Exploring Transdermal Drug Delivery of Buspirone through Microemulsions in Conjugation with Microneedles

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Abstract

The objective of the present studies was to develop and evaluate buspirone microemulsion in conjugation with microneedle pre-treatment for transdermal delivery as an alternate to oral delivery. Transdermal delivery was investigated to increase permeation of Buspirone, increase residence time in skin and to provide sustained drug release from a non-irritating dosage form. Microemulsions were formulated using safe excipients by phase titration method and evaluated for globule size, viscosity, pH, conductivity, refractive index and time for 80% drug release, ex-vivo permeation and retention study with and without microneedle pre-treatment. W/O microemulsion of buspirone, displayed nano sized globules with adequate zeta potential for stability and was able to sustain the drug release for 24 h. W/O microemulsion of buspirone when applied after microneedle pre-treatment was found to be significantly better as compared to topical solution with and without microneedle pre-treatment as evidenced by the skin permeation and retention studies as well as fluorescent microscopy. The favourable results point out to a plausibility of using transdermal delivery as an alternate route with possible dose reduction of the drug.

Keywords: Buspirone, microemulsion, microneedle pre-treatment, anxiety, transdermal drug delivery

Introduction

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed, it is a universal human emotion, closely allied with appropriate fear and presumably serving psychobiologically adaptive purposes [1]. Buspirone hydrochloride (BUH) from azapirone class and a centrally acting anxiolytic agent is primarily used for treatment of general anxiety disorder. At post synaptic seratonergic receptors in raphe nucleus, it acts as partial agonist and at pre-synaptic seratonergic receptors as agonist in brain hippocampalregion [2]. It is as potent as the benzodiazepines, but does not produce the sedation or motor impairment effect [3]. Extensive hepatic first pass metabolism in liver and intestine by cytochrome p3A4 is reported after oral administration resulting in very low bioavailability (about 5%) [4].

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Transdermal drug delivery (TDD) is a safe, potential and highly promising mode of drug delivery to avoid first pass metabolism. TDD is the non-invasive delivery of medications to the circulatory system from the surface of skin-the largest and most accessible organ of human body-through its layers. In a transdermal system, the major two challenging goals are the maintenance of the desired constant drug concentration at the skin surface for a suitable length of time and ensuring drug permeation so that adequate drug can reach the systemic circulation. Controlled drug deposition within targeted skin layers can be achieved by modulation of drug formulations and its delivery modalities [5].

Microemulsions are an excellent delivery vector for both hydrophilic and lipophillic drugs. They offer numerous advantages such as increased drug solubility, increased bioavailability, controlled drug release. They enhance the transdermal drug delivery by increased dermal accumulation of the drug [6,7]. Microneedles are also a promising

modality for transdermal drug delivery. They are convenient, painless, and less invasive alternative to injection & can be used for administering drugs, large proteins and peptides, antibiotics, vaccines with low manufacturing cost [8,9]. In contrast to oral delivery, microneedles avoid first pass effect and offer the benefit of immediate cessation of drug administration in case of an adverse effect or overdose. There is also no molecular size limitation, no molecular electrical charge requirement, and no specific formulation pH constraint. These two modalities can also be used in conjugation to optimize the drug delivery [10, 11]. Microneedle pre-treatment was reported to enhance the percutaneous permeation of hydrophilic compounds [12]. Solid microneedles can increase the permeability of a drug formulation by creating micro-holes across the skin. Commercially solid microneedles are available as Dermaroller®. They are arrays of steel or titanium microneedle routinely used by cosmetologists and dermatologists. The micropores created by the application of microneedles repairing and resealing was apparent at 8–24 h post application [13].

Researchers have investigated transdermal delivery of buspirone but its hydrophilicity is the reason for limited transdermal permeation. Studies have investigated matrix-type transdermal formulation to enhance the bioavailability and improve the patient compliance as well as reservoir-based transdermal therapeutic system (TTS) for buspirone [14,15]. Buspirone could be delivered transdermally with success but a quick onset of action cannot be achieved with such systems. The transdermal delivery of buspirone hydrochloride was investigated across hairless mouse skin with the combined use of iontophoresis and terpene permeation enhancers [16]. The iontophoretic delivery required an instrumental setup, has its own limitations and cannot be method of choice for prolonged use. One of the studies explored the microemulsion formulation of buspirone and revealed that components of the formulation influenced the drug permeation significantly. However, it again failed to show a fast onset of action and displayed a significant lag time [17].

