In vivo studies were carried out on wistar rats and the samples were analyzed for glucose content by Accu-check blood glucose meter. Inclusion complex showed significant blood glucose reduction as compared to drug alone. Microemulsion system provides viscous consistency for the topical application, which delivered the drug in sustained or controlled manner and prolonged delivery as compared to conventional dosage form.

Key words: Diabetes mellitus; Pseudo-ternary phase diagrams; Controlled drug delivery system

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INTRODUCTION

To date, microemulsions have been used to deliver drugs via the percutaneous, ocular, oral and parenteral routes, with reports of improved absorption and bioavailability for a number of compounds. Microemulsions are attractive vehicles for drug delivery because of their ease of formulation, thermodynamic stability and solubilization properties. This report describes the topical delivery of microemulsion system, which composed of non-irritating, pharmaceutically acceptable ingredients. The purpose of this investigation was to study the feasibility of transdermal delivery of glipizide [1, 2].

Microemulsions are quaternary systems composed of an oil phase, a water system, surfactants and a cosurfactant [3]. These spontaneously formed systems possess specific physicochemical properties such as transparency, optical isotropic, low viscosity and thermodynamic stability. The observed transparency of these systems is due to the fact that the maximum size of the droplets of the dispersed phase is not larger than one-forth of the wavelength of visible light-approximately 150 nm. Droplet diameter in stable microemulsions is usually within the range of 10-100 nm (100-1000 °A), which means that the term 'microemulsion' is misleading and these systems are actually nano-sized emulsions. Many studies have shown that microemulsion formulations possessed improved transdermal and dermal delivery properties, mostly *in vitro* [4-13], and several *in vivo* [14-16]. The intradermal permeation rate of a lipophilic drug was significantly increased from microemulsion as compared to commercial macroemulsions. In macroemulsion (emollient liquids, cream, lotions etc.), the free mobility of the active material between the internal (disperse) phases to external (continuous) phase within the structure of the formulated system is limited due to the strong interactions between the surfactants that form the interfacial membrane film. In microemulsion, the cosurfactant lowers the interfacial tension of the surfactant film, resulting in a more flexibility and dynamic layer system [4, 10, 13]. The drug in this energy-rich system can diffuse across the flexible internal surfactant film between the phases, a thermodynamic process that increases partitioning and diffusion into the stratum corneum. An excellent and comprehensive review on the role of microemulsion in percutaneous penetration of drugs was recently published by Kreilgaard [17].

Glipizide is a drug of second generation non-insulin dependent diabetes mellitus. The partition coefficient values indicated that glipizide could partition well into the skin and it did not show any skin metabolism. The objective of present work is to develop, characterize and evaluate microemulsion for topical delivery of glipizide in sustained or controlled manner and prolonged delivery as compared to conventional dosage form. Glipizide, an effective antidiabetic drug which requires controlled release owing to its short biological half life of 2-4 h, was tried to deliver by microemulsion.

MATERIALS AND METHODS

Materials

Glipizide was procured as a gift sample from M/s micro labs Ltd. (Bangalore, India), oleic acid was purchased from LOBA chemie, propylene glycol was supplied from Merck (Mumbai, India) and Tween-80, menthol, Potassium dihydrogen phosphate, Sodium dihydrogen phosphate were purchased from CDH, Central Drug House Pvt. Ltd, (New Delhi, India).

Preparation of Microemulsion

Microemulsion was prepared as per the method described by Chen *et al*, 2004, with slight modification. In brief oily phase was prepared by dissolving glipizide (0.05%) in 1 ml of dimethyl formamide, used as a cosolvent and added in the mixture of oleic acid (6%) and propylene glycol (10%) with continuous stirring on magnetic stirrer. The aqueous solution of tween-80 (20%) was added drop wise in oil phase with continuous stirring at ambient temperature. Menthol as a permeation enhancer was added to a final drug-loaded o/w microemulsion formulation at the level of 1% w/w. Various microemulsion formulations were prepared for optimization of process variables.

Analytical method for estimation of glipizide

Glipizide content in the microemulsion was estimated by an UV spectrophotometric method based on the measurement of absorbance at 275 nm in phosphate buffer of pH 7.4. The method

was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range 0-25 µg/ml. When a standard drug solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 1.2%, respectively.

Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of microemulsion, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of Tween-80 to propylene glycol. For each phase diagram at a specific surfactant/cosurfactant weight ratio, the ratios of oil to the mixture of surfactant and co--surfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixture of oil, surfactant and cosurfactant at certain weight ratios were diluted with water dropwise, under moderate magnetic stirring. After being equilibrated, the mixture was assessed visually and determined as microemulsions, crude emulsion or gels. No attempts were made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemulsions. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90°.

Characterization of microemulsions

The developed formulations were characterized for pH value, viscosities, average droplet size and polydispersity index.

The pH values of microemulsion were determined at 25°C using a µ-pH system-361, Systronics and the viscosity of various microemulsions was measured at 25°C using a Brookfield viscometer. The refractive index was determined at 25°C using a WAY-2S digital Abbe refractrometer.

The average droplet size and polydispersity index of the microemulsion was determined by photon correlation spectroscopy. The measurement was obtained at 635 nm using a 15 mW solid state laser. Sample was suitably diluted with distilled water to avoid multi-scattering phenomena. The droplet size of the diluted microemulsion was not significantly changed.

***In vitro* permeation studies**

Abdominal skin of swiss albino male mice was used in the permeation experiments. Mice (30-35 g.) were anesthetized slightly by ether and hairs were removed from the abdominal skin. The mice were sacrificed and the abdominal skin of the mice was separated. The subcutaneous fat is removed, and then the skin were washed and examined for integrity. The skins were placed in a refrigerator at 4°C overnight and then used for the experiments. The permeation experiments were performed in diffusion cell with a recirculating water bath and 12 diffusion cells. The skins were clamed between the donor and the receptor chamber of diffusion cells. The cell has an effective diffusion area of 2.8 cm^2 and a 7 ml cell volume. The receptor chamber was filled with freshly prepared solution of water-ethanol 4:1 v/v to solubilized glipizide and to ensure sink conditions. The solution of 20% ethanol was used to solubilize glipizide. The receptor chambers were thermostat at 37°C and the solution in the receptor chambers was stirred continuously at 300 rpm. The formulations (1.5 g) containing glipizide were kept in the donor chamber. At appropriate time interval, 0.5 ml of the solution from receptor chamber was removed for UV determination and replaced immediately with an equal volume of fresh solution of 20% ethanol. Cumulative corrections were made to obtain the total amount of glipizide permeated at each time

interval. The cumulative amount of drug permeated through mouse skins was plotted as a function of time [18].

Skin irritation studies

Three young rabbits of white strain were taken for skin irritation studies. Hair on the back area (approximately 6 cm^2 area) of each rabbit was removed by hair removing cream. Developed formulations were applied to the shaved area, and then rabbits were secured. On one side of the back, a control microemulsion and aqueous solution (without drug) were applied for each day. The animal were observed and evaluated for any sign of erythema or oedema for a period of 7 days.

***In vivo* studies**

Protocol design

The *in vivo* experimental protocol was approved by the institutional Ethics Committee Male Spargue-Dawley albino rats, 100-150g weight, were selected for *in vivo* studies. Animals were randomly divided in to 4 groups. Each group comprised of 4 animals.

For the induction of diabetes, rats were fasted for 24 h and blood glucose level of each group was assessed to obtain the fasting blood glucose levels. Alloxan at dose of 100 mg/kg b w in water for injection was administered by intravenous route to each rat and blood glucose level was measured by using digital glucometer (Accque check) after 24 h. Rats showing 200-250% increase in fasting blood glucose levels were selected for study.

The optimized formulation of microemulsion (ME-3) was made with different animal dose concentrations (600 µg/kg, 800 µg/kg and 1000 µg/kg) in 0.5 ml of microemulsion and applied topically to last three groups. Hairs were shaved in 2 cm^2 areas with the help of hair removing cream in interscapular region and treatment was provided topically on shaved area. First group was treated orally with plain glipizide suspension at a dose equivalent to 800 µg/kg in PBS (pH 7.4). Blood samples were collected using heparinized sterile capillaries from the orbital sinus of each rat at appropriate time interval for 24 h. The blood glucose level was measured immediately using glucometer.

Stability of microemulsions

The chemical and physical stability of microemulsion with glipizide were evaluated via phase separation by mechanical stress study and residual drug content.

Microemulsions were stored at 8°C, RT, 45°C, and 60°C for 6 months, respectively. Then the phase separation and residual content of glipizide were investigated to judge the optimal storage temperature monthly.

