

Research Article

Benzyl Benzoate-Loaded Microemulsion for Topical Applications: Enhanced Dermatokinetic Profile and Better Delivery Promises

Gajanand Sharma,¹ Geeta Dhankar,¹ Kanika Thakur,¹ Kaisar Raza,^{2,3} and O. P. Katare^{1,3}

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Abstract. Benzyl benzoate (BB) is one of the oldest drugs used for the treatment of scabies and is recommended as the “first-line intervention” for the cost-effective treatment of the disease. Though a promising candidate, its application is reported to be associated with irritation of the skin and eye, resulting in poor patient compliance. Hence, the present study aims to develop BB-loaded topical microemulsion for the safer and effective delivery of BB. Pseudo-ternary phase diagrams with BB as the oily phase itself, along with Tween 80 as surfactant, and mixture of phospholipid and ethanol as the co-surfactant along with aqueous solution as the external phase were constructed and various compositions were formulated. The optimized formulation was characterized for particle-size, zeta-potential, drug-content, globule-morphology pH, and refractive-index, whereas evaluated for skin permeation, retention, compliance, and dermatokinetics. The nanosized formulation offered threefold higher drug permeation *vis-a-vis* plain drug solution across LACA mice abdominal skin. The drug retention of the selected formulation was nearly twice of that from the marketed product, assuring depot formulation and sustained release. The skin histopathology revealed the non-irritant nature of the formulation, as no changes in the normal skin histology were observed. The dermatokinetic studies confirmed better permeation and enhanced skin bioavailability of BB to epidermis as well as dermis *vis-à-vis* the conventional product. The results indicate that the developed lipid-based microemulsion hydrogel can alleviate the concerns associated with BB and can provide a better and safer delivery option in substantial amounts to various skin layers.

KEY WORDS: colloidal delivery; dermal availability; drug delivery; hydrogel; nanoemulsion; retention; scabies.

INTRODUCTION

Scabies, a highly contagious skin infection, is one of the most common pruritic skin disorders (1). It is common worldwide but is more conspicuous in the areas with poor sanitation and overcrowding. The global prevalence of scabies is around 300 million cases, with larger variations between countries (2,3). It is a neglected parasitic disease and a major health problem of the developing countries. The World Health Organization (WHO) has issued alarming figures of incidence as 9.7–13% in India (9.7 and 13%), 23–30% in children below 6 years in Bangladesh (23–30% in under 6 years old in slum areas), 4.3% in Cambodia, and 0.7% in Malawi. This disease is caused by the mite *Sarcoptes scabiei*, which burrows into the skin and consumes epidermis and sera of the host, resulting in

inflammation, allergy-like reactions, and pruritic lesions (4–7). The currently available treatment options for scabies range from topical scabicides like permethrin, pyrethroids, crotamiton, and lindane to oral medications like antihistamines (8). Benzyl benzoate (BB) is one of the oldest drugs used for the treatment of scabies and is considered as the preferred treatment option for the same (9). The mechanism of action of BB is not well understood, but it is assumed that it acts on the nervous system of the parasite (*S. scabiei*), resulting in its death (10,11). Benzyl benzoate is the ester of benzyl alcohol and benzoic acid. It occurs as a white solid or colorless oily liquid having a faint aromatic odor. It is completely insoluble in water and glycerol with miscibility in alcohol, chloroform, ether, and oils (11).

Topical BB therapy has been widely used in adults and also in diluted form for children, babies, and lactating mothers (2). *In vitro* results advocate the superiority of BB in killing scabies mites to permethrin and are more beneficial in severe crusted scabies (12–14). The most common adverse event associated with the use of BB is initial severe burning sensation following local application of concentrated lotion (25% v/v) (13). Other adverse effects include blister formation, crusting, itching, oozing, reddening, and scaling of skin, after repeated use (13,14). Henceforth, clinically, the use of topical

¹ Division of Pharmaceutics, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies, Panjab University, Chandigarh, 160 014, India.

² Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Bandar Sindri, Dist. Ajmer, Rajasthan 305 817, India.

³ To whom correspondence should be addressed. (e-mail: drkaisar@curaj.ac.in; katare@pu.ac.in)

BB is not very patient-compliant owing to the associated side effects. Thus, to circumvent the side effects, a suitable carrier system is needed which can suit this very promising drug molecule and revitalize its usage.

Colloidal systems such as microemulsions have been investigated as drug delivery and targeting systems possessing a potential to modify bioavailability, stability, and side effects of various drugs (15–20). Microemulsions (ME) are colloidal dispersions having droplet size in the range of 10 to 100 nm (18,21). These are composed of oil phase, surfactant, co-surfactant, and aqueous phase in an appropriate ratio. As topical delivery vehicles, MEs tend to increase the local or systemic delivery of drugs through a number of mechanisms including enhancement in the solubility of poorly soluble drugs and by the modification of diffusional barrier of the skin, depending upon the composition of ME (22). MEs provide deeper penetration in the skin layers which results in an effective and controlled delivery of the active agents (23). Micro-emulsified hydrogel-based systems have been selected as the carriers in the present study as they possess oil-like consistency which is suited for easy application on to the infected area including scalp. Pluronic® F-127 (PF-127) has been employed as a gelling agent to achieve the desired ease of application. F-127 is a nonionic surfactant composed of polyoxyethylene-polyoxypropylene copolymers which undergoes sol-gel transition, depending on its concentration and temperature (24,25). Due to its reverse thermal gelation behavior, good solubilization capacity, optical properties, and low toxicity, it appears to have potential application as topical drug delivery system (26). This characteristic has allowed PF-127 to be used as a carrier for various routes of administration including oral, topical, intranasal, vaginal, rectal, ocular, and parenteral routes (27).

Thus, the main objective of the present study was to effectively load BB into the microemulsion based hydrogel for better delivery of the drug to the desired site in a safe and effective manner.

