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Original Article

FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF IBUPROFEN LOADED MICROEMULSION SYSTEM USING D-OPTIMAL MIXTURE DESIGN

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ABSTRACT

Objective: The purpose of this study was to develop, optimize and characterize a stable microemulsion, with an improvement of the solubility of a poorly aqueous soluble drug, ibuprofen.

Methods: Various oils (oleic acid, cottonseed oil, olive oil, argan oil, and labrafac® WL 1349), surfactants (tween® 80, tween® 40, tween® 20) and co-surfactants including polyethylene glycol 400, ethanol, 1-butanol, and propylene glycol were selected after solubility studies. Then, pseudo-ternary phase diagrams with surfactant/co-surfactant ratio of 1:2, 1:1, 2:1 and 3:1 were constructed and a D-optimal mixture design method was used to optimize the ibuprofen loaded microemulsion. The optimized microemulsion was evaluated for several characteristics including globule size, zeta potential, pH, conductivity, refractive index and stability studies.

Results: Optimized microemulsion obtained was composed of oleic acid (6.88% w/w), tween® 80/1-butanol (3:1, 63.11% w/w) and water (30.00% w/w). The results obtained showed an average globule size of 117.5 nm, a zeta potential of-6.47 mV and a transmittance of 96.95±0.77%. The optimized formulation showed an improvement in the solubility of ibuprofen with unchanged characteristics for one month.

Conclusion: The use of pseudo-ternary phase diagrams and mathematical modeling allows to obtain an optimal microemulsion with perfect stability for 1 mo and a better solubilization capacity of ibuprofen.

Keywords: Microemulsion, Ibuprofen, Mixture design, Surfactant, Co-surfactant

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INTRODUCTION

Ibuprofen, a phenyl propionic acid derivative, is one of the best tolerated non-steroidal anti-inflammatory drugs to treat pain caused by headaches, toothaches, back pain, rheumatoid arthritis and slight injuries [1, 2]. However, ibuprofen has several disadvantages following oral administration, including gastrointestinal irritation, dizziness, headache, peptic ulcer and shows low gastrointestinal absorption due to its low solubility and dissolution rate in water [3]. To reduce these side effects, various dermal dosage forms of ibuprofen, such as patch [4] and gels [5], have been studied.

Recently, many microemulsions have been developed as a vehicle for transdermal delivery of active ingredients. They have also been used to increase the absorption of ketoprofen [6], piroxicam [7], apomorphine [8], celecoxib [9], clotrimazole [10] and lidocaine [11]. Indeed, the microemulsion is a recent colloidal system that becomes a promising vehicle for topical administration and bioavailability enhancer for poorly water-soluble active pharmaceutical ingredients [12-14]. Therefore, a microemulsion-based system may be a new beneficial approach for the topical application of ibuprofen.

Microemulsions are homogeneous and thermodynamically stable mixtures of two immiscible phases such as oil and water. They are translucent and stabilized by an interfacial film composed of conjugated surfactant and co-surfactant. The droplet size varies between 20 and 200 nm [15].

These systems have several advantages such as spontaneous formation, reduction of therapeutic dosage and consequent side effects, enhanced drug solubility, controlled release of the active ingredient, and good thermodynamic stability [16-18]. They have been widely studied as drug delivery approach for a wide range of active ingredients because the formulation contains a higher concentration of drug due to the high solubilizing capacity associated with high thermodynamic activity towards the skin [19, 20].

In this work, an attempt has been made to construct a microemulsion for a poorly water-soluble drug, ibuprofen. After examining the appropriate formulation excipients, we selected a D-optimal mixture design to statistically optimize the components of the ibuprofen-loaded microemulsion system.

Based on solubility studies, pseudo-ternary phase diagrams were constructed with different surfactant/co-surfactant ratios. The resulting formulation was optimized by experimental design and characterized for various physicochemical properties.

MATERIALS AND METHODS

Reagents

Ibuprofen ($C_{13}H_{18}O_2$) as a model drug was donated by Pharma5 Pharmaceutical Company (Morocco). Labrasol® (Caprylocaproyl polyoxylglycerides) and labrafac® lipophile WL 1349 (propylene glycol dicaprylocaprate) were received as gift samples from Gattefosse (SAS, France). Tween® 20 (Polyoxyethylene sorbitan monolaurate), tween® 40 (Polyoxyethylene sorbitan monopalmitate), tween® 80 (Polyoxyethylenesorbitan monooleate), polyethylene glycol with an average molecular weight of 400 (PEG 400), propylene glycol (PG), cottonseed oil, methanol and 1-butanol were procured from Sigma-Aldrich GmbH (Germany). Oleic acid was purchased from Fluka Chemie AG (Switzerland). A certified high-purity Moroccan argan oil (Argania spinosa L.) and olive oil, were selected for the study and purchased from SOMAPROL Company (Morocco). Ethanol 96% w/w was purchased from Prolabo (French). Freshly distilled and filtered water was used throughout the study.

Selection of microemulsion components

The selection of the oil phase was based on the maximum solubility of ibuprofen. Several naturals or synthetics oils such as oleic acid, cottonseed oil, olive oil, argan oil and labrafac® WL 1349 were used for solubility studies.

Surfactants are chosen according to their HLB value, the maximum solubility of ibuprofen and the absence of toxicity. Several surfactants including tween® 80, tween® 40, tween® 20 and labrasol® were tested.

Co-surfactants were selected for their ability to facilitate microemulsions formation under a minimum of surfactants and for their solubilizing tendency. Several co-surfactants including polyethylene glycol 400 (PEG 400), alcohol (ethanol and 1-butanol) and propylene glycol (PG) were screened.

Solubility studies in the various components (oils, surfactants, co-surfactants) were determined by adding an excess amount of ibuprofen to conical flasks containing 5 ml of each vehicle. The resulting mixture was placed for 24h in shaking water bath assembly (GFL1083, Germany) maintained at constant temperature (25 °C). Then, each sample was centrifuged for 30 min at 12 000 rpm (industria epf12, Argentina) to remove the undissolved drug. The supernatant was diluted with methanol and ibuprofen content was quantified by UV spectrophotometer (Shimadzu UV 2450; Japan) at a wavelength of 220 nm [21]. Blank solutions were prepared by diluting each ingredient in methanol and the interference was canceled out from the absorbance of the solubility test. The spectrophotometric method was specific and linear in the range between 10 and 80 µg/g (R^2=0.989). Repeatability was determined from six analysis by 20 µg/g ibuprofen solution in phosphate buffer (pH 7.2). The relative standard deviation obtained was below 5%.

