ORIGINAL ARTICLE

Development, evaluation, and optimization of flurbiprofen nanoemulsions gel using quality by design concept

G. Aiswarya, Kh. Hussan Reza[1], R. Kumaravel Rajan[2]

Department of Chemistry, Shree Devi college of pharmacy, Mangalore, Karnataka, [2] Department of Research and Developement, Aavishkar Oral Strips Pvt.Ltd, Hyderabad, Telengana, [3] Department of Pharmaceutics, C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India

The objective of this study was to formulate topical gel loaded nanoemulsions containing flurbiprofen using volatile oil. The gel released the drug at a controlled rate to the targeted site. Flurbiprofen (log $P = 4.09$) in generally used for transdermal treatment of rheumatoid arthritis and osteoarthritis. Selection of the oil phase, surfactant, and cosurfactant was done by individual screening method with the aid of pseudo-ternary phase study. International Conference on Harmonisation Q8 guidelines was applied using 3^2 factorial designs coupled with response surface methodology. The formulations were prepared by using spontaneous emulsification method. The selected formulation from various statistical and other studies was investigated. It was found that selected formulation showed an optimum *in-vitro* data. Later the optimized formulation obtained within the tentative design space was incorporated in the gel and compared with the marketed formulation. The result suggested the optimized formulation with good potential for transdermal delivery of the drug than the marketed formulations.

Key words: *Design space, flurbiprofen, in-vitro diffusion, nanoemulsions, transdermal drug delivery*

INTRODUCTION

The current research work in all technical and biomedical fields is based on nanosize.[1] Nanoemulsions are defined as transparent dispersions of oil and water stabilized as an interfacial film of surfactant and cosurfactant molecules having droplet size less than a micron. Nanoemulsions are thermodynamically stable dispersed system.[2] The droplet size of the nanoemulsions may range within 100-600 nm or less.[3] The long-term stability, ease of preparation and high-drug solubilization property, make nanoemulsions a promising tool for drug delivery.[4,5] Nanoemulsions were previously proved to be potential drug delivery tool for ocular, pulmonary, nasal, vaginal, and parental.[6-10] In recent times, they have shown promising potential as transdermal drug delivery in delivering drug across the skin than other conventional transdermal delivery system.

Nanoemulsions can be prepared by high-pressure homogenization, microfludization, ultrasonication, and phase inversion method.[11-13] Out of the various techniques high-pressure homogenization and ultrasonication are most preferred for laboratory preparation.[14] The spontaneous emulsification is the technique that is recently followed for the preparation of nanoemulsions. The process largely depends upon factors such as surfactant nature, interfacial - bulk viscosity, interfacial tension, and phase titration region.

The transdermal drug delivery system can be used to deliver anti-inflammatory drugs across the skin for the treatment of acute and chronic pain and inflammation. Nonsteroidal anti-inflammatory drugs are in use to reduce the pain and inflammation.[15] Flurbiprofen (log $P = 4.09$) is generally recommended for the treatment of rheumatoid arthritis, osteoarthritis[16,17] and also has analgesic and anti-pyretic activity.[18] Prolonged administration of the drug generally results in gastrointestinal bleeding and gastric ulcer.[19] Thus,

Address for correspondence:
Prof. G. Aiswarya,
Department of Chemistry, Shree Devi College of Pharmacy, Kenjar, Mangalore - 574 142, Karnataka, India.
E-mail: aiswarya.nath@gmail.com

Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.150035

the transdermal route of administration increases patient compliance avoiding first-pass metabolism and maintains plasma drug level for a prolonged period.

Thus, the objective of the work was to formulate a topical gel loaded with nanoemulsions containing the flurbiprofen as a topical gel showing controlled, targeted release of the drug for the given period. Oil phase, surfactant, and cosurfactant were selected by pseudo-ternary phase study. The volatile oil was selected for the study due its short chain and high-drug solubility nature. Concept of quality by design was implemented for design a quality product and its manufacturing process for consistently deliver the intended performance of the product. Information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls. Design of experiment (DOE) used here was based on 3^2 factorial designs coupled with response surface methodology. The optimizing formulation screened and prepared by using spontaneous emulsification method. The evaluation of the preparation was based on the study of viscosity, turbidity, particle size, stability, and diffusion. The optimized formulation from the targeted design space was incorporated into the gel.