With this background, we envisaged the microneedle assisted transdermal delivery of buspirone-loaded microemulsion. It is hypothesized that a dual enhancement in systemic availability of buspirone is expected via microneedle pre-treatment and followed by w/o microemulsion application. Buspirone is expected to reach the systemic circulation steadily with no loss of drug by hepatic first pass effect. It also presents a possibility of dose reduction of drug and thus minimizing side effects.

Material and Methods

Materials

Buspirone hydrochloride was gift samples from Hangzhou Pharma&Chem Co., Ltd. China. Isopropyl Myristate, Tween 80, Propylene Glycol, PEG 200 were purchased from Chemdyes corporation, Rajkot, India. Span 80 was purchased from Suvidhanath laboratories, Baroda, India. Transcutol was purchased from Ozone international, Mumbai, India. Dermaroller (DNS 0.5mm 192 needles) purchased from Biogenesis, Mumbai , India.

Analytical Method

The detection and quantification of buspirone was done with a modified high-performance liquid chromatography (HPLC) method. The analysis was performed at room temperature on a reverse-phase C18 Hypersil C-18 column (150×4.5 mm i.d., 5 μm) with UV detection at 240 nm. The mobile phase used was acetonitrile:methanol (65:35) isocratic at a constant flow rate of 1.0 mL/min [18].

Preparation of microemulsion - Screening of ratio of surfactant to co-surfactant

Oils, surfactants and cosurfactants were selected on the basis of preliminary studies. Tween 80, Span 80, Transcutol P and Isopropyl myristate were selected for the formulation development. The ratio

of surfactant to cosurfactant was optimized by pseudoternary phase diagrams. Samples containing different weight ratios of oil: Smix (1:1, 2:1, 1:2) were initially prepared. Phase studies were carried out by adding aqueous phase to the mixture while stirring. After each successive addition of aqueous phase, resulting system was examined for clarity and transparency. The endpoint of microemulsion domain at a given ratio was determined when the system became turbid after addition of aqueous phase. The phase behaviour of the system was mapped on phase diagrams with the apices representing water, oil and Smix using chemix software. The transparent and homogenous area enclosed by the line connecting the endpoints was considered as the microemulsion domain. The pseudo ternary phase diagram showing maximum microemulsion region was taken as the criteria for selection. The drug loading was kept constant in all the batches at 10mg/gm [19].

Optimization of formulation

A Circumscribed Central Composite design was employed to optimize microemulsion formulation. Design-Expert version 10 software (Stat-Ease Inc., Minneapolis, MN) was used for generation of central composite design matrix, statistical analysis of data and optimization of microemulsion based on desirability criteria. The design was employed to study the effect of independent variables, i.e. concentration of water (X1) and concentration of Smix (X2) on dependent variables globule size (Y1), viscosity (Y2) and time for 80% drug release (Y3). The process variables were kept at their optimal levels during the preparation of formulation. A statistical model incorporating interactive and polynomial terms was utilized to evaluate responses.

$$Y = b0 + b1X1 + b2X2 + b11X11 + b22X22 + b12X1X2$$

Where Y is the dependent variable, b0 is the arithmetic mean response of the thirteen runs, b1 and b2 are the estimated coefficients for the factors X1 and X2 respectively. Analysis of variance (ANOVA) was used to ensure the significance of experimental model. Response surface plots showing the effect of independent variables on dependent variables were generated and the optimized batch was selected from the design space [20].

Characterization of microemulsion

The pH of the formulated microemulsion was measured using a calibrated pH meter (Welltronix, PM100). Rheological studies were performed with a temperature controlled Brookfield rheometer using spindle no. 96 at 25°C. Percent transmittance of the developed microemulsion at 630 nm was measured using UV visible spectrophotometer (UV-Vis, 1700, Shimadzu, Japan) with distilled water as reference. Refractive index of the w/o microemulsion was assessed using an Abbe type thermostated refractometer. The microstructure of the microemulsion was confirmed by conductivity measurement.

The size and zeta potential determination were performed using photon correlation spectroscopy with in built Zetasizer (Model: Nano ZS, Malvern Instruments,Worcetershire, UK) at 633 nm. Helium-neon gas laser having intensity of 4 mW was the light source. The equipment was programmed to provide 18 mm laser width. Mean value of triplicate measurements was considered. Electrophoretic mobility (mm/s) was measured using small volume disposable zeta cell and converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation [21].