Construction of pseudo-ternary phase diagrams

As described in previous studies [22, 23], phase diagrams were constructed by titration method to obtain appropriate components and their concentration ranges allowing a large microemulsion surface.

The surfactant was mixed with co-surfactant in fixed weight ratios (1:2, 1:1, 2:1 and 3:1). Aliquots of each surfactant and co-surfactant mixture (Smix) were then mixed with oil at room temperature. For each phase diagram, the ratios of oil to Smix were varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Then, 100 µl of distilled water was added at room temperature to each mixture. The amount of water is varied to provide a water concentration in the range of 9 to 90% w/w. The samples were vigorously mixed with a vortex shaker for 2 min and kept at room temperature for 24 h to reach equilibrium. The macroscopic changes were noted.

The microemulsion domain was determined by visual observation of two parameters: fluidity and clarity. No test was made to recognize the microemulsion structures between oil in water (O/W), water in oil (W/O), or bicontinuous type microemulsions.

After equilibration for 24 h, the mixtures were assessed macroscopically and determined as being microemulsions, liquid coarse emulsions, gels, and phase separation. Gels (transparent i.e. microgels (MG) or opaque i.e. emulgels (EG)) were claimed for those highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90°.

According to these diagrams, selected oil, surfactant and co-surfactant were used for the preparation of Ibuprofen loaded microemulsions.

Preparation of Ibuprofen microemulsions

The appropriate oil, water and Smix weight ratios used in the microemulsions were chosen from the constructed phase diagrams. According to the Smix ratios used, the existence areas were calculated.

Ibuprofen representing 5 % (w/w) of total formulation weight was added to the oily phase, composed of the chosen oil and Smix, and vortexed together until the drug was completely dissolved. Water was added dropwise with continuous mixing. This mixture was kept for 24 h at 25 °C, in a shaking incubator, to attain equilibrium.

Thermodynamic stability studies

After microemulsion region identification in the phase diagram, appropriate ibuprofen microemulsion formulations (F1 to F17) were selected for thermodynamic stability test.

The selected formulations were centrifuged at 6000 rpm for 30 min. Several tests are carried out on the stable formulations after centrifugation, including appearance, pH and refractive index. Three complete cycles between the temperatures 2 °C (in a refrigerator) and 50 °C (in a hot air oven) with storage at each temperature for not less than 48 h, were done. The stable formulations at these temperatures were selected for further studies.

Optimization of ibuprofen microemulsion by D-optimal experimental design

After determination of the concentration range of various components leading to stable microemulsion formulations, a D-optimal design was applied for optimization of the final formulation.

A D-optimal mixture design [24] with 3 independent variables (percent content of oil, surfactant/co-surfactant mixture, and water) was used for carrying out the optimization study.

The experimental matrix was constructed with 19 experimental trials including 8 model points, 5 replicates, 5 lack of fit and one center analysis point. The drug content in every batch was kept constant at 5 % (w/w). The responses studied were globule size and transmittance.

Design Expert® software (Stat-Ease Inc., Minneapolis, USA) was used for design construction and making interpretations by fitting suitable mathematical polynomial equations.

The statistical study for the chosen model involves the calculation of adequate precision (signal-to-noise ratio). It compares the range of predicted values at design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination. For the model used to be able to fit the data and can reliably be used to interpolate, the adjusted R-squared and predicted R-squared should be within approximately 0.20 of each other. All the responses observed for 19 runs were fitted to various models using response surface methodology. The ANOVA test was conducted to identify the p-values and to determine if the model explains a significant portion of the variance.

The contour plots and response surfaces were generated from the software. Confirmation of the model is done with 3 other random points to validate that the model can predict actual outcomes at optimal settings determined from the analysis. The optimized microemulsion was achieved by converting each response into an individual desirability function. The target was to formulate an adequate microemulsion in order to maximize stability with reduced globule size.

Characterization of the drug-loaded microemulsion

Microemulsions aspect

The homogeneity, clarity and optical transparency of drug-loaded microemulsions were studied by visual examination on a black background at room temperature.

Globule size and zeta potential determination

Globule size, polydispersity index, and Zeta potential were determined at 25 °C, by dynamic light scattering analyses using Zetasizer 3000HS (Malvern Instruments, France).

pH

The pH of the various formulations was measured at room temperature (25 °C) with a glass electrode pH meter (Bante 920, Bante Instruments L. China). The pH meter was calibrated before each use with a buffer solution of pH 4.0, 7.0, and 9.0.

Transmittance test

The percent transmittance of microemulsions was determined using UV spectrophotometer (Shimadzu UV 2450; Japan) at a wavelength of 650 nm using distilled water as the reference [25].

Refractive index

Stability tests of ibuprofen microemulsions were evaluated by the refractive index. For that, one drop of Microemulsion was placed on a slide and refractive indices were measured by using Abbe refractometer BK-R2S (Biobase, China).

Electrical conductivity

The electrical conductivity of the optimized microemulsion was determined simultaneously by Malvern Zetasizer at 25 °C. Based on

electrical conductivity, the phase system of the optimized microemulsion was determined.

Drug solubility studies

The drug was added in excess to the optimized microemulsion formulation as well as each individual ingredient of the formulation. After continuous stirring for 24 h at room temperature, the undissolved ibuprofen was removed by centrifugation at 12 000 rpm for 30 min. Ibuprofen concentration in the supernatant was measured by spectrophotometry UV method as mentioned above. Drug solubility of optimized microemulsion was compared with respect to its individual ingredients.

Stability studies

Optimized microemulsion containing ibuprofen was kept under cold condition (4±2 °C), controlled room temperature (25±2 °C) and at accelerated storage (40±2 °C) for 30 d. After, the microemulsion was analyzed for phase separation, percent transmittance, globule size, refractive index, and pH. To evaluate the percentage of the drug, under the stability testing, for optimized microemulsion containing 5% w/w ibuprofen, an assay with UV spectrophotometry at 220 nm was performed.