MATERIALS AND METHODS

Flurbiprofen was obtained from Sri Radhe Sales, Gujarat, India. Turpentine oil, castor oil, eucalyptus oil, propylene glycol (PG), and Carbopol-934 were purchased from Loba Chemie, Mumbai. The other ingredients such as Tween 20, Tween 40, Tween 80, and isopropyl myristate (IPM) were acquired from S.d.Fine Chemicals, Mumbai. All other chemicals and solvents used were of analytical grade.

Screening of the oil phase

Selection of oil phases was done by determining relative solubility of the drug in the oil phase.[20] The various oils used namely – peppermint oil, eucalyptus oil, IPM, castor oil, and water. Excess of the drug was added to 2 ml of oil in a 5 ml capacity stopper vials. Then, it was kept in isothermal shaker at room temperature for 24 h period. Sample was then removed, and the amount is centrifuged. Supernatant liquid obtained was filtered through membrane filter of pore size 45 μm. The concentration of flurbiprofen was determined spectrophotomertically at 247 nm.

Screening of surfactant

The surfactants Tween 20, Tween 40, and Tween 80 were chosen for the purpose of screening. A 15% w/w solution of the selected surfactants was prepared using distilled water. A volume of 2.5 ml of the solution was taken in each of the three glass bottles. To it added the oil phase screened previously in dropwise manner with vigorous vortex till the solution turns cloudy. The surfactant that can solubilize maximum amount of oil without turning cloudy was selected as surfactant.[21]

Screening of cosurfactant

Different alcohols used for screening of cosurfactant are ethanol, isopropyl alcohol (IPA), butanol, and propanol. Screening was done by using pseudo-ternary phase study. Nanoemulsions area was examined for the screening of surfactant. They were assed at a fixed surfactant–cosurfactant ratio (Smix ratio) at 1:2 by keeping the surfactant unchanged, replacing the cosurfactant. Greater the size of nanoemulsions area obtained from pseudo-ternary phase study, greater is the efficiency of the cosurfactant.[3]

Selection of surfactant–cosurfactant mass ratio in the formulation of nanoemulsions

The screened surfactant was blended with cosurfactant in different ratios as Smix ratio ranging from 1:0, 1:1, 1:2, 1:3, 1:4, and 1:5. The Smix was selected with decreasing concentration of surfactant and increasing concentration of cosurfactant. The selected Smix ratio was further treated with oil at different ratios of 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:2, and 1:1. The pseudo-ternary phase diagram used for selecting the best ratio of Smix and oil.[3]

Drug–excipients interaction study

Drug–excipients interaction study involved the investigation using infrared (IR) spectrophotometer and differential scanning calorimeter (DSC).

Infrared spectrophotometry

Infrared study was carried out for plain drug and drug–excipients mixture using Fourier transform IR (FTIR) (Shimadzu FTIR-8101) based on KBr disc method. The study was carried out within the frequency range of 4000-400/cm with a resolution of 4/cm.

Differential scanning calorimeter study

The DSC study was done using Netzsec DSC 204, Tokyo, Japan. The samples were heated in a sealed aluminum pans at a rate of 10°C/min in a 30-300°C temperature range under nitrogen flow of 40 ml/min.

Design of experiment and optimization of nanoemulsions

The optimization of nanoemulsions was done by 3^2 full factorial designs. Various softwares such as Design Expert® (8.0.4, Statease Inc 2021 East Hennepin Avenue, suit 480, Minneapolis, MN -55413.) and DOE++® (Relia Soft, New No. 16, Old No. 21, Cenotaph 1st Street, Alwarpet, Chennai - 600 018) were used for the purpose. In a factorial design, general features are considered at various levels. The effect that can be given by the factors and their interaction are assured by maximum efficacy in the design. For the current DOE two factors were chosen. They are X_1 and X_2 selected as Smix and Oil phase respectively as they were the critical quality attributes (CQA) affecting final formulation. Three

levels of concentration for each selected attributes was taken as discussed in Table 1a and b.