Mechanical stress study

The different formulations were centrifuged (Remi centrifuge) at 2000 rpm for different time interval and noted down the volume of phase separation of formulation [19].

Residual drug content

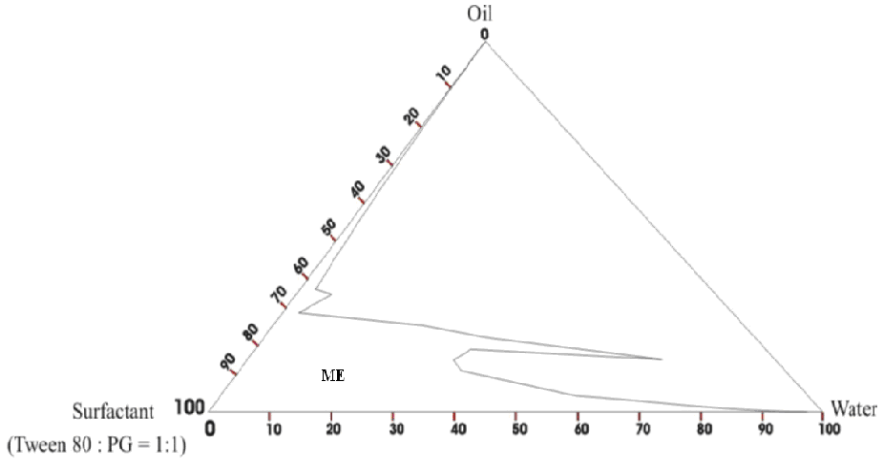
Each formulation (1 gm) was assessed for drug content after storing for 2, 4, and 6 months at 8°C, RT, 45°C, 60°C. After that formulations were transferred into a beaker containing 10 ml methanol. The content of the beaker were stirred for 30 minutes and then kept for 24 h for extraction of drug. After 24 h the content of beaker were transferred into centrifuge tube and centrifuged at the 3000 rpm for 10 min, supernatant was separated and filtered. Then 0.1 ml of

the supernatant was diluted appropriately with saline phosphate buffer (pH 7.4) and assayed spectrophotometrically for drug content. The clarity, phase separation and concentration of glipizide were investigated to judge the optimal storage temperature in each 2nd month of six months study.

RESULTS AND DISCUSSION

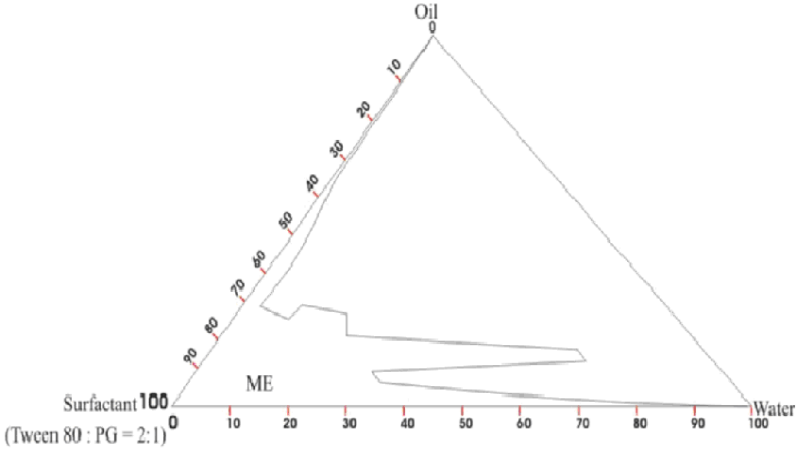
Preparation of phase diagram and microemulsion formulations

The studied systems composed of safe constituents including oleic acid, propylene glycol, tween-80 (non-ionic surfactant) and water. Non–ionic surfactant was selected because they are generally less toxic, produce less skin irritation. The constructions of phase diagrams make it easy to find out the concentration range of components for the existence range of microemulsions.