In-vitro diffusion study

In vitro drug release studies were carried out in a two-compartment Franz diffusion cell. The donor compartment contains the formulation equivalent to 10.0 mg of buspirone; the drug diffuses into the receptor phase (20ml of phosphate buffer pH 7.4) through a semipermeable cellulose acetate membrane (himedia LA401, molecular weight cut off – 12000 to 14000 daltons) previously activated.

The apparatus was kept under thermostatic conditions at 32°C and under constant slow-speed stirring. At predefined time points, aliquots were withdrawn from the receptor compartment for analysis of drug content and replaced by an equivalent receptor solution [22].

Ex – Vivo Permeation and Retention Study

Rats (Wistar strain) 6–8 weeks old weighing 120–150 g were humanely killed by ether inhalation. The study was conducted on depilated full thickness abdominal rat skin. The skin sample was rinsed with phosphate buffer saline multiple times and clamped between the donor and receptor chamber of vertical Franz diffusion cell with stratum corneum on upper side and dermal side flushing to the receptor media. The skin was allowed to stabilize with receptor media for 0.5 h. The effective diffusion area was 2.8 cm2. The receptor chamber was filled with freshly prepared phosphate buffer pH 7.4. The diffusion cell was maintained at 32˚C using a re-circulating water bath and the solution in the receptor chamber was stirred continuously at slow speed. The formulation equivalent to 1.0mg of buspirone was gently placed in the donor compartment. In a subset of such experiments, pretreatment with microneedles was given wherein the Dermaroller (0.5 mm titanium microneedle roller array) was rolled over the entire region before formulation application. At suitable time intervals, aliquots of the solution was removed from receptor compartment and replaced immediately with an equal portion of fresh PBS. The samples were analysed by HPLC. Ex-vivo permeation and retention study was performed with and without microneedle pre-treatment [22].

Fluorescent Microscopy

To visualize the penetration of buspirone loaded microemulsion into the skin tissues, formulation containing fluorescent dye, rhodamine B was prepared replacing the drug with fluorescent marker. Formulation containing fluorescent dye was applied on the back of wistar rat and humanely sacrificed after 2 h. In another animal, the microneedle pretreatment was given by rolling over Dermaroller (0.5 mm titanium microneedle roller array) over the back of the animal. The excised skin was cryodermatomed at -20˚C and sections were cut and observed under with an Olympus fluorescence microscope (BX51, Japan) at exposure of 10S [22].

Results and Discussion

Isopropyl myristate was selected as the oil phase for the microemulsion development because it has been extensively explored for transdermal drug delivery with excellent permeation capabilities and is well tolerated [17]. Tween 80 and span 80 in ratio 1:2 were chosen as combination of surfactants as mixed surfactants yield more stable emulsions due to formation of a complex interfacial barrier. Transcutol P was selected as co-surfactant for its amphiphillic nature, excellent permeation enhancement capabilities and exhibits good solubility for the drug as well. The pseudo – ternary phase diagrams are an efficient platform to optimize the component levels in microemulsions which will yield a stable microemulsion. From the pseudo – ternary phase diagrams it was observed that all the ratios of surfactant and co-surfactant in form W/O microemulsion easily. The surfactant: co-surfactant in 2:1 ratio showed formation of stable W/O microemulsion with maximum microemulsion region. Hence 2:1 ratio of surfactant: co-surfactant was regarded as optimum surfactant to co-surfactant ratio for further optimization studies. The conductivity studies confirmed the formation of w/o microemulsion [7].

A central composite circumscribed design was used for the optimization of microemulsion formulation with respect to key performance characteristics. The water content and surfactant content were chosen as independent variables because earlier studies have also reflected about the choice of microemulsion components and HLB values as significant in transdermal delivery of buspirone [17]. The key performance characteristics which were chosen as response variables were emulsion globule size, viscosity and time for 80% drug release. The globule size of

microemulsion is a critical parameter as it determines not only its stability but also influences its permeation enhancement. It has been reported that the nanometric size of globules of microemulsion helps it to easily blend with lipid mantle of the cell membrane and ensure intracellular drug transport. The microemulsion components also act as fluidizers for epithelial membranes to enhance the translocation of the drug [6]. Microemulsions are having intermediate viscosity; they are neither too fluid nor too viscous as semisolids. Viscosity is one major factor impeding the drug partitioning across the phases to ultimately reach the deeper skin layers. Hence, an optimum viscosity is required for the formulation to be retained at the site of application at the same time ensuring that formulation is not a barrier to drug release. The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behaviour. The table 2 shows the experimentally determined response variables of the optimization batches. This design elucidates the main effects and interaction effects of the independent variables on the dependent variables. Fig 2, 3 and 4 are the response surface plots generated for the dependent variables.