Method of preparation of nanoemulsions

The method involved in the preparation of nanoemulsions is generally termed as spontaneous emulsification or aqueous phase titration. The probe sonicator with a probe of $^1/_4$ inches or 6 mm in diameter having the frequency of 20 KHz (Sonics and Material Inc., USA) was used for the size reduction. An apparatus was set to carry out the emulsification and sonication spontaneously at a controlled temperature by providing cold water jacket, to reduce heat production by the probe sonication. As per the process initial measured amount of oil and Smix were taken in a measuring cylinder, and the water phase added from the burette followed by occasional sonication. The whole setup was placed in an ice cold water bath. After the selection of the best formulation by optimization, it is incorporated in the gel.

Preparation of the gel involved the Carbopol-940 dispersed in sufficient quantity of water. After complete dispersion, Carbopol-940 solution was kept in the dark for 24 h. Thereafter the emulsion incorporated. The other ingredients such as IPA and PG were added. Finally, triethanolamine was added to form a thick gel. The formula for the gel is given in Table 2.

Turbidity

Turbidity of the colloidal substances and the dispersed systems has a direct correlation to the particle or droplet size of the dispersed phase. The turbidity for the selected nanoemulsions was measured using Turbidmeter (Deep Vision, India) and expressed in terms of nephelometric turbidity unit.

Table 1a: Factors with levels for 20 ml emulsion

Independent variables	+1 (high)	0 (medium)	−1 (low)
X_1 (oil)	2 ml	1.5 ml	1 ml
X_2 (Smix)	8 ml	9 ml	10 ml

Viscosity

The viscosity of the nanoemulsions for optimization was measured by Brookfield DV-E viscometer-LV2 (Brookfield Engineering Laboratory, USA). The spindle size 62 and rpm of 60 was used for the study. The viscosity expressed in terms of centipoises.

Particle size analysis

The droplet size of the optimized formulation was measured by using Zetasizer (Nanoseries, Malvern Instrument, UK). The instrument generally works by photon correlation spectroscopy that measures the light scattering caused by the Brownian motion of the particles or droplets.

***Ex-vivo* diffusion study**

The *ex-vivo* diffusion study was carried out using Franz diffusion cell using the rat skin for the study. The process involved preparation of the rat skin and transdermal diffusion study.

Preparation of rat skin

Male Albino Wistar rat was scarified by aspiration of ethyl ether and abdominal skin was carefully excised. Hair was removed by using hair remover. The subcutaneous tissue wiped out using IPA to remove the adhering fat. The cleaned skin was washed with distilled water. The preservation of the skin was done at 4°C.[22]

Diffusion study

The diffusion study was performed using Franz diffusion cell. The donor compartment of volume 17 ml filled with saline

Table 2: Formula for preparation of gel

Ingredients	Amount (g)
Flurbiprofen	5
Carbopol-934	1
IPA	10
PEG	10
PG	10
TEA	0.5
Distilled water	100

IPA: Isopropyl alcohol, PEG: Polyethylene glycol, PG: Propylene glycol, TEA: Triethanolamine

Table 1b: Factors with the interaction and formulation code

Interaction		Oil	Smix	Water	Percentage	Percentage	Percentage	Code
A	B	(ml)	(ml)	(ml)	of oil	of Smix	of water	
+1	+1	2	10	8	10	50	40	F_1
+1	0	2	9	9	10	45	45	F_2
+1	−1	2	8	10	10	40	50	F_3
0	+1	1.4	10	8.6	7	50	43	F_4
0	0	1.4	9	9.6	7	45	48	F_5
0	−1	1.4	8	10.6	7	40	53	F_6
−1	+1	1	10	9	5	50	45	F_7
−1	0	1	9	10	5	45	50	F_8
−1	−1	1	8	11	5	40	55	F_9

phosphate buffer of pH 7.4 and rpm of 600 rpm was used for the study. A slight modification in the apparatus was made for study diffusion of nanoemulsion samples. Test tube with an open end was taken, and the skin was tied to the even end. Later it was placed on the diffusion cell such that the skin touches the diffusion fluid. 1 ml of the nanoemulsion was placed in a test tube, and the study was done for 24 h. The sampling was done at the required interval. The diffusion study was carried out for the nine formulations. The optimized formulation was subjected to the release kinetics study. Further, the optimized formulation was incorporated into the gel and the diffusion study was compared with the optimized nanoemulsion and marketed the gel.

Stability studies

The stability study is an important criterion to be considered for the evaluation of nanoemulsions. Nanoemulsions are characterized by its high stability than the other dispersed systems. The various stability studies conducted for nanoemulsions include: (1) Thermodynamic stability studies, and (2) accelerated stability studies.