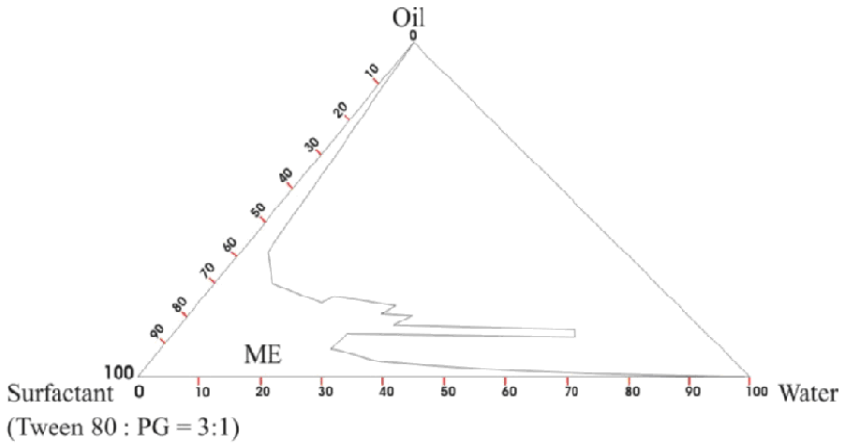
Pseudo - ternary phase diagram of the oil - surfactant - water system at 1:1 weight ratios of Tween 80 to propylene glycol at 25°C. ME represents microemulsion regions.

Figure 1. Phase diagram for microemulsion. Percent concentration of oil phase oleic acid, surfactant & cosurfactant in 1:1 ratio for the preparation of phase diagram



Pseudo - ternary phase diagram of the oil - surfactant - water system at 2:1 weight ratios of Tween 80 to propylene glycol at 25°C. ME represents microemulsion regions.

Figure 2. Phase diagram for microemulsion. Percent concentration of oil phase oleic acid, surfactant & cosurfactant in 2:1 ratio for the preparation of phase diagram



Pseudo - ternary phase diagram of the oil - surfactant - water system at 3:1 weight ratios of Tween 80 to propylene glycol at 25°C. ME represents microemulsion regions.

Figure 3. Phase diagram for microemulsion. Percent concentration of oil phase oleic acid, surfactant & cosurfactant in 3:1 ratio for the preparation of phase diagram

The pseudo-ternary phase diagrams with various weight ratio of tween-80 to propylene glycol are described in figs. 1-3. The translucent microemulsion region is presented in phase diagrams. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsion was observed. The gel area showed the transparent and high viscosity region. The rest of the region on the phase diagram represents the turbid and conventional emulsion based on visual observation. No liquid crystalline structure was observed using cross polarizer. The area of microemulsion isotropic region changed slightly in size with the increasing ratio of surfactant to cosurfactant. A similar result was obtained from an ethyl laurate based microemulsion system with tween-80 as surfactant, propylene glycol and ethanol as cosurfactant [20].

Table 1: Compositions of the selected microemulsion formulation

Component (%)	ME1	ME2	ME3	ME4	ME5	ME-B	ME-E	ME-IPA	ME-IPM
Glipizide	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Oleic acid	2	4	6	8	10	6	6	6	-
Tween-80	20	20	20	20	20	20	20	20	20
Propylene glycol 10	10	10	10	10	-	-	-	10	
Water	67.95	65.95	63.95	61.95	59.95	63.95	63.95	63.95	63.95
n-butanol	-	-	-	-	-	10	-	-	-
Ethanol -	-	-	-	-	-	10	-	-	
Isopropyl Alcohol -	-	-	-	-	-	-	10	-	
Isopropyl myristate	-	-	-	-	-	-	-	-	6

Abbreviations: ME-1, Microemulsion having 1 ml oleic acid as oil phase; ME-2, Microemulsion having 2 ml oleic acid as oil phase; ME-3, Microemulsion having 3 ml oleic acid as oil phase; ME-4, Microemulsion having 4 ml oleic acid as oil phase; ME-5, Microemulsion having 5 ml oleic acid as oil phase; ME-IPM, Microemulsion having 3 ml Isopropyl Myrestate as oil phase; ME-B, Microemulsion having 3 ml n-Butanol as cosurfactant; ME-E, Microemulsion having 3 ml Ethanol as cosurfactant; ME-IPA, Microemulsion having 3 ml Isopropyl Alcohol as cosurfactant.

Various microemulsions were selected from 2:1 phase diagram (Table 1). Tween 80 was added into oily phase in the construction of phase diagram. A relatively long time (about 2-3 h) was

required to obtain transparent microemulsion under magnetic stirring when microemulsions contained 20% tween-80, 10% propylene glycol and 6% oleic acid. However, when tween 80 was solubilized into aqueous phase, and then the aqueous phase was added to oily phase contain propylene glycol and oleic acid; the clear microemulsion could be obtained quickly. But the order of the addition of tween 80 did not change the physicochemical properties of the microemulsions. So in order to reduce the equilibrium time, tween 80 was added to water in preparation of drug loaded microemulsions.