MATERIALS AND METHODS

Materials

Free gift samples of Phospholipon S100 were obtained from M/s Phospholipid GmBH (Nattermannalle, Germany). BB, Tween 20, Tween 80, Span 20, and Span 80 were procured from M/s S.D. Fine chemicals Ltd. (Mumbai, India), while absolute ethanol was procured from M/s Bengal Chemicals Ltd. (Kolkata, India). Pluronic® F-127 was procured from BASF Corporation, NJ, USA. All other chemicals and reagents used in the study were of analytical grade and double distilled water was employed in the study.

Construction of Pseudo-Ternary Phase Diagrams

Pseudo-ternary phase diagrams were constructed employing aqueous titration method at a temperature of 37°C. For screening of surfactants, pseudo-ternary phase diagrams were constructed employing the surfactant (Tween 80/Tween 20/Span 20/Span 80) in the mass ratio of 5:1 with the co-surfactant (mixture of phospholipid and ethanol in the mass ratio of 1:5). BB itself was used as the oil and various clear ratios of oil and S_{mix} (mixture of surfactant and co-

surfactant from 1:9 to 9:1 mass ratio) were titrated against water, till the development of haziness. Similarly, various ratios of aqueous phase and S_{mix} were prepared and titrated against oil. On the basis of the area of emulsification, the selection of surfactant was made. After selecting the surfactant out of the studied ones, five different phase diagrams were constructed with the varying ratios of surfactant to co-surfactant (S_{mix}) varying from mass ratio of 1:1 to 5:1 as, depicted in Table I. The physical state of the microemulsion was marked on the pseudo ternary diagrams with one axis representing aqueous phase, other representing the oil, and the third representing the fixed S_{mix} ratio. The monophasic/microemulsion region was identified as the area in the diagram, where clear and transparent formulations were obtained on visual inspection (28).

Selection of Formulations on the Basis of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams were analyzed to identify the microemulsion region. From each diagram constructed, different formulations/points were selected on the basis of minimum amount of emulsifier used in the formulations.

Preparation of Microemulsion

In brief, the non-aqueous phase (ethanolic solution) consisting of phospholipid, ethanol and Tween 80 was heated to 50–60°C with continuous stirring (15 min) in order to obtain a homogenous solution of surfactant and phospholipid (29). The temperature of the above system was allowed to decrease gradually and BB (equivalent to 20%, w/w) was added to it as the oily phase. The required amount of aqueous phase was slowly added under continuous stirring to fetch a clear yellow microemulsion.

Characterization Studies

According to the ME regions from the phase diagrams, eight ME formulations were prepared and characterized for the following parameters:

Emulsion Attributes

The formulations were kept randomly for a stipulated period of time (45 days) under the given experimental conditions (37°C) to assess the sustenance of the characters of the product. The formulations were observed for various

Table I. Composition of Various Ternary Phase Diagrams on the Basis of Ratio of Surfactant to Co-surfactant Used

S. Number	Pseudo-ternary diagram code	Oil, ratio of surfactant to co-surfactant (S_{mix})
1	Diagram 1	BB, Tween 80:co-surfactant (1:1)
2	Diagram 2	BB, Tween 80:co-surfactant (2:1)
3	Diagram 3	BB, Tween 80:co-surfactant (3:1)
4	Diagram 4	BB, Tween 80:co-surfactant (4:1)
5	Diagram 5	BB, Tween 80:co-surfactant (5:1)

macroscopic characters like creaming, cracking, and phase separation.

Drug Content

The amount of the drug present in the developed systems was determined by extracting the drug in ethanol and subsequent analysis by UV spectrophotometer (UVS-2700, Spectro UV-vis Dual beam, Labomed Inc., USA).

Micromeritics and Zeta Potential

The mean droplet size and size distribution of prepared microemulsion globules was assessed using DLS technique (Malvern Zetasizer™, M/s Malvern Instruments Co., Worcestershire, UK). The same equipment was used to determine the zeta potential of the prepared formulations. The value of three subsequent observations was reported as the mean result.

Globule Morphology

Morphology and structure of the globules of drug-loaded microemulsions was determined using transmission electron microscopy (TEM) installed at Central Instrumentation Laboratory (CIL), Panjab University, Chandigarh, India, and microphotographs were clicked at suitable magnifications.

Measurement of pH

As the formulations were topical, pH measurement was essential to ensure the non-irritating nature of the formulations. The pH of the undiluted formulations was determined using L1-120, Cyber Scan 510 pH meter (Eutech Instruments Pvt. Ltd., Singapore).

Refractive Index

The optical density of the prepared system was measured using Abbe Refractometer (M/s Shijiazhuang Optical Instrument Factory, Xiamen, China) at room temperature. Refractive index (RI) of the formulations was reported while taking water as reference.

Development of Secondary Topical Vehicle

Pluronic® F-127 was employed to gel the selected microemulsions to make it rheologically acceptable for topical applications. Pluronic® F-127 equivalent to (3.6%, w/v) was dissolved at 4°C for 24 h, and after complete solubilization, this aqueous phase was added in the oily phase to give ME gel.

Ex Vivo Permeation Studies

The animal protocols of the study were approved by the Institutional Animal Ethical Committee (IAEC) of Panjab University, Chandigarh, India, with reference no. 5622/PS. The permeation studies were conducted on excised abdominal skin of LACA mice (30,31). In brief, the animals were sacrificed by cervical dislocation. The skin was removed from the animal with the help of surgical scissors, and hair

was removed from the separated skin with the help of surgical blade no. 24. The skin thickness was measured (0.31 to 0.32 mm) with the help of a vernier caliper, and dye test was used to test the integrity of the skin. The excised skin was wiped to remove residual adhering fat and further and washed with PBS (pH 6.8). The prepared skin was wrapped in aluminum foil and stored in a deep freezer at -30°C for further use. (32,33) Prior usage methylene blue dye test was performed to assess the skin integrity (34). *Ex vivo* skin permeation studies were conducted on Franz diffusion cells (PermeGear, Inc., Hellertown, PA, USA). The excised skin was mounted between the donor and receptor compartment exposing an effective area of 3.14 cm² with sink volume of 30.0 mL (35). The receptor compartment contained absolute ethanol as diffusion medium to maintain sink condition and its temperature was maintained at 37±0.5°C. The prepared formulations (5 mg; equivalent to 1 mg of drug) were applied onto the skin in the donor compartment. Pure drug (oil 1 mg) was also applied onto the skin of donor compartment for the comparison purpose. The prepared formulations were uniformly spread using flat end of spatula so as to maintain intimate contact with skin (36, 37). At suitable time intervals (0–24 h), aliquots of 1 mL each were withdrawn through the sampling port and replaced with equal amount of sink medium to maintain constant receptor compartment volume (23). The samples were analyzed spectrophotometrically, after appropriate dilutions.