Thermodynamic stability studies

The selected formulation was subjected to different thermodynamic stability studies tests to assess their physical stability.[23] The process involved 3 cycles, initially heating and cooling cycles were carried out for 6 times, followed by alternately heating and cooling at 40°C and 4°C respectively, this is followed by centrifugation at 3500 rpm for 30 min. Each cycle was observed for changes in the formulation due to phase separation.

Accelerated stability studies

Accelerated stability studies were performed on optimized formulation. Three batches of the nanoemulsions were taken in glass vials and were kept at a temperature of 30°C, 40°C, and 60°C at ambient humidity condition.[24] The samples were withdrawn for studying drug content as per standard procedure mentioned in International Conference on Harmonisation (ICH) Q1 guidelines. The amount of the drug degraded at each time interval was calculated. Order of the reaction was determined by graphical method. The degradation rate constant (*K*) was determined for each temperature.

RESULT AND DISCUSSION

Screening of oil

Flurbiprofen is lipophilic in nature with log $P = 4.09$. Thus, the drug gets dissolve in lipophilic solvent. As the dissolved drug can only permeate across the skin, solubility of the drug in the oil phase is an important criterion. Out of the oils used for the screening, peppermint oil showed highest solubility as shown in Figure 1. The oils selected were castor oil (long chained) and IPM (long and branched chain) on the other hand eucalyptus oil and peppermint oil which were short chained. Peppermint oil contains menthol as the chief constituent

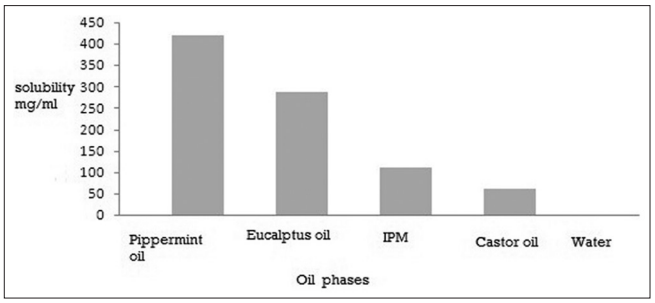

Figure 1: Screening of the oil phases

which is a short chained cyclic compound. In general, short chain oils are capable of overcoming the problems related to the stability of nanoemulsions including Ostwald's ripening due to its steric property.[25] Peppermint oil showed highest solubility of the drug in it. Thus peppermint oil is selected for formulating nanoemulsion.

Screening of surfactant

The difficulty related with the formulation of nanoemulsions is the toxicity due to excipients. Generally, a large amount of surfactant are needed to be added for stabilizing the system, which often results in the local toxicity when applied to the skin. Thus, selection of the surfactant is an important criterion to be considered. Nonionic surfactants produce less toxicity than other anionic or cationic surfactants for which the irritant property is reported. Along with low toxicity, lower critical micelle concentration is also reported. Another preferable condition to be taken into account is the selection of surfactant of proper hydrophilic–lipophilic balance (HLB) value. Generally, hydrophilic surfactants and cosurfactants were taken into considerations as it reduces the energy to form nanoemulsions. The required HLB value of the surfactant to form nanoemulsions should be >10.[26]

Thus, for screening of surfactant nonionic surfactants were only considered. Various nonionic surfactants that were used include - Tween 40, Tween 80, and Tween 20. These surfactants were known to produce less effect to the skin surface pH as they were uncharged. Moreover, Tween family of surfactants was approved by Food and Drug Administration as generally regarded as safe.

The study for the selection of the surfactant involved the solubilization of the selected oil phase in the surfactant solutions. The one which showed the highest solubilization is selected as the surfactant. The study from Figure 2 showed Tween 20 having highest solubilization capacity. The reason behind this may be due to the smaller chain length of Tween 20 than the others. By chemical nature, Tween 20 is lauric acid derivative having shorter chain length than Tween 80 and Tween 40 which are oleic acid and palmatic acid derivative having longer chain length than that of Tween 20. Thus, due to shorter chain length Tween 20 shows greater penetration into the oil phase that enables greater solubilization, which was selected as the surfactant.