Statistical analysis

Data were done in triplicate and expressed as the mean±standard deviation. Statistical data were analyzed by the Student's t-test at the level of p=0.05.

RESULTS AND DISCUSSION

Solubility study of ibuprofen

The solubility of poorly water-soluble ibuprofen in various oils, surfactants, and co-surfactants was analyzed to screen the components for microemulsion. The results are shown in table 1.

Table 1: Solubility of ibuprofen in various solvents saturated at 25 °C

Phase type	Vehicle	Solubility* mg/g
Oil	Labrafac® WL1934	88.8±15.3
	Oleic acid	232.1±9.1
	CottonSeed oil	77.1±17.2
	Argan oil	89.7±12.3
	Olive oil	73.6±5.4
Surfactant	Tween® 80 (HLB=15.0)	277.5±11.7
	Tween® 40 (HLB=15.6)	210.3±10.3
	Tween® 20 (HLB=16.7)	187.0±7.2
	Labrasol® (HLB=14.0)	218.1±19.4
Co-surfactant	PEG 400	214.9±18.0
	Propylene glycol	183.8±16.1
	Ethanol	203.2±8.1
	1-Butanol	218.5±6.3

*(mean±SD, n=3)

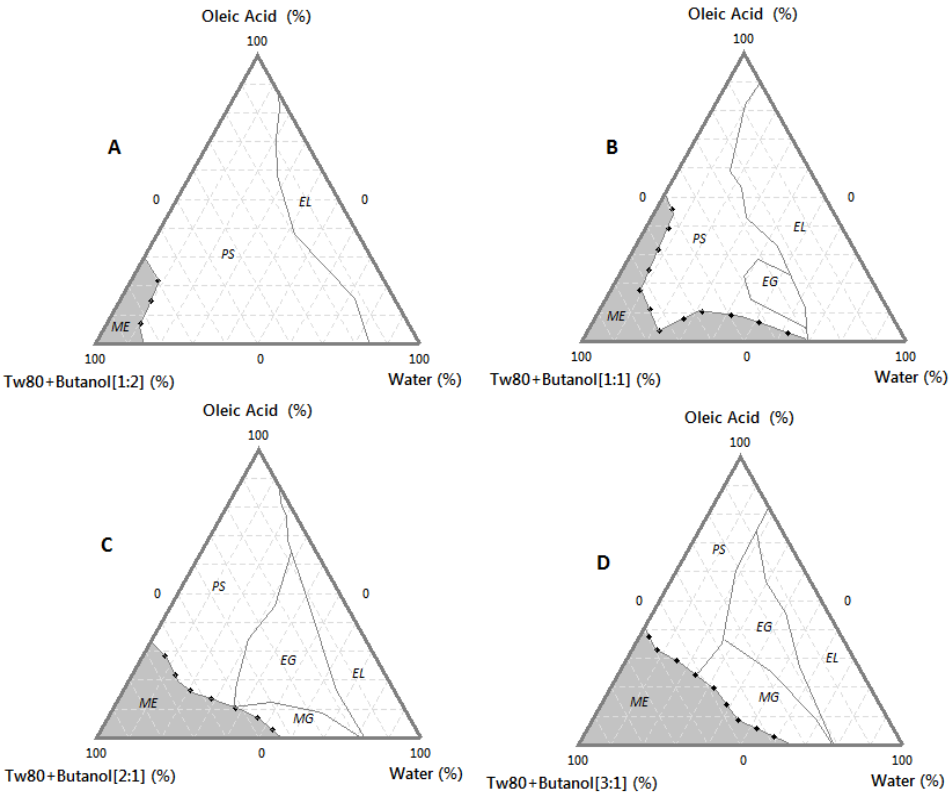

Fig. 1: Pseudo-ternary phase diagrams with 1:2 (A), 1:1 (B), 2:1 (C) and 3:1 (D) Surfactant/Co-surfactant ratios, Abr.: ME: microemulsion; EG: emulgel; MG: microgel; EL: emulsion liquid; PS: phase separation

The solubility of the ibuprofen in various studied oils was higher in the oleic acid (232.1±9.1 mg/g) than in the labrafac® WL1934, the cottonseed oil, the olive oil, and argan oil. Amongst surfactants, tween® 80 showed the maximum solubility (277.5±11.7 mg/g) followed by labrasol®, tween® 40 and tween® 20.

Butanol showed the highest solubility among the co-surfactants (218.5±6.3 mg/g), followed by polyethylene glycol 400, ethanol, and propylene glycol.

According to the solubility of ibuprofen, oleic acid should be the most appropriate oil for the development of microemulsion. It was also reported that oleic acid was a powerful enhancer of skin permeability for dermal delivery since it could increase the fluidity of the lipid portion of the stratum corneum [26, 27].

Tween® 80 is a non-toxic surfactant that can be used in topical delivery. With an HLB of 15, it tends to create an o/w microemulsion system.

In addition, alcohol has a high solubilization capacity [22]. Therefore, 1-Butanol was chosen as a co-surfactant, for further evaluation.

Pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed without ibuprofen to obtain appropriate concentration ranges of components and find out the areas of microemulsions.

The phase diagrams containing tween® 80 as a surfactant, oleic acid as an oil and 1-butanol as a co-surfactant with various weight ratios values (1:2, 1:1, 2:1, and 3:1) are described in fig. 1.

The analysis of phase behavior allowed classifying four different regions based on visual observation: translucent microemulsion, gel, liquid turbid emulsion and phase separation.

An example representing the different phases is presented in fig. 2. According to several studies, it has been reported that a wide range of surfactant compositions produce stable systems [21, 28-31].