The variables that showed a significant effect on (p-value < 0.05) on globule size of microemulsion were both the water content and Smix content. The results indicate that as the water content is raised the gobule size is increased and with increasing Smix concentration the globule size becomes finer. Higher surfactant levels reduce the interfacial tension and result in finer emulsion [6]. The effect of Smix was more pronounced on viscosity. The smix content in the microemulsion is significantly larger as compared to coarse emulsions and the surfactants that have been used are highly viscous. Hence, the variation in surfactant content influences the viscosity significantly in comparison to water content, which is present as the dispersed phase. Similarly, it was seen that effect of Smix dictated the time for drug release. The water content had an insignificant influence on drug release. As the surfactants in the microemulsion increase, they help the drug to partition between the phases more efficiently and got dissolved in the release media [17, 23]. The following reduced polynomial equations were generated for the response variables based on the significance (Table 3).

Size = + 95.0 + 344.6*X1 – 265.2*X2 – 358.2*X1X2 + 215.5*X12

Viscosity = + 256.0 + 6.8*X1 + 57.3*X2 + 10.8*X22

Time for 80% release = + 97.0 – 21.0*X2 – 8.7*X1X2

Based on defined constraints for each independent variable, the Design Expert software generated a design space and suggested formulations with maximum desirability. Optimization was done based on the desirability, which focused on minimizing globule size and time for drug release, and maximizing viscosity. The overlay plot depicts the design space for the desired characteristics of formulation.

An optimized solution (WO1) was generated from the design space which was expected to have desirability closest to 1. The composition of the formulation and its characterization are shown in table 4.

The optimized formulation (WO1) was subjected to various evaluation parameters which showed fine globule size, optimum viscosity, pH similar to skin, minimal conductivity in nature due to presence of water in minor phase. Refractive index showed uniformity in microemulsion structure. % Transmittance indicated microemulsion was clear and transparent. Time for 80% drug release showed formulation does not provide any hindrance to drug release. The time taken for 80% drug release was close to 1 hour.

The Ex-vivo study of the buspirone solution and optimized batch (WO1) without microneedle pre-treatment and with microneedle pre-treatment were carried out for 24 hours. The % of drug in receptor medium at 6hrs and 24hrs and drug retained in skin after 24 hrs is given in table 5. The aqueous drug solution and WO1 applied topically showed 20% and 41.5% drug translocation (receptor phase + retained in skin) respectively after 24 hours. The microneedle pretreatment increased the dermal translocation to 29.35% and 62.10% in a course of 24 hours.

The optimized formulation WO1 showed higher drug permeation and retention as compared to aqueous drug solution. The increase observed was almost two fold. Microneedle pre-treatment significantly increased drug permeation and retention for all formulations. The enhanced permeation as observed with microemulsion in comparison to aqueous solution was around two fold. The skin deposition when compared after microneedle pretreatment was increased to around 3 fold in comparison to aquesous solution [24]. The mass balance studies conducted showed not more than 6% drug loss confirming the validity of the studies.

The study showed higher flux over 24 hrs in WO1 after microneedle pre-treatment as compared to drug solution without and after microneedle pre-treatment through excised rat skin (table 6). The results clearly indicate that microneedle pretreatment created micropores or microchannels through which a hydrophilic molecule could be translocated across the dermal barrier with ease and reach the systemic circulation [12]. The statistical analysis between the various treatment modalities were explored by multiple comparisons and the significance values markedly advocate the superior results of buspirone microemulsion in conjugation with microneedle pretreatment (table 7).

In order to compare the skin penetration ability of microemulsion with and without microneedle pre-treatment, fluorescent microscopy was carried out. The images showed higher and deeper fluorescence intensity in WO1 applied after microneedle pre-treatment in skin as compared to microemulsion applied without microneedle pre-treatment of skin. Thus, evidencing that the skin penetration of microemulsion (WO1) applied after microneedle pre-treatment in skin is superior to microemulsion applied without microneedle pre-treatment of skin [11]. The micropores are also visible with higher fluorescent densities in localized regions. The fluorescent marker studies in the animal model affirmed the earlier results of exvivo skin permeation and retention studies.