Optimization of process variables and characterization of microemulsion

Preparation of glipizide microemulsion involves various process variables, out of which the followings were selected (Table 2):

(a) Effect of the oil concentration.
(b) Effect of cosurfactant concentration
(c) Effect of different oil.

Oleic acid is used as oil phase in 2, 4, 6, 8, 10% concentration of formulation content and then 6% (ME-3) obtained in clear form and have higher cumulative percent release than others. The different cosurfactants like butanol, ethanol and isopropyle alcohol are used in place of propylene glycol but clarity and cumulative percent release was found to be expected with propylene glycol. Oleic acid and isopropylene myriatate were used as oil phase, in which oleic acid has clear appearance and better cumulative percent release than isopropylene myristate. The average droplet size and polydispersity index of developed optimized microemulsion was found to be138±4.5 nm and around 0.45 respectively.

Table 2: Optimization of process variables

S.No.	Formulation	Appearance	pH	Viscosity	Cumulative drug release (%)	Polydispersity	*R.I.
Effect of oil concentration							
1	ME-1	Milky	5.28±0.04	76.8±0.6	47.20±0.32	0.28±0.012	1.3128
2	ME-2	Opalescent	5.54±0.03	91.4±0.8	91.25±0.52	0.325±0.028	1.3621
3	ME-3	Clear	5.41±0.03	104.2±0.5	96.31±0.26	0.149±0.021	1.3506
4	ME-4	Milky	5.82±0.03	140.6±0.6	70.00±0.34	0.180±0.032	1.4001
5	ME-5	Milky	6.03±0.02	358.4±0.8	52.50±0.46	0.210±0.015	1.2986
Effect of cosurfactant							
1	ME-B	Milky	5.62±0.04	169.8±0.3	42.6±0.54	0.192±0.031	1.3622
2	ME-E	High milky	5.94±0.03	328.5±0.5	54.2±0.38	0.098±0.042	1.4151
3	ME-IPA	Opalescent	5.41±0.03	210.5±0.7	84.0±0.45	0.134±0.035	1.3121
Effect of oils							
1	ME-3	Clear	5.41±0.03	104.2±0.5	96.31±0.26	0.149±0.021	1.3506
2	ME-IPM	Opalescent	5.38±0.04	280.7±0.4	53.86±0.47	0.141±0.011	1.3628

** Referective index*

***In vitro* permeation studies**

The permeation profile of glipizide through mouse skins was observed by *in vitro* permeation studies (Table 2). A steady increase in permeation of glipizide in the receptor chambers with time was observed. The permeation profile of microemulsion follows zero order release kinetics. A significant increase in permeation of glipizide was observed from microemulsion as compared to aqueous solution at the end of experiments. This phenomenon may be result of the depletion of the driving concentration in the donor chamber.

Microemulsion containing a lower amount of tween-80 and propylene glycol provided higher flux. The content of surfactant mixture in microemulsions affected the skin permeation flux of glipizide significantly. This may be due to an increased thermodynamic activity of the drug in microemulsion at the lower concentration of surfactant and cosurfactant [21]. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug in to the skin [22]. The thermodynamic driving force for the release reflects shows the relative activities of the drug in different phase [23]. Since drug can be release from the internal phase to external phase and then from external phase to the skin, the relative activities may monitor the skin permeation flux. In addition, the surfactant and cosurfactant may exist in each phase, so glipizide can partly solubilized in external phase. The depletion of glipizide may be from the external phase because of the permeation in to the skin can be supplemented by the release of glipizide from the internal phase. Zero order release kinetics shows the sustained, controlled and prolonged delivery of glipizide from the microemulsion. This may also be the main mechanism of permeation of glipizide in to the skin from the microemulsion. The permeation rates of glipizide from microemulsion accorded with the Fick's first diffusion law [24, 25].

Skin irritation studies

The irritation studies did not show visible irritation after application of microemulsion for 7 days on the skin of rabbits. No erythema or oedema was observed on the skin of rabbits. Only rubefaction appeared on the skin of some rabbits occasionally on the third or forth days and disappeared on the sixth or seventh day. The encapsulation of drug in microemulsion might reduce the skin irritation induced by glipizide. Thus the microemulsion system for the transdermal delivery of glipizide is viable and could improve patient compliance.