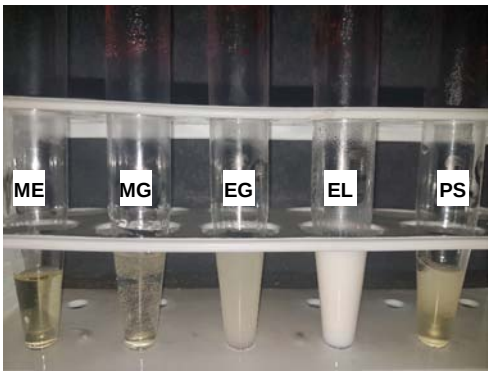

Fig. 2: Example of phase behavior prepared

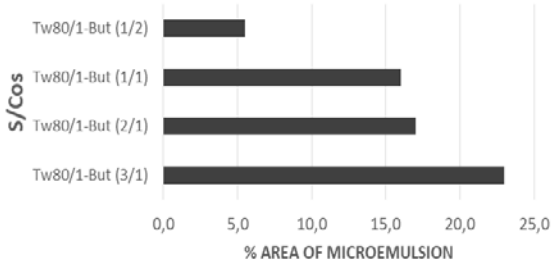

Fig. 3: Existence area of microemulsion formulated with different tween 80/1-butanol ratios in the pseudo-ternary phase diagrams

However, when the surfactant concentration was below 55% in the different phase diagrams, some instability and phase separation were observed, indicating that at lower surfactant concentrations there could not be a balance between high proportions of aqueous and oily phases. The existence areas of microemulsion made with different S/Cos ratios are presented in fig. 3.

The area of translucent microemulsion region increase in size by increasing the ratio of surfactant to co-surfactant, for microemulsions with tween® 80 to 1-butanol ratios like 1:2, 1:1, 2:1 and 3:1 shown in fig. 1. Also, the system containing tween® 80 as surfactant and 1-butanol as co-surfactant at Smix (3:1) provide a stable and broad microemulsions area and permit incorporation up to 30% w/w of oil. This can be explained by the improvement of micelles formation with an increase of the Smix ratio, which favors the solubilization capacity of the microemulsions [12]. In view of these results, the Smix ratio of 3:1 was selected for further optimization.

For a dermal application, several reports show that the highest skin flux and permeability coefficient are observed for the formulation containing a maximum amount of water. Also, the increase of surfactant content generates skin toxicity [29, 32, 33]. For that, limited proportions of Smix (3:1) (70-50%), oil (25-5%) and water (20-40%) were selected for test thermodynamic stability of the dispersion of ibuprofen in the microemulsion.

Ibuprofen microemulsions stability

Dispersion stability studies were performed in pre-selected formulations containing Smix 3:1 Tween®80 to butanol (70-50%), oil (25-5%) and water (20-40%). The formulations, labeled F1 to F17 (fig. 4), are chosen along the lines of dilutions. Ibuprofen is added to each microemulsion at a fixed concentration of 5% w/w.

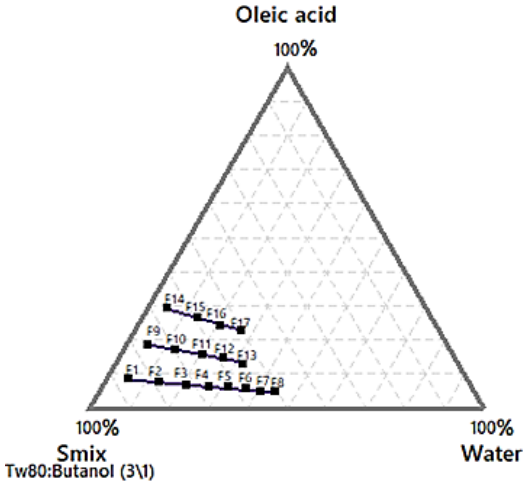

Fig. 4: Formulations F1 to F17 for dispersions stability studies

The results of the physicochemical analysis, attained before and after submitting samples to temperature variations, are listed in table 2.

Before thermal treatment, samples were submitted to centrifuge at 6000 rpm for 30 min at 25 °C. Then, it was observed that the majority of the samples were clear and no phase separation was observed after visual inspection.

After centrifugation, the samples F7, F8, F13, F16, and F17 showed phase separation (PS) and were therefore eliminated. Thereafter, all the formulations did not show any type of instability after the heating-cooling cycle. They appeared as a single translucent phase when visually observed.

The results presented in table 2 show that all the systems are within the required physiologic pH range accepted for dermal preparations (4.0–7.0 pH units) [10]. Refractive index measurements for all tested formulations remains constant and were in the range expected for transparent isotropic systems [23].

The microemulsions remain stable and clear, indicating their sufficient capacity for the solubilization of the drug without precipitation or blurriness, thus confirming their thermodynamic stability [34].

Experimental design

Through the results of the dispersion stability study (table 2), a concentration of a Smix of less than 55% results in instability of the preparation. Other results confirm that a high concentration of surfactant and oleic acid could cause toxicity and skin irritation [26]. Hence, seventy percent (70% w/w) Smix (3:1), which corresponds to 52.5% (w/w) of tween® 80, and fifteen percent (15% w/w) of oil were selected as safe maximum concentrations.

The microemulsion region corresponding to minimum surfactant content from the pseudo-ternary phase diagram plotted between oleic acid, water and tween 80/1-butanol (3:1) was selected for optimizing the formulation. Oil content ranging from 5 to 15% (w/w), Smix content ranging from 55 to 70% (w/w) and water content ranging from 20 to 30% (w/w) were chosen as a design space for investigating experimental trials.

Thereafter, a 3-factor, 3-level D-optimal mixture statistical experimental design was employed for formulation optimization.

The mathematical response surface methodology involved 19 experimental runs comprising various combinations of oil, water, and Smix and studying their effects on studied response variables as the globule size and transmittance (table 3). The average globule size of the experimental formulations was between 109.3 and 202.5 nm, which corresponds to the usual microemulsion droplet size range [15].

Using the normal probability plot that indicates whether the residues follow a normal distribution, the plots were found normally distributed and looked like straight lines and outliers were not encountered.

Prediction point analysis

Validation of the statistical model was performed by characterizing the formulations prepared according to 3 random points predicted formulations. The results of the checkpoint analysis are shown in table 4. The average prediction point analysis is compared to the two-sided prediction interval with an alpha risk of 0.05.

As shown in table 4, no outliers were found based on the Studentized residual analysis. The observed values for response variables were in close agreement with predicted values as the confirmation experiments are within the confirmation node's prediction interval. Therefore, it can be inferred that obtained mathematical model equations are valid for predicting the response values.