Screening of cosurfactant

Cosurfactants are generally short to medium chain alcohols. Cosurfactant is used to reduce the interfacial tension and to enhance mobility of the hydrocarbon tail of surfactants.[27] Alcohols such as ethanol, propanol, IPA, butanol, and octanol were reported to be used in the preparation of nanoemulsions and microemulsions as cosurfactants. All cosurfactants studied were pharmaceutically acceptable. For the screening of nanoemulsions pseudo-ternary phase study was carried out. The area of the nanoemulsions region as obtained from ternary phase study was considered as the main criteria. Greater the area greater the capability of the cosurfactant to stabilize it.[19] For the purpose of the study, all the cosurfactants are mixed with a surfactant at a definite ratio of 2:1. Various pseudo-ternary phase diagram obtained using ethanol, propanol, IPA, and butanol. It was clear from the study that the ethanol in Figure 3 had a greater area of nanoemulsions formation. Thus, ethanol was selected as the cosurfactant.

Effect of surfactant and cosurfactant mix in the formation of the nanoemulsions

The formation of the nanoemulsions depends upon the components of the system. Formulation of the nanoemulsions zone is depicted by the help of phase diagram study. Peppermint as the oil phase, Tween 20 and the ethanol as the surfactant and cosurfactant respectively were screened for the formulation. The ternary phase study was done by using various ratios of surfactant and cosurfactant as 1:0, 1:1, 1:2, 1:3, 1:4, and 1:5, respectively mixed with the oil in different ratios ranging as oil: Smix ratio of 1:9, 1:8, 1:4, 1:5, 1:2, 4:1, and 9:1. The ratio 1:9 of oil Smix was found to produce stable nanoemulsions for almost all the Smix. The Smix ratio 1:2 showed the highest area of nanoemulsions followed by 1:1 as obtained from Figure 4. The Smix ration of 1:0 showed poor flow property resulting information of nanogel with poor flow property. Even a phase study was done using only cosurfactant, ethanol without any combination of the surfactant as 0:1. It was observed that such a composition resulted in phase separation, which justified that cosurfactant alone cannot act as a stabilizing agent but can be an aid to enhance the stability of the system. It was concluded that a high amount of Smix is required for stabilizing the nanoemulsions. Thus from the study we obtained that 5-10% oil, 40-50% of Smix were suitable to form nanoemulsions in various combination. The optimization of the combination was done by using 3^2 factorial designs.

Drug–excipient interaction study

Test for interaction between drug and excipients are necessary for the integrity of the formulation. Studies involve FTIR study and DSC study. The FTIR study data obtained is illustrated in Figure 5.

Infrared study shows characteristic peak of flurbiprofen at around 1700/cm and around 2920/cm due to its characteristic

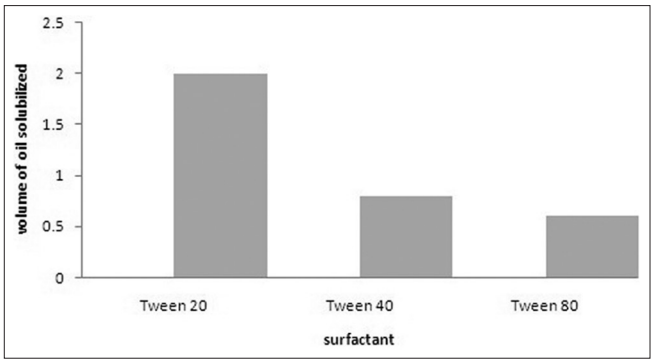

Figure 2: Screening of the surfactant phases

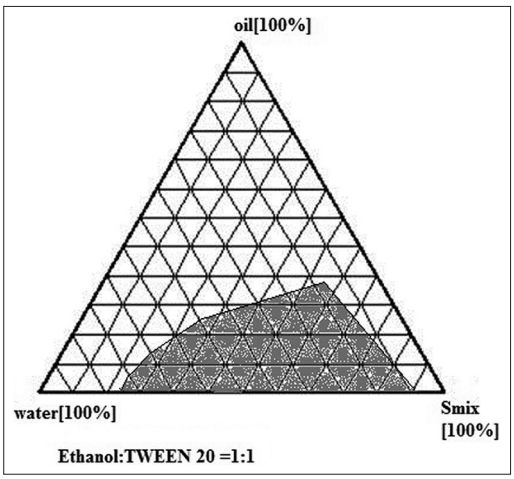

Figure 3: Ternary phase diagram for the screened ethanol as cosurfactant