Drug Retention Studies in Skin

After the completion of skin permeation studies, skin tissue mounted on the diffusion cell was removed carefully and washed with distilled water thrice to remove the excess amount of the formulation. The skin was dried under the folds of tissue paper and subsequently weighed. After proper drying, the skin was washed with tissue homogenizer. The homogenate suspension thus obtained was mixed with 20 mL chloroform-methanol mixture (2:1 v/v) and shaken for 2 h at 37°C for extraction of BB. The supernatant was filtered through a 0.45-µm membrane filter (M/s Millipore, MA, USA) and spectrophotometrically analyzed for the drug content.

Dermatokinetic Modeling

The excised skin of the LACA mice was used for the studies. The skin tissue was prepared as discussed in Section 2.7, and the protocol was in consonance with Section 2.8. At the respective sampling times (0–5 h), the whole skin was removed from the Franz cell and washed thrice to remove any adhering formulation. Portion of the washed skin was soaked in hot water (60°C) for 30 s to facilitate the detachment of epidermis from dermis. Both the sections were separated by means of surgical blade and chopped into small pieces, separately, and macerated in 5 mL of chloroform-methanol mixture (2:1 v/v) for 24 h for complete drug extraction. After filtration through a membrane (0.45 µm), the filtrate was analyzed using UV spectrophotometer. The obtained data

were fitted into one-compartment model, as per the Eq. 1(38)

$$C_{\text{Skin}} = \frac{K_p \cdot C_{\text{max}}^{\text{Skin}}}{(K_p - K_e)} (e^{-K_p t} - e^{-K_e t}), \quad (1)$$

where C_{skin} is the concentration of drug either in epidermis or dermis at time t , K_p is the dermal permeation constant, $C_{\text{max}}^{\text{Skin}}$ is the maximum concentration achieved either in epidermis or dermis, and K_e is the skin elimination constant. Win-Nonlin Ver 5.0 software was employed to compute the various dermatokinetic parameters *viz.* K_p , $C_{\text{max}}^{\text{Skin}}$, K_e , $T_{\text{max}}^{\text{Skin}}$ (time required to achieve $C_{\text{max}}^{\text{Skin}}$ and area under the curve (AUC_{0-12 h}) using the Wagner-Nelson method (32, 38).

Skin Compliance Studies

Male LACA mice (4–6 weeks old; 15–20 g) were divided into three groups, each group consisting of three animals; hairs on the dorsal side of mice were removed with the help of a 0.1-mm animal hair clipper. The skin was wiped thrice with a saline-wetted cotton swab, and after drying, the formulations (0.5 g; equivalent to 0.1 g of BB) were topically applied on the hair skin by uniform spreading within the area of 4 cm² once a day for 7 days. Group 1 and group 2 animals received topical application of conventional cream and ME hydrogel, respectively. Group 3, on the other hand, was treated only with saline, which served as the control. Prior to each daily application, the formulation remaining on skin was removed carefully and swabbed with saline soaked cotton, and the new dosage was applied. On completion of study, the animals were then sacrificed by cervical dislocation; skin samples were removed and fixed in 10% formalin solution. The skin samples

obtained were stained with hematoxylin and eosin. The stained skin samples were observed microscopically for the histopathological changes (31).

STABILITY STUDIES

All the prepared microemulsion formulations were subjected to stability studies at various temperatures *viz.*, 4±2, 30±2, and 45±2°C for 45 days in sealed ampoules. After every 7 days, the drug content was determined, as per the method described in drug content studies.

Statistical Analysis

The results were statistically analyzed using one-way ANOVA followed by *post hoc* analysis using Student's *t* test. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Screening of Oils, Surfactants, and Co-Surfactants for ME

Selection of oily phase is the most crucial step for the formulation of microemulsions. BB itself is oily in nature and is being used in the concentration range of 10–20%; hence, in the present study, it was used oil as such. Despite all this, it is also reported to possess cosolvency properties (39).

From the pseudo-ternary phase diagrams as portrayed in Fig. 1, it is clearly vivid that Tween 80 was able to offer better emulsification region *vis-à-vis* other employed non-ionic surfactants. However, there were no issues of miscibility of the drug with the studied surfactants. On the basis of maximum emulsification, Tween 80 was selected