Table 2: Physico-chemical results for dispersions stability studies

Formulation	% Oil	% S/Cos (3:1)	% Water	Centrifugation	Aspect cycle heating cooling		pH*		Refraction index*	
					Before	After	Before	After	Before	After
F1	9.1	81.8	9.1	N	TL	TL	4.8*±0.2*	4.6*±0.1*	*1.431±0.005*	*1.433±0.006*
F2	8.3	75.0	16.7	N	TL	TL	4.9*±0.3*	4.8*±0.1*	*1.434±0.008*	*1.430±0.008*
F3	7.7	69.2	23.1	N	TL	TL	4.7±0.1	4.9*±0.3*	*1.446±0.01*	*1.439±0.002*
F4	7.1	64.3	28.6	N	TL	TL	4.6*±0.3*	4.6±0.2	*1.439±0.003*	*1.437±0.012*
F5	6.7	60.0	33.3	N	TL	TL	4.7*±0.3*	4.5*±0.1*	*1.441±0.003*	*1.448±0.002*
F6	6.3	56.3	37.5	N	TL	TL	4.4*±0.1*	4.8*±0.3*	*1.439±0.001*	*1.445±0.004*
F7	5.9	52.9	41.2	PS	–	–	–	–	–	–
F8	5.6	50.0	44.4	PS	–	–	–	–	–	–
F9	18.2	72.7	9.1	N	TL	TL	4.9*±0.2*	5.1*±0.1*	*1.440±0.002*	*1.440±0.005*
F10	16.7	66.7	16.7	N	TL	TL	5.0*±0.1*	4.9±0.1	*1.436±0.006*	*1.431±0.004*
F11	15.4	61.5	23.1	N	TL	TL	4.5*±0.2*	4.6*±0.2*	*1.442±0.001*	*1.435±0.002*
F12	14.3	57.1	28.6	N	TL	TL	4.8*±0,1*	4.8*±0.1*	*1.442±0.005*	*1.441±0.001*
F13	13.3	53.3	33.3	PS	–	–	–	–	–	–
F14	27.3	63.6	9.1	N	TL	TL	5.2*±0.1*	5.0*±0.1*	*1.425±0.001*	*1.429±0.007*
F15	25.0	58.3	16.7	N	TL	TL	4.4*±0.1*	4.6*±0.1*	*1.435±0.002*	*1.432±0.002*
F16	23.1	53.8	23.1	PS	–	–	–	–	–	–
F17	21.4	50.0	28.6	PS	–	–	–	–	–	–

*(mean±SD, n=3), N: normal; TL: translucent liquid; PS: phase separation.

Table 3: Experimental runs

Formulation code	Independent variables			Response variables	
	A: Oil (%)	B: S/Cos (%)	C: Water (%)	Globule size (nm)	Transmittance (%)
ME1	9.7	60.3	30.0	131.6	94.2
ME2	5.0	65.0	30.0	110.1	98.0
ME3	15.0	65.0	20.0	126.8	94.0
ME4	11.7	61.5	26.8	128.2	96.7
ME5	10.0	65.0	25.0	125.8	95.2
ME6	6.6	66.6	26.8	116.8	98.2
ME7	8.3	68.4	23.4	130.8	97.5
ME8	15.0	55.0	30.0	191.6	94.2
ME9	10.0	65.0	25.0	126.5	96.8
ME10	11.7	66.5	21.7	132.4	94.3
ME11	5.0	65.0	30.0	109.3	98.7
ME12	5.0	70.0	25.0	126.2	98.5
ME13	9.7	60.3	30.0	122.1	97.3
ME14	14.4	58.0	27.6	202.5	92.0
ME15	15.0	55.0	30.0	193.2	94.1
ME16	15.0	65.0	20.0	128.3	93.5
ME17	5.0	70.0	25.0	128.9	98.4
ME18	10.0	70.0	20.0	122.2	96.0
ME19	15.0	60.4	24.6	118.9	92.3

Table 4: Prediction points analysis results

Formulation code	Independent variables			Observed value		Predicted value		Studentized residual		95% prediction interval	
	Oil (%)	S/Cos (%)	Water (%)	Globule size (nm)	Transmittance (%) *	Globule size (nm)	Transmittance (%)	Globule size	Transmittance	Globule size (nm)	Transmittance (%)
ME20	15.0	60.0	25.0	149.3	92.7±1.5	146.17	92.48	0.343	0.308	[127.41-192.63]	[91.53-94.39]
ME21	10.5	69.5	20.0	116.0	95.17±0.89	118.93	95.04	-0.353	0.174	[98.42-147.15]	[94.07-96.98]
ME22	5.0	67.5	27.5	108.7	97.17±1.29	108.92	97.52	-0.022	-0.488	[91.24-134.3]	[96.55-99.45]

*(mean±SD, n=3)

Response variables modeling

The quadratic model was the highest order polynomial suggested for globule size prediction. The mathematical model equation in terms of coded factors, that permits comparing the factor coefficients to identify their relative impact, is provided in Eq. (1).

Globule size = 215.57 A+167.43 B+111.28 C–268.26 AB+128.97 AC–128.78 BC (1)

The F-value of 10.86 and the p-value of 0.0003 imply that the result of the globule size model is significant. The predicted R^2 of 0.6028 is in reasonable agreement with the adjusted R^2 of 0.7325. The high value of adequate precision (10.993) indicates an adequate signal. So, this model can be used to navigate the design space.

For the transmittance percent, who's an important indicator for the quality of emulsification [35, 36], the linear model was the best-fitted model suggested for prediction of % transmittance responses. The mathematical model equation in terms of coded factors is provided in Eq. (2).

% Transmittance = 88.25 A+98.57 B+98.52 C(2)

The transmittance model F-value of 34.66 and p-value<0.0001 implies the model is significant. The closeness of predicted R^2 (0.7511) to the adjusted R^2 (0.7890) suggests goodness of fit to the data. The high value of adequate precision (12.976) depicts adequate model discrimination and adequacy of the signal.

The influence of components on globule size and transmittance has been shown in using the contour plot and response surface in fig. 5. The data indicate that response values are strongly affected by variations in the studied mixture components. The results obtained from this study show that the lower oil level, the smaller of globules size. Responses vary more significantly with the variation of Smix. The increase of oil up to 15% while leaving the proportion of Smix higher than 65%, allows smaller sizes than 130 nm. The factor coefficients also show that the simultaneous increase in oil and water causes an increase in globule size values.