Table 1: Central Composite Design Variables

Independent variables	***Variable levels***			***Dependent Variables***
	Low Level (-1)	**Central Level (0)**	**High Level (+1)**	globule size (Y_1)
% Water (X_1)	3	6	9	viscosity (Y_2)
% Smix (X_2)	30	40	50	time for 80% drug release (Y_3)

Table 2: Randomized central composite design matrix with experimentally determined response variables.

Central Composite Design Matrix (Coded Values) and Response Variables for Microemulsion					
Runs	% Water (X_1)	% Smix (X_2)	Globule size (nm)	Viscosity (cps)	Time for 80% drug release (min)
T1	0	0	97	202	110
T2	0	1.41421	95	256	97
T3	1	-1	39	368	78
T4	1	1	95	256	97
T5	0	0	56	308	75
T6	-1	-1	20	248	105
T7	-1	1	35	323	65
T8	0	-1.414	95	256	97
T9	0	0	1509	215	135
T10	-1.41421	0	468	195	123
T11	0	0	986	267	85
T12	0	0	95	256	97
T13	1.41421	0	95	256	97

Table 3: Summary of results of multiple regression analysis for response Y1, Y2 and Y3

Dependent Variable	*globule size (Y_1)*		*viscosity (Y_2)*		*time for 80% drug release (Y_3)*	
	P value	Coefficient	P value	Coefficient	P value	Coefficient
Intercept	-	95.0	-	256.0		97.0
X_1	0.0001	344.6	0.0117	6.8	0.5373	– 1.6
X_2	0.0005	– 265.2	< 0.0001	57.3	< 0.0001	– 21.0
$X_1 X_2$	0.0007	– 358.2	0.8665	0.5	0.0406	– 8.7
X_1^2	0.0025	215.5	0.6208	– 1.1	-	-
X_2^2	0.0946	90.7	0.0016	10.8	-	-

Table 4: Composition and characterization of optimized w/o microemulsion (WO_1) of buspirone

Composition of Optimized Formulation (WO_1)	
Ingredients	**Quantity (gm)**
Buspirone (gm)	0.1
Water (gm)	0.8
Tween 80 (gm)	1.11
Span 80 (gm)	2.22
Transcutol (gm)	1.66
IPM (gm)	q.s up to 10.0 gm
Characterization	
Globule size (nm) (PDI)	130.2 (0.360)
Zeta potential (mV)	-13.4
Viscosity (cps)	320.15 ± 10.84
pH	6.9
Conductivity (ohm)	3.4
Refractive index	4.8
%Transmittance	98.94 ± 1.98
Time for 80% drug release (min)	70

Table 5: *Ex-Vivo* Permeation and Skin Retention Studies of Buspirone Formulation

Formulation	***% Drug in Receptor Medium***		***% Drug Retained Into The Skin***	***% Drug Unabsorbed***	***% Drug Loss***
Time (hrs)	**6**	**24**	**24**	**After 24hrs**	
WO_1 without microneedle pre-treatment	7.58	14.22	27.26	55.42	3.1
WO_1 after microneedle pre-treatment	16.11	20.98	41.12	35.94	1.96
Aqueous Drug Solution (10mg/ml) without microneedle pre-treatment	3.52	5.85	15.05	75.45	5.65
Aqueous Drug solution (10mg/ml) after microneedle pre-treatment	5.48	9.39	19.94	68.51	6.16

Table 6: Steady State Flux and Permeation Coefficient after 24 Hrs

Formulation	***Flux over 24 Hrs ($\mu g/cm^2 .h$)***	***Permeation Coefficient (cm/h)***
WO_1 without microneedle pre-treatment	50.07	2.50
WO_1 after microneedle pre-treatment	72.86	3.64
Drug solution without microneedle pre-treatment	20.32	1.01
Drug solution after microneedle pre-treatment	32.62	1.63

Table 7: ANOVA of Skin Permeation and Retention Study

Tukey's multiple comparisons test	***Summary of significance***	***Adjusted P Value***
Topical Sol vs. Topical sol + MN	ns	0.3625
WO_1 vs. WO_1 + MN	*	0.0132
WO_1 vs. Topical Sol	*	0.0134
WO_1 vs. Topical sol + MN	ns	0.1256
WO_1 + MN vs. Topical Sol	***	0.0003
WO_1 + MN vs. Topical sol + MN	**	0.0012