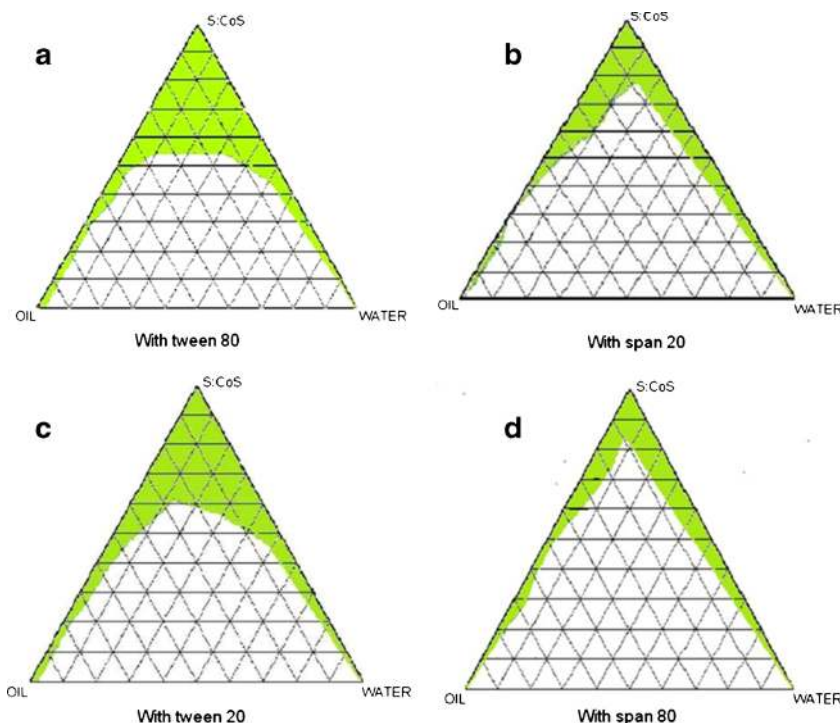


Fig. 1. Pseudo-ternary phase diagrams employing different surfactants as: **a** Tween 80; **b** Span 20; **c** Tween 20 and **d** Span 80

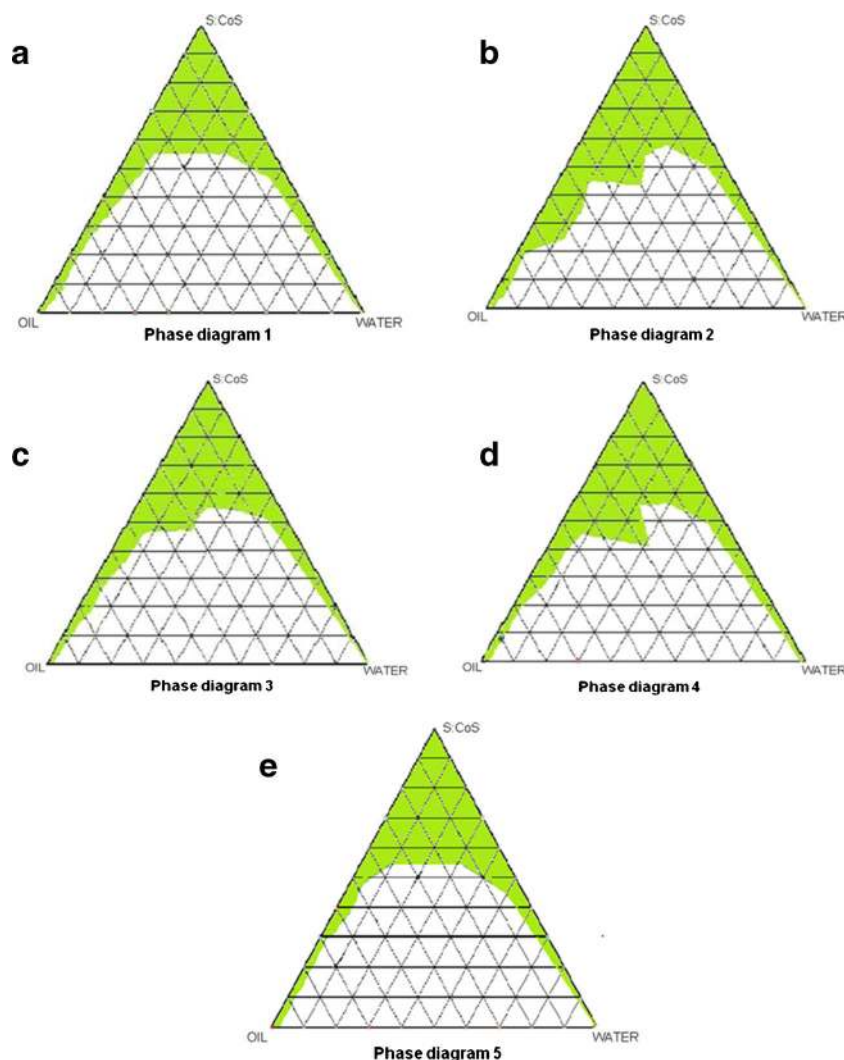


Fig. 2. Various pseudo-ternary phase diagrams with surfactant to co-surfactant ratios as: **a** 1:1; **b** 2:1; **c** 3:1; **d** 4:1 and **e** 5:1

as the surfactant, as higher concentrations of BB (20%) can be loaded with use of this emulsifier. Tween 80 is a well-established non-ionic surfactant for its biocompatibility and better emulsification characteristics (17). In addition to this, there are various studies which report that Tween 80 when incorporated in microemulsion enhances the skin permeation of drugs (36,38).

On the other hand, presence of co-surfactant is not mandatory, but its incorporation reduces the interfacial tension which further enhances the stability of microemulsions (37,40). In the present study, phospholipid S100 along with ethanol was used as the co-surfactant with an aim to exploit the biocompatibility of phospholipid and the permeation enhancement from ethanol (36,37,41). Ethanol has the capability to reduce the rigidity of the phospholipid condensed films, which is needed for the microemulsion globule formation (41,42). The presence of co-surfactant ultimately decreases the bending stress of the interface and imparts sufficient flexibility to take up different curvatures required to form ME over a wide range of composition (43).

Construction of Ternary Phase Diagram and Selection of Formulations

The relationship between the phase behavior of a mixture and its composition can be established with the aid of a phase

Table II. The Compositions of the Selected Formulations

Formulation code	Percentage of oil	Percentage of aqueous phase	Percentage of emulsifier
Formulation from 1, 3, and 5 pseudo-ternary phase diagrams			
G11	20	24	56
G21	15	28	57
G13	20	25	55
G23	15	27	53
G15	15	29	56
G25	20	24	56
Formulation from two to four pseudo-ternary phase diagrams			
G12	15	25	60
G14	15	27	58