At high levels of Smix above 65%, any variation of oil or water in the experimental field results in reduced size. This can be explained by better emulsification due to the reduction of the oil/water interfacial tension, which makes it possible to reduce the size of the globules of microemulsions [37]. These results are consistent with previous research in which the addition of surfactant to the microemulsion system causes condensation and stability of the interfacial film, while the addition of the co-surfactant causes expansion of the interfacial film [38, 39]. Smaller globule size is an important parameter affecting microemulsion stability, skin penetration and hence *in vivo* efficacy [40].

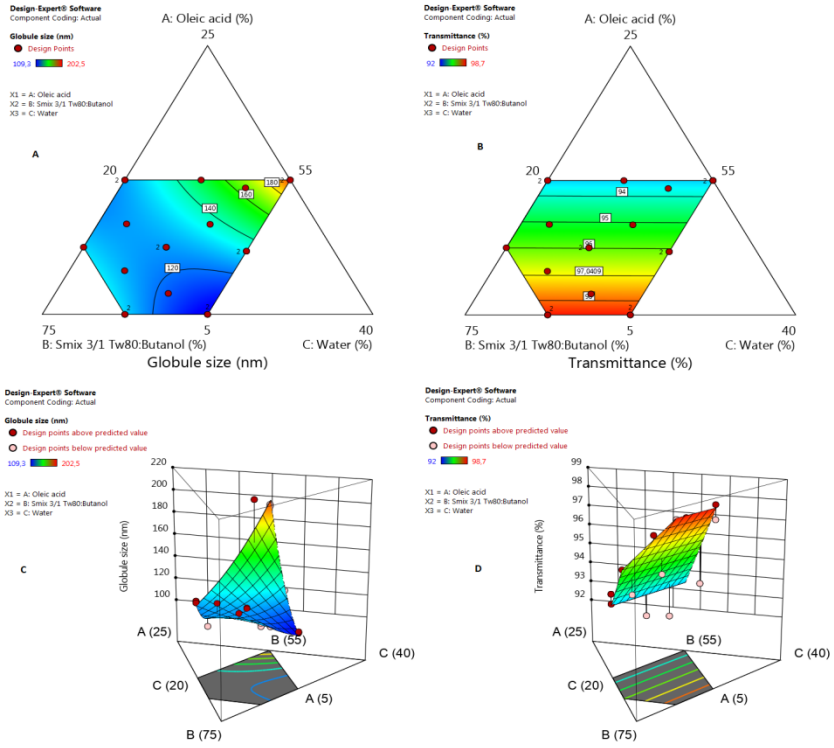

Fig. 5: Design contour plot (A and B) and response surface graphs (C and D) for globule size and transmittance responses

On the other hand, the transmittance of the microemulsion systems was maximum at low oil content levels. Due to the linear transmittance model, the effect of each component is measured by the difference between each coefficient. The effect of the component, therefore, corresponds to the amount of the response when the component goes from its low to its high level. With the t-test applicable to the difference between the estimated coefficients of the mixture, the value p<0.0001 for the oil coefficient shows that the variation of the transmission response is significantly sensitive to the variation of oil. As already discussed, the transmittance of microemulsion systems increased with decreasing oil content and increasing surfactant mixture due to the efficient emulsification of oil into smaller droplets [25, 37, 41].

Optimized formulation

The optimal values of mixture components were obtained by numerical optimization based on the criterion of maximizing transmittance percent and minimizing globule size. Through the solutions presented by Design Expert® software, the optimized solution was chosen on the basis of desirability function. The goal criteria of the formulation component are to minimize Smix content and maximize water proportion. The oil content is kept in the range between 5 and 15% w/w. This objective will, in the case of a skin formulation, increase the flow rate and the coefficient of permeability while maintaining a good tolerance with respect to the skin [26, 30, 42].

Two solutions, presented in table 5, were obtained. On the basis of the highest desirability score, the microemulsion with a percentage (w/w) of oil, surfactant/co-surfactant (3:1) and water of 6.88%, 63.11%, and 30% respectively, was chosen as an optimized microemulsion.

Characterization of optimized formulation

Macroscopic aspect

Ibuprofen loaded optimized microemulsion appear as translucent, slightly yellowish and easily flowable liquid. The preparation was perfectly homogeneous and no phase separation or precipitation was observed.

Physico-chemical characteristics

According to the results obtained (table 6), optimized microemulsion possessed a mean globule size of 117.5 nm, near to the predicted value of 113.59 nm, while the polydispersity index (PDI) was found to be 0.253. This PDI indicates a small distribution width and a low polydispersion of the system. This confirms the homogeneity and stability of the preparation [43, 44].

Table 5: Solutions for optimized formulation

Solutions number	Oil %	Smix %	Water %	Predicted size (nm)	Predicted transmittance (%)	Desirability
1	6.88	63.11	30.00	113.59	97.57	0.777
2	8.33	61.66	30.00	121.76	96.82	0.767

Table 6: Physico-chemical results of the optimized microemulsion

	Globule Size (nm)	Transmittance* (%)	Zeta potential (mV)	Conductivity (mS/cm)	Refractive index*	pH*
Optimized ME	117.5	96.95±0.77	-6.47	0,034	1.439±0.002	5,1±0.2

*(mean±SD, n=3)

The zeta potential was found to be-6.47 mV. The microemulsion globules, therefore, have a slightly low negative zeta potential, which indicates the stability of the system since no aggregation is expected [32, 45]. The intensity distribution of globules size and the zeta potential are shown in fig. 6. The transmittance value (96.95%) is close to predicted values (97.57%). This result confirms the validation of the model and the efficient emulsification of the different components [24, 46].