*- Significant difference between treatment ($p<0.005$), ns- No significant difference between treatments

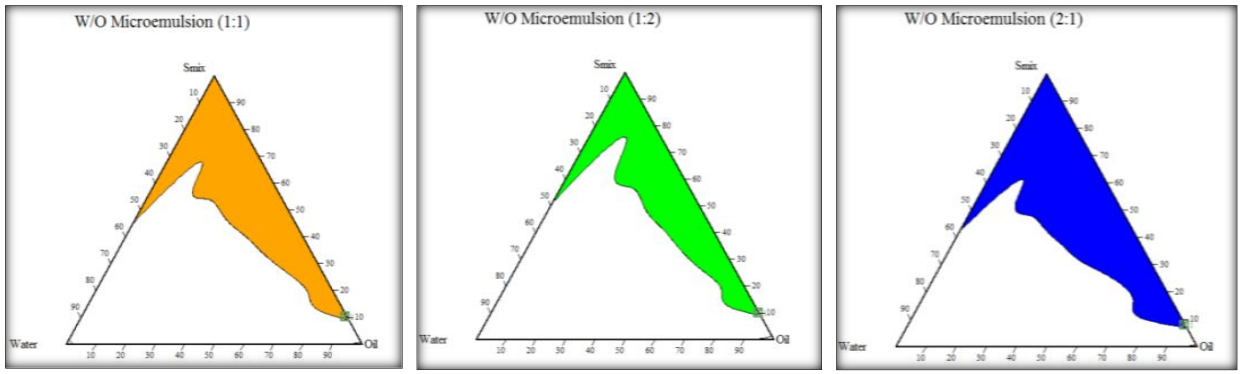

Figure 1. Pseudoternary phase diagrams for optimization of surfactant to –cosurfactant ratio.