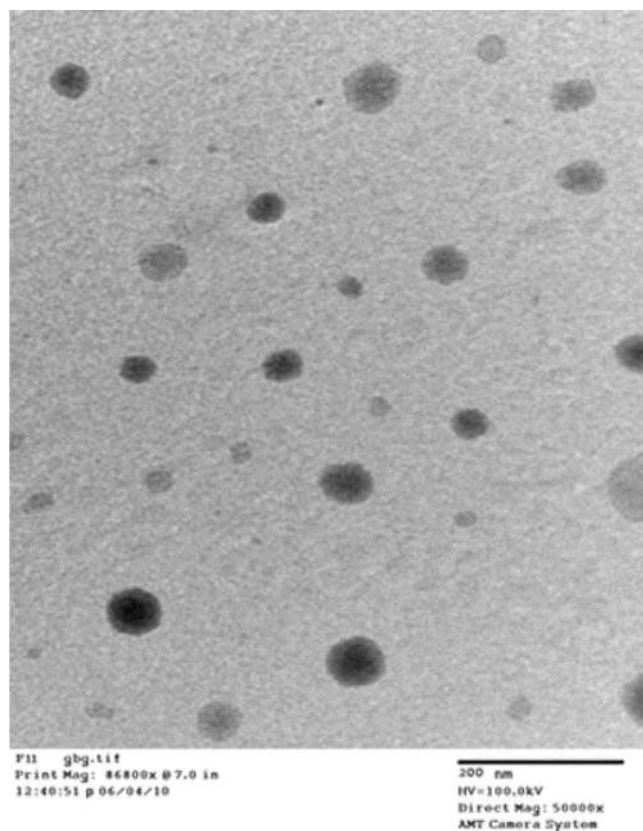
Table III. Physicochemical Characteristics of Studied ME Formulations

Formulation code	Drug content (%)	Globule size (nm)	Zeta potential (mV)	RI
G11	99.51	104.8	0.218	1.438
G21	99.78	136	0.398	1.418
G12	99.81	446.9	0.0416	1.437
G13 (o)	99.62	–	–	1.442
G23	99.66	2378	0.258	1.436
G14	99.91	240	0.148	1.450
G15	99.99	235.1	0.239	1.425
G25 (o)	99.49	–	–	1.432

(o) not taken for studies as were are unstable, *RI* refractive index

diagram (44). Figure 2 shows the obtained phase diagrams from all the S_{mix} compositions.

There are no reports of the proper basis of selecting different microemulsion formulations from a phase diagram, as hundreds of formulations can be prepared from microemulsion region of a single diagram (17). Two formulations were selected from pseudo-ternary phase diagrams 1, 3, and 5, and one point each was selected from pseudo-ternary phase diagrams 2 and 4. The points were selected on the basis of fixed amount of oil to be taken (20 and 15%), as drug was acting as oil phase. The formulae and codes of the selected formulations have been shown in Table II.

**Fig. 3.** TEM photograph of BB-loaded microemulsion (50,000 \times)

Characterization of Microemulsions

The results of the characterization studies indicated successful development of microemulsion formulations of BB with optimum characteristics. The dispersed systems of ME formulations were found to be macroscopically identical, *i.e.*, homogeneous, transparent without any precipitates, optically isotropic, and yellow colored. The physicochemical parameters of microemulsions are tabulated in Table III. The drug content of all eight formulations was in the range of 99.49 to 99.99% with mean value of 99.72%. The average size of the ME globules ranged from 104.8 to 2378 nm, while the PDI value was found in the range of 0.0416 to 0.398. Higher values of drug content assured successful material transfer of the active drug during various formulation processes. The zeta potential values were found to be near zero, which may be ascribed to the non-ionic nature of the surfactant and co-surfactant used (*i.e.*, Tween 80 and phospholipid S100). However, such components are reported to provide better stability to the formulation against any ionic interactions. Out of all the formulations, the mean diameter of G11 was found to be the smallest *i.e.*, 104.8 nm. The zeta potential was found to be 0.218 mV for the selected formulation. This may be attributed to the non-ionic nature of both surfactants and co-surfactant used. They do not impart any charge to the droplet surface and provide better stability to the microemulsion against any ionic interactions. The refractive index values were of the studied formulations and were found to range between 1.418 and 1.450 against water. The consistency in RI values of

Table IV. Permeation Profile and Drug Retention of BB from Various Formulations ($n=3$)

Formulation code	Percentage of BB permeated (% \pm SD)	Permeation flux (μ g/cm ² /h \pm SD)	Amount retained (% \pm SD)
μ 11	67.45 \pm 0.100	8.59 \pm 0.51	0.920 \pm 0.010
G21	38.37 \pm 0.243	5.09 \pm 0.44	0.470 \pm 0.023
G12	51.44 \pm 0.121	6.83 \pm 0.42	0.468 \pm 0.031
G23	50.75 \pm 0.089	6.73 \pm 0.23	0.695 \pm 0.042
G14	47.55 \pm 0.203	6.31 \pm 0.12	0.557 \pm 0.033
G15	60.35 \pm 0.229	8.01 \pm 0.51	0.530 \pm 0.019

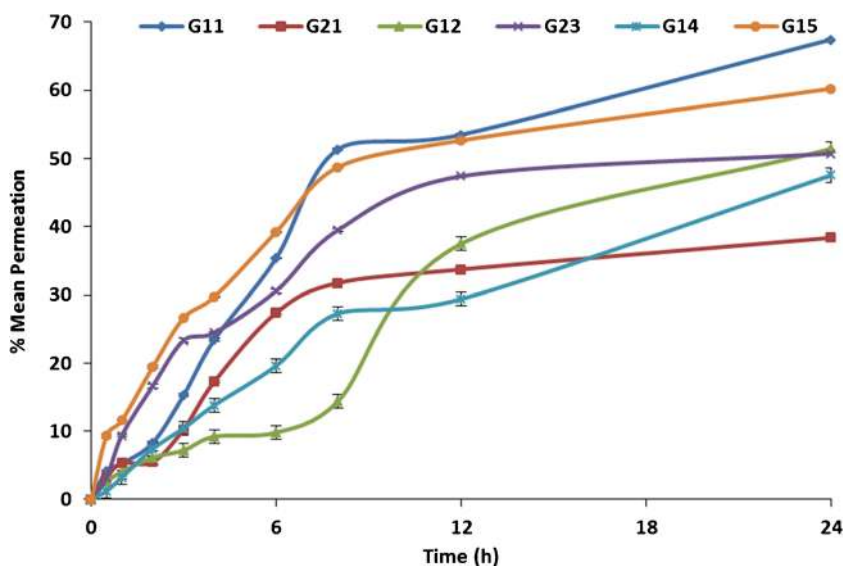


Fig. 4. Plot between mean percentage of BB permeated *versus* time from various tested formulations. Each cross bar indicates average value \pm SD ($n=3$)

all developed formulations is an indication of regular microemulsion structure (44).