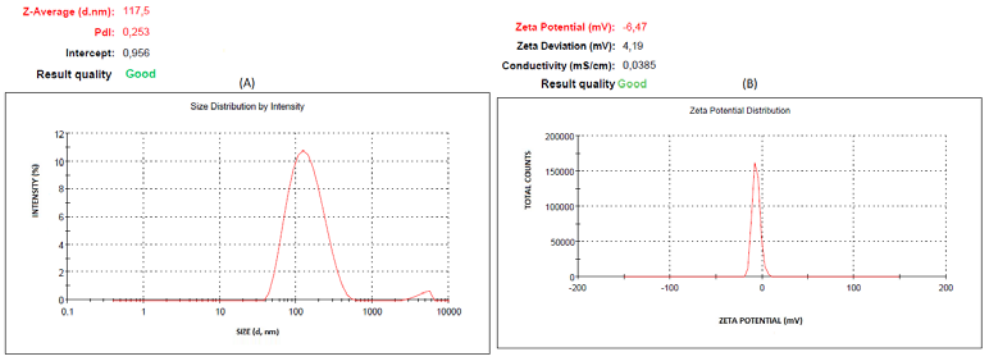

Fig. 6: Size distribution (A) and Zeta potential distribution (B) of the optimized microemulsion

Regarding the pH of the optimized microemulsion, the value obtained was ascribed to the weak acidic character of the drug molecule (pKa: 4.50). Nevertheless, the pH of microemulsions studied is acceptable for dermal application because it is compatible with the skin's functionalities [21, 47].

The value of conductivity is greater than the conductivity of the distilled water, which allows us to predict the oil-in-water type of our microemulsion.

Ibuprofen solubility

The solubility of the drug in the microemulsion indicates an enhanced solubility of ibuprofen in the optimized formulation (i.e. 298.2±21.3 mg/g) when microemulsion was compared to its respective individual ingredients. The results showed ibuprofen solubility in oleic acid and tween 80/butanol 3:1 of 232.1±9.1 mg/g and 281.1±24.4 mg/g respectively. Due to the presence of both lipophilic and hydrophilic domains, microemulsions are adaptable delivery systems that improve the solubilization of lipophilic drugs and enhance their bioavailability [15].

Stability studies

Stability studies were carried out to detect any changes in pH, refractive index, globule size, transmittance, and drug content. Results of temperature stability studies on the optimized microemulsion are reported in table 7. Results obtained indicated that the optimized Ibuprofen based microemulsion was stable for one month.

Table 7: Results of stability tests of optimized microemulsion after 30 d of storage

Temperature test (°C)	Phase separation	% transmittance*	Globule size (nm)	pH*	Refractive index*	% of Assay*
4±2 °C	No	90±1.2	122.3	5.0±0.1	1.435±0.003	97.1±2.4
Room Temperature	No	89±0.6	117.9	5.0±0.4	1.442±0.005	96.2±3.5
Accelerated (40±2 °C)	No	88±1.8	127.3	5.2±0.2	1.431±0.001	95.4±2.9

*(mean±SD, n=3)

The clarity of all optimized microemulsion was preserved as indicated by the refractive index measurements throughout the storage period. No significant change in percent drug content value was found. The results showed that the drug was uniformly distributed throughout the formulations and drug loss was minimum during the preparation of the formulation. The closeness of the globule size and the high transmittance percent despite a slight increase in size during the temperature change, confirms the good emulsification of the system due to the low interfacial tension that exists between the two phases of the microemulsion.

CONCLUSION

During this microemulsion formulation containing 5% (w/w) ibuprofen, the appropriate components and their optimal concentration ranges were obtained using pseudo-ternary phase diagrams. Concentrations of the main components were optimized by D-optimal mixture design. The formulation was considered as optimized ibuprofen loaded microemulsion consisting of 6.88 % oleic acid, 63.11 % tween® 80/butanol (3:1) and 30% of water. Modeling of responses indicates that the size of the globules of the microemulsion is smaller with augmentation of surfactant mixture whereas emulsification is more efficient at a low level of oil.