Evaluation of hypoglycemic effect

Table 3 shows reduction in blood glucose level in sustained manner after topical administration of glipizide from various microemulsion formulations. When pure glipizide suspension was administered orally, a maximum reduction in blood glucose level was observed within 2 h. The microemulsion formulation containing the dose of 1000 µg/kg was shown significant reduction in blood glucose level but in sustained manner as compared to oral glipizide suspension in PBS (pH 7.4). From the reported literature, it was well defined that a 25 % reduction in blood glucose levels is considered hypoglycemic effect. Results of present study revealed that glipizide topical microemulsion formulation is more effective as compared to conventional formulation because it provide reduction in glucose level with controlled manner.

Table 3: Reduction in blood glucose level

S.No.	Time (h)	% Reduction in blood glucose level (mean±SE, n=4)*			
		Plain glipizide (800µg/kg, Oral)	ME-3 (600µg/kg, Topical)	ME-3 (800µg/kg, Topical)	ME-3 (1000µg/kg, Topical)
1	1	44±2.3	09±1.3	11±1.5	20±1.8
2	2	48±1.6	12±1.2	15±1.3	29±1.5
3	3	39±1.4	20±1.8	23±2.1	38±1.7
4	4	25±2.1	19±2.1	22±2.2	42±2.3
5	5	27±2.1	18±1.1	23±1.4	40±1.5
6	6	11±1.7	16±1.6	22±1.2	40±2.3
7	7	09±1.5	15±1.4	21±1.8	39±1.6
8	8	07±1.4	14±2.3	20±1.3	38±1.4
9	24	-	06±1.5	05±2.2	04±2.1

** Results show means ±SE between four rats in each group.*

Stability of microemulsion

Phase separation of microemulsion after centrifugation was not observed in ME-2, ME-3, ME-5 and ME-IPM microemulsions (Table 4).

Table 4: Percent phase separation of microemulsion on centrifugation

S. No.	Centrifugation time (min)	% phase separation									
		ME-1	ME-2	ME-3	ME-4	ME-5	ME-B	ME-E	ME-BE	ME-IPA	ME-IPM
1	10	-	-	-	-	-	2	-	-	-	-
2	20	3	-	-	-	-	8	-	-	10	-
3	60	10	-	-	8	-	21	-	3	15	-
4	90	42	-	-	12	-	35	-	14	38	-

Table 5 shows the results of residual drug content of microemulsions which indicated maximum loss of drug at 45°C and 60°C and room temperature while only 10-15% of drug loss occurs at 8°C. Thus 8°C may be more favorable temperature for storage of microemulsion.

Table 5: Effect of storage condition on residual drug content of formulations ME-2, ME-3 and ME-IPM formulation

Time (months)	Temperature	Residual drug content (%)		
		(ME-2)	(ME-3)	(ME-IPM)
Initial	RT	100	100	100
2	8° C	97.9±1.2	98.3±1.8	96.9±1.9
4	8° C	93.9±2.5	92.0±2.3	88.9±2.3
6	8° C	83.4±1.8	88.3±1.6	79.4±1.6
2	RT	98.7±0.9	98.8±2.9	97.7±3.1
4	RT	94.3±1.9	93.9±3.1	89.3±2.1
6	RT	89.2±2.1	86.1±2.9	76.2±1.4
2	45° C	98.6±1.4	97.4±3.4	96.6±2.8
4	45° C	91.8±1.5	92.4±1.5	89.4±1.7
6	45° C	88.1±2.1	82.8±4.1	78.5±2.2
2	60°C	97.5±1.1	97.6±2.2	96.5±1.5
4	60°C	92.4±1.4	92.8±1.9	89.4±1.7
6	60°C	87.5±2.5	81.8±2.1	78.5±2.2

CONCLUSION

Microemulsion system provides viscous consistency for the topical application, which delivered the drug in sustained or controlled manner and prolonged delivery as compared to conventional dosage form. From the present study it was concluded that microemulsion may have number of advantages such as enhance drug solubility, good thermodynamic stability, ease of manufacturing and enhance the effect on transdermal ability. Apart from that, glipizide microemulsion system may be the most convenient topical formulation for the patient unable to take drug orally.

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