Morphology and Structure

The optimized formulation was viewed microscopically through TEM. The image depicts the spherical nature of the globules, revealing that the globules are covered by additional coating as shown in Fig. 3.

Ex Vivo Skin Permeation Studies

The percutaneous permeation parameters of the tested ME formulations are depicted in Table IV. The permeation behavior of the developed formulations was assessed by analyzing the permeation flux values as well as graphic relationship of the amount released with respect to time, as depicted in Fig. 4.

The order of amount of drug permeation was found to be as follows:

G11 ($67.45 \pm 0.10\%$) > G15 ($60.35 \pm 0.23\%$) > G12 ($51.44 \pm 0.12\%$) \approx G23 ($50.75 \pm 0.09\%$) > G14 ($47.55 \pm 0.20\%$) > G21 ($38.37 \pm 0.24\%$), while the order of drug permeation flux was observed as:

G11 ($8.59 \pm 0.51 \mu\text{g cm}^{-2} \text{h}^{-1}$) > G15 ($8.01 \pm 0.51 \mu\text{g cm}^{-2} \text{h}^{-1}$) > G12 ($6.83 \pm 0.01 \mu\text{g cm}^{-2} \text{h}^{-1}$) > G23 ($6.73 \pm 0.23 \mu\text{g cm}^{-2} \text{h}^{-1}$) > G14 ($6.31 \pm 0.12 \mu\text{g cm}^{-2} \text{h}^{-1}$) > G21 ($5.09 \pm 0.44 \mu\text{g cm}^{-2} \text{h}^{-1}$).

It was observed that the maximum drug permeation and maximum permeation flux values were for G11 (Tween 80=28%, PL=23.33%, and ethanol=4.67%), while the similar formulation G21 (Tween 80=38%, PL=15.83%, and ethanol=3.16%) offered very low permeation and poor permeation flux values. Despite from same phase diagram 1, differences in composition as well as oil amount (20% in G11 *vis-à-vis* 15% in G21) are the regions for difference in permeation profiles. The next higher permeation flux after G11 was observed for G15 (Tween 80=46.67%, PL=7.78%, and ethanol=1.55%) which was selected and obtained from ternary phase diagram 5. Interestingly,

two contradicting observations were found to be prevalent in this composition. Firstly, it contained the maximum amount of Tween 80 (46.67%), out of all the studied compositions, which is reported to emulsify sebum and extract skin lipids that enhance the thermodynamic coefficient of the drug, allowing it to penetrate into the cells more effectively (45,46). On the other hand, the amount of phospholipid was observed to be least in G15 (7.78%). Hence, it was inferred that the permeation effect from phospholipid was far greater than that from Tween 80. This relatively lower permeation flux can be ascribed to the lower concentration of phospholipid present *vis-à-vis* the formulation G11. The lower amount of phospholipid seems to be more responsible and the deciding factor than the increased amount of surfactant in determining the drug permeation profile.

In general, it was observed that the enhancement in drug permeation was most affected by the total amount of surfactant(s) (cumulative effect of Tween 80, phospholipid, and ethanol), followed the amount of BB. The findings are in consonance with the previous reports indicating the substantial effect of surfactant amount and type on the skin permeation of drugs (47,48).

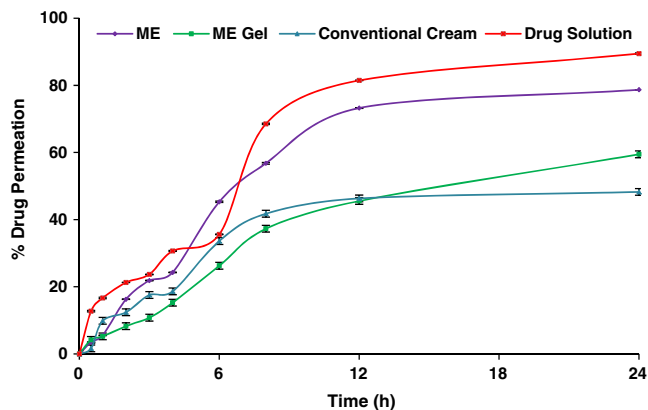


Fig. 5. Percutaneous permeation profile of the selected formulation and other reference products. Each cross bar indicates average value \pm SD ($n=3$)

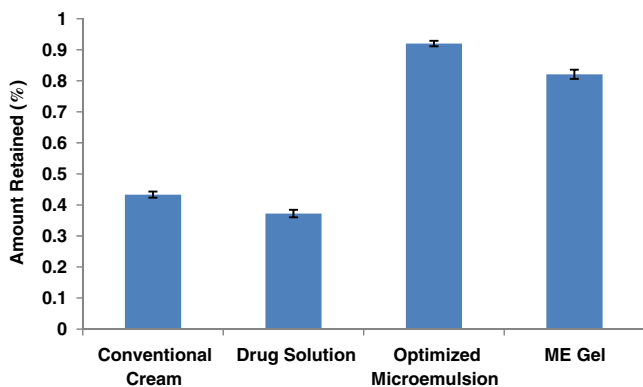


Fig. 6. Bar diagram showing the mean percent drug retention in the skin from various formulations. Each *cross bar* indicates average value \pm SD ($n=3$)

Drug Retention Study

The results of the study are displayed in Table IV. In this study, drug retention analysis was carried out after 24 h for six remaining formulations. The highest drug retention was observed in G11, followed by G23. Hence, the formulation G11 offered the maximum permeation flux, highest percentage permeation, as well as the desired drug retention, hence, was selected as the final formulation for further evaluation.