The results indicate that the studied microemulsion may act as a promising vehicle for topical delivery of ibuprofen. A study on skin permeability and *in vitro* release of ibuprofen will validate a new drug delivery system.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman Med J 2010;25:155-61.
2. Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology 2009;17:275-342.
3. Irvine J, Afrose A, Islam N. Formulation and delivery strategies of ibuprofen: challenges and opportunities. Drug Dev Ind Pharm 2018;44:173-83.
4. Lewis F, Connolly MP, Bhatt A. A pharmacokinetic study of an ibuprofen topical patch in healthy male and female adult volunteers. Clin Pharmacol Drug Dev 2018;7:684-91.
5. Nagai N, Tanino T, Ito Y. Pharmacokinetic studies of gel system containing ibuprofen solid nanoparticles. J Oleo Sci 2016;65:1045-53.
6. Aliberti ALM, de Queiroz AC, Praca FSG, Eloy JO, Bentley M, Medina WSG. Ketoprofen microemulsion for improved skin delivery and *in vivo* anti-inflammatory effect. AAPS PharmSciTech 2017;18:2783-91.
7. Xing Q, Song J, You X, Xu D, Wang K, Song J, *et al.* Microemulsions containing long-chain oil ethyl oleate improve the oral bioavailability of piroxicam by increasing drug solubility and lymphatic transportation simultaneously. Int J Pharm 2016;511:709-18.
8. Priano L, Albani G, Brioschi A, Calderoni S, Lopiano L, Rizzone M, *et al.* Transdermal apomorphine permeation from microemulsions: a new treatment in Parkinson's disease. Mov Disord 2004;19:937-42.
9. Cao M, Ren L, Chen G. Formulation optimization and ex vivo and *in vivo* evaluation of celecoxib microemulsion-based gel for transdermal delivery. AAPS PharmSciTech 2017;18:1960-71.
10. Hashem FM, Shaker DS, Ghorab MK, Nasr M, Ismail A. Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. AAPS PharmSciTech 2011;12:879-86.
11. Dogrul A, Arslan SA, Tirnaksiz F. Water/oil type microemulsion systems containing lidocaine hydrochloride: *in vitro* and *in vivo* evaluation. J Microencapsulation 2014;31:448-60.
12. Kawakami K, Yoshikawa T, Hayashi T, Nishihara Y, Masuda K. Microemulsion formulation for enhanced absorption of poorly soluble drugs. II. *In vivo* study. J Controlled Release 2002;81:75-82.
13. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Delivery Rev 2012;64:175-93.
14. Tole K, G Deshmukh. Design and characterization of microemulsion gel for transdermal drug delivery system of duloxetine hydrochloride. Asian J Pharm Clin Res 2018;11:157-61.
15. Talegaonkar S, Azeem A, Ahmad F, Khar R, A Pathan S, Iqbal Z. Microemulsions: a novel approach to enhanced drug delivery. Recent Pat Drug Delivery Formulation 2008;2:238-57.
16. Narang AS, Delmarre D, Gao D. Stable drug encapsulation in micelles and microemulsions. Int J Pharm 2007;345:9-25.
17. Jaiswal PL, Darekar AB, Saudagar RB. A recent review on nasal microemulsion for the treatment of cns disorder. Int J Curr Pharm Res 2017;9:5-13.
18. Sanjaymitra PvSS, Ganesh GNK. Dissolution and solubility enhancement strategies: Current and novel prospectives. J Crit Rev 2018;5:1-10.
19. Chen H, Chang X, Du D, Li J, Xu H, Yang X. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm 2006;315:52-8.
20. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. Int J Pharm Invest 2012;2:140-9.
21. Djekic L, Primorac M, Filipic S, Agbaba D. Investigation of surfactant/cosurfactant synergism impact on ibuprofen solubilization capacity and drug release characteristics of nonionic microemulsions. Int J Pharm 2012;433:25-33.
22. Gustmann PC. Development of Brazil nut oil microemulsion as vehicle for levamisole. J Appl Pharm Sci 2017;7:92-8.
23. Junyaprasert VB, Boonme P, Songkro S, Krauel K, Rades T. Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions. J Pharm Pharm Sci 2007;10:288-98.
24. Shah N, Seth A, Balaraman R, Sailor G, Javia A, Gohil D. Oral bioavailability enhancement of raloxifene by developing microemulsion using D-optimal mixture design: optimization and *in vivo* pharmacokinetic study. Drug Dev Ind Pharm 2018;44:687-96.
25. Mandal S, Das Mandal S, Chuttani K, Subudhi bb. Mucoadhesive microemulsion of ibuprofen: design and evaluation for brain targeting efficiency through intranasal route. Braz J Pharm Sci 2015;51:721-31.
26. Zhu W, Yu A, Wang W, Dong R, Wu J, Zhai G. Formulation design of microemulsion for dermal delivery of penciclovir. Int J Pharm 2008;360:184-90.
27. Hua L, Weisan P, Jiayu L, Ying Z. Preparation, evaluation, and NMR characterization of vinpocetine microemulsion for transdermal delivery. Drug Dev Ind Pharm 2004;30:657-66.
28. Fanun M. Celecoxib solubilization in nonionic microemulsions. J Dispersion Sci Technol 2010;31:241-7.
29. Hu L, Hu Q, Yang J. Enhancement of transdermal delivery of ibuprofen using microemulsion vehicle. Iran J Basic Med Sci 2014;17:760-6.
30. Xavier Junior FH, Vauthier C, Morais ARV, Alencar EN, Egito EST. Microemulsion systems containing bioactive natural oils: an overview on the state of the art. Drug Dev Ind Pharm 2016;43:1-15.

31. Mauludin R, SF Bt Mohamad, T Suciati. Formulation and characterization of ascorbyl palmitate loaded o/w microemulsion. Int J Pharm Pharm Sci 2014;6:294-8.
32. Kumar A, Kushwaha V, Sharma P. Pharmaceutical microemulsion: formulation, characterization and drug deliveries across skin. Int J Drug Dev Res 2014;6:1-21.
33. Kilor V, N Sapkal, G Vaidya. Design and development of novel microemulsion based topical formulation of hesperidin. Int J Pharm Pharm Sci 2015;7:142-8.
34. Desai SA, Mohite RA, Hajare A. Screening of safflower oil microemulsion for enhancing bioavailability of lovastatin. Int J Pharm Sci Res 2015;6:28-49.
35. Date AA, Nagarsenker MS. Design and evaluation of microemulsions for improved parenteral delivery of propofol. AAPS PharmSciTech 2008;9:138-45.
36. Date AA, Nagarsenker MS. Parenteral microemulsions: an overview. Int J Pharm 2008;355:19-30.
37. Shah KA, Joshi MD, Patravale VB. Biocompatible microemulsions for fabrication of glyceryl monostearate solid lipid nanoparticles (SLN) of tretinoin. J Biomed Nanotechnol 2009;5:396-400.
38. Kale NJ, Allen LV. Studies on microemulsions using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as cosurfactants. Int J Pharm 1989;57:87-93.
39. Yati K, Srifiana Y, Putra F. Effect of optimization of tween 80 and propylene glycol as a surfactant and cosurfactant on the physical properties of aspirin microemulsion. Int J Appl Pharm 2017;9:127-9.
40. Wang X, Xue M, Gu J, Fang X, Sha X. Transdermal microemulsion drug delivery system for impairing male reproductive toxicity and enhancing efficacy of tripterygium wilfordii hook f. Fitoterapia 2012;83:690-8.
41. Abd Allah FI, Dawaba HM, Ahmed AM. Preparation, characterization, and stability studies of piroxicam-loaded microemulsions in topical formulations. Drug Discoveries Ther 2010;4:267-75.
42. Subramanian N, Ghosal SK, Moulik SP. Topical delivery of celecoxib using microemulsion. Acta Pol Pharm 2004;61:335-41.
43. Biruss B, Valenta C. The advantage of polymer addition to a non-ionic oil in water microemulsion for the dermal delivery of progesterone. Int J Pharm 2008;349:269-73.
44. Pereira Lachataignerais J, Pons R, Panizza P, Courbin L, Rouch J, Lopez O. Study and formation of vesicle systems with low polydispersity index by ultrasound method. Chem Phys Lipids 2006;140:88-97.
45. Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of clopidogrel. Iran J Pharm Res 2010;9:327-34.
46. Shah P, Swarnkar D, Parikh R. Development and characterization of microemulsion containing antihypertensive agent using factorial design. J Pharm Bioallied Sci 2012;4(Suppl 1):69-70.
47. Djekic L, Martinovic M, Stepanovic Petrovic R, Micov A, Tomic M, Primorac M. Formulation of hydrogel-thickened nonionic microemulsions with enhanced percutaneous delivery of ibuprofen assessed *in vivo* in rats. Eur J Pharm Sci 2016;92:255-65.