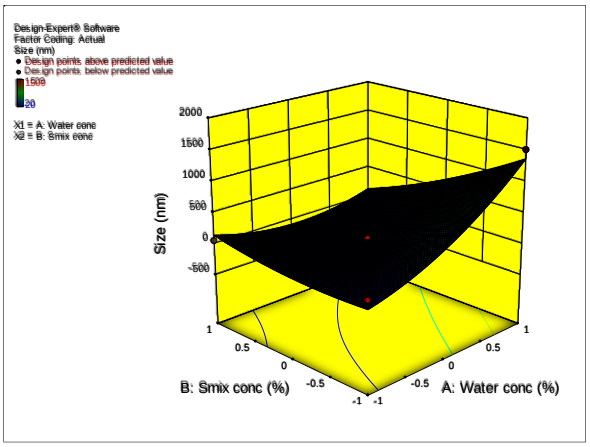

Figure 2. Response Surface Plot for globule size

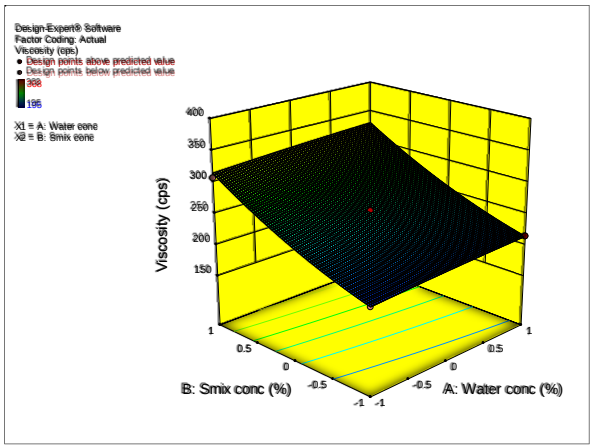

Figure 3. Response Surface Plot for viscosity

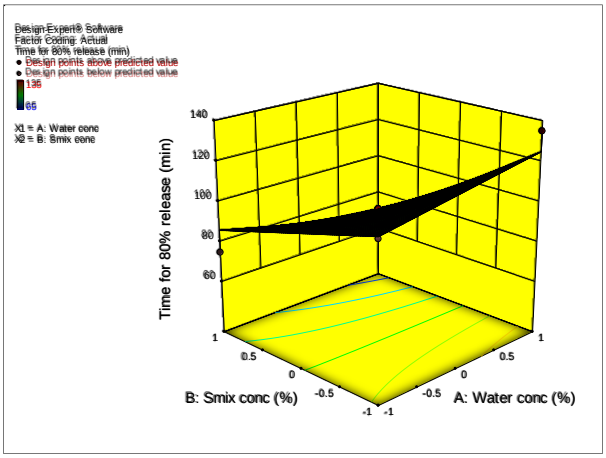

Figure 4. Response Surface Plot for time for 80% drug release

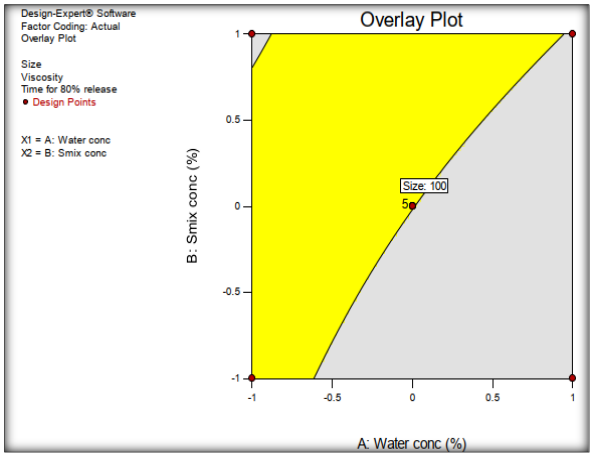

Figure 5. Overlay plot

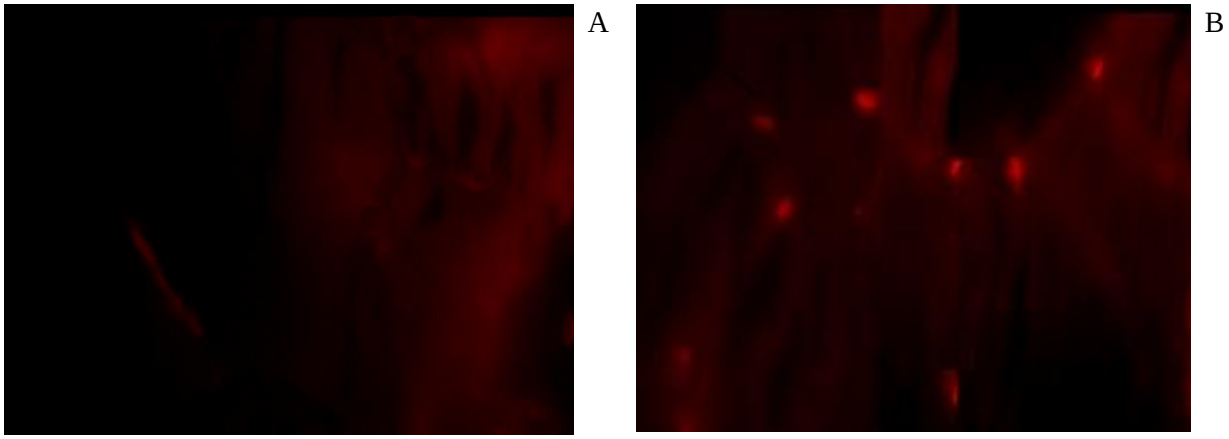

Figure 6. Fluorescent Microscopy of Rat Skin After Treatment With W/O Microemulsion (A) Without Microneedle Pre-Treatment (B) After Microneedle Pre-Treatment

Conclusion

The preliminary developmental studies of water in oil microemulsion revealed a possibility of transdermal delivery of hydrophilic buspisrone. However, in order to achieve a quick onset of action and ensure steady diffusion of the drug over a prolonged period of time, preatreatment with microneedles was done in conjugation. This presents an excellent opportunity for exploring transdermal delivery as an alternative route because it ensures a quick onset of action as well as steady release over a prolonged period of time as drug is deposited into the dermal layers of the skin. Since, buspirone was a substrate for hepatic metabolism and had a very low bioavailability because of first pass metabolism. This could translate to dose reduction, better safety profile, higher patient compliance and perhaps a more cost effective treatment modality.

Conflict of Interest

The authors declare no conflict of interest.

Disclaimer

The views, thoughts and opinions expressed in this review belong solely to the authors, and not necessarily to the author's employer, organization, committee or other group or individual.

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