Comparative Evaluation of the Optimized ME Formulation

Ex Vivo Skin Permeation Study

The *ex vivo* skin permeation profiles of microemulsion, microemulsion hydrogel, commercial cream, and drug solution through excised abdominal skin of mice are as shown in Fig. 5. The release behavior of the selected formulation was assessed by analyzing the flux value as well as the graphic relationship.

The order of release flux obtained is in the following order: drug solution ($11.98 \mu\text{g cm}^{-2} \text{h}^{-1} \pm 0.92$)

>microemulsion ($8.95 \mu\text{g cm}^{-2} \text{h}^{-1} \pm 0.77$) >microemulsion gel ($7.59 \mu\text{g cm}^{-2} \text{h}^{-1} \pm 0.61$) >conventional cream ($6.96 \mu\text{g cm}^{-2} \text{h}^{-1} \pm 0.53$). The permeation flux of drug solution was the highest, followed by microemulsion and microemulsion hydrogel which was found to be higher as compared to the conventional cream ($p < 0.05$). The surfactants in ME act as permeation enhancers and reduce the barrier of stratum corneum. Additionally, ME acts as the nanocarrier for drug providing a large surface area for drug transfer to skin and high drug concentration within the upper layers of the skin which results in a higher concentration gradient as the driving force for topical drug delivery.

Drug Retention Studies in Skin

The drug retention studies were performed to compare the above mentioned formulations of BB. Figure 6 shows the drug retention profile of various formulations of BB. The optimized formulation had three times higher drug deposition capacity than commercial cream and plain drug solutions, respectively. This might be due to the faster release observed for the drug from G11 compared to conventional cream and drug solution, thereby more drug penetrates into the skin by which more amount of drug retains in the skin. Hence, it can be inferred from drug retention study that the optimized formulation, G11, is an efficient delivery vehicle for the safe and effective treatment of scabies.

Dermatokinetic Modeling

Figure 7a, b shows the distribution of drugs in the epidermis and dermis of the LACA mice skin. Delivery of BB in the skin layers by ME gel was found to be significantly greater ($p < 0.05$) than that from the conventional cream. Table V gives the numeric values of $AUC_{0-12 \text{ h}}$, $C_{\text{max}}^{\text{Skin}}$, skin penetration rate constant (K_p), $T_{\text{max}}^{\text{Skin}}$, and skin elimination rate constant (K_e). It is vivid from the results that the biological half-life of the drug was enhanced by ME in both the skin layers, and T_{max}

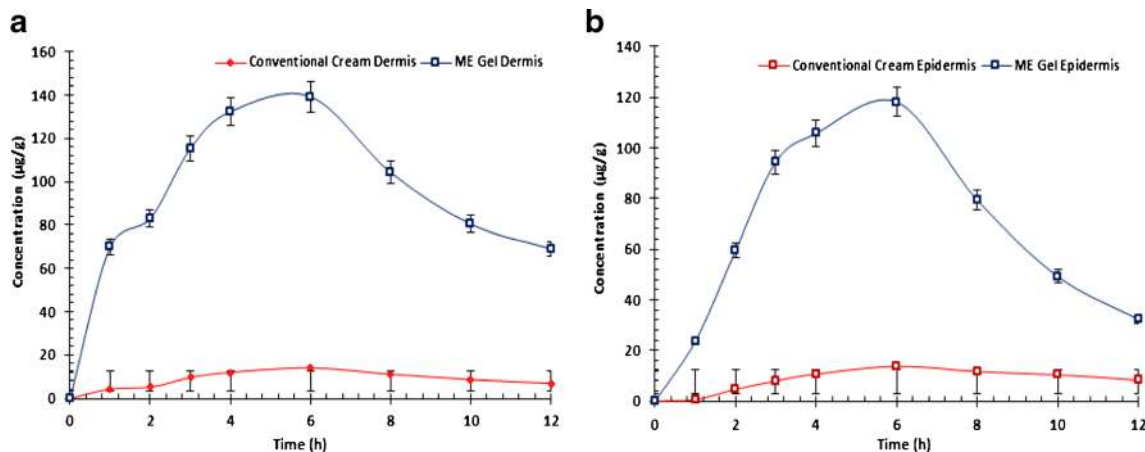


Fig. 7. Dermatokinetic profile showing the drug concentration in **a** the dermis and **b** the epidermis, at various time points. Each *cross bar* indicates average value \pm SD ($n=3$)

Table V. Dermatokinetic Parameters (Mean±SD) of BB-Loaded Topical Formulations in the Epidermis and Dermis of LACA Mice ($n=3$)

Dermatokinetic parameters	ME Gel		Conventional cream	
	Epidermis	Dermis	Epidermis	Dermis
$AUC_{0-12\text{ h}}$ ($\mu\text{g cm}^{-2}\text{ h}$)	740.36±286.75	1049.056±174.17	771.83±156.66	559.27±295
C_{max} ($\mu\text{g cm}^{-2}$)	61.16±6.68	83.68±4.23	35.24±5.6	36.02±9.52
T_{max} (h)	4.45±0.99	4.61±0.39	7.86±2.39	5.71±0.75
K_{a} (h^{-1})	3.07±554.99	3.13±37.29	4.41±35.06	3.96±142.45
K_{e} (h^{-1})	3.09±558.98	3.26±39.04	6.84±66.43	3.95±141.8

was reduced drastically. C_{max} , in both the layers, and AUC in the dermis was significantly enhanced. The results unequivocally confirmed that the ME has the potential to enhance the delivery of BB and increase its topical bioavailability *vis-à-vis* the conventional product.

Skin Compliance Studies

Photomicrographs of the skin samples treated with ME hydrogel showed the absence of any marked changes in the normal histology vouching better tolerability of the ME-based formulation as depicted in Fig. 8a-c. This may

be attributed to the encasement of the drug within the biocompatible components *viz.*, phospholipid and surfactant; however, the mice skin treated with the conventional cream showed marked irritation such as infiltration of inflammatory cells, epidermis thickening, and peeling of the epidermis from the dermis which is considered evidence of irritation, thus indicating its non-compliant nature.

STABILITY STUDIES

The drug content (Table VI) and physical attributes (Table VII) of optimized formulation were studied at 4

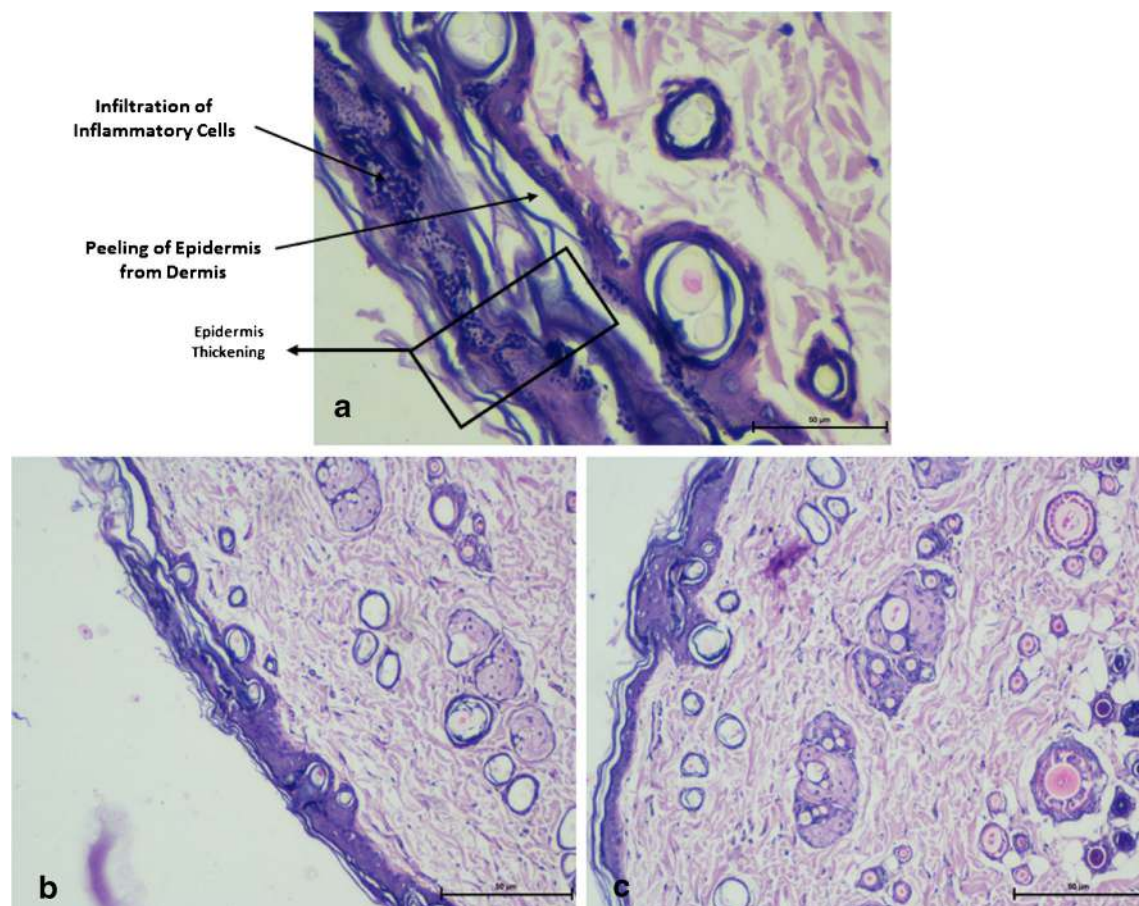


Fig. 8. Microphotographs of skin sections treated with **a** conventional cream, **b** ME hydrogel, and **c** saline solution (control)

Table VI. Drug Content of Optimized Formulation at Various Temperatures ($n=3$)

S. Number	Days	Percent drug content remaining		
		4±2°C	30±2°C	45°C±2°C
1	1	99.51±0.009	99.51±0.005	99.51±0.008
2	7	99.24±0.010	99.34±0.007	99.32±0.004
3	14	99.01±0.007	99.16±0.009	99.27±0.006
4	28	98.76±0.011	98.76±0.010	99.13±0.007
5	35	98.45±0.010	98.59±0.011	98.09±0.010
6	45	98.41±0.009	98.52±0.010	98.97±0.011
Net loss	1.10±0.009	0.99±0.023	0.54±0.007	

±2, 30±2, and 45±2°C for a period of at least 8 weeks. After every 7 days, percentage drug content was determined as per the method described in drug content studies of preliminary microemulsion formulations. The mentioned results indicate that the optimized formulation was stable at all the temperatures for 45 days; however, at 30±2 and 45±2°C, the microemulsion was found to be stable for more than 45 days. These results indicate that the optimized microemulsions need not to be refrigerated for enhancing its shelf-life and can be stored at room temperature.

Conclusions

In the present study, it may be stated the nanocarrier-like microemulsion-based hydrogel has immense potential to combat the problem related to the drug. In this study, this nanocarrier has appeared as one of the best biocompatible topical carrier, with noticeable threefold higher percutaneous permeation of benzyl benzoate through the stratum corneum and enhanced passive skin-targeting characteristics with respect to the conventional formulation. Furthermore, the microemulsion-based hydrogel presented the excellent ability of slow release and no irritation, which provides a probability for clinical application. Dermatokinetic modeling revealed the presence of sufficient concentration of drugs in the dermis layer of the skin. In a nutshell, the present studies corroborated that the developed nano-sized microemulsion-based hydrogel system can serve as efficient promoter of benzyl benzoate by increasing its availability at the site of action

Table VII. Physical Attributes of the Optimized Formulation

S. Number	Days	Physical stability		
		4±2°C	30±2°C	45°C±2°C
1	1	√	√	√
2	7	√	√	√
3	14	√	√	√
4	28	√	√	√
5	35	√	√	√
6	45	√	√	√
Net result	>45 days	>45 days	>45 days	

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors confirm that this article's content has no conflicts of interest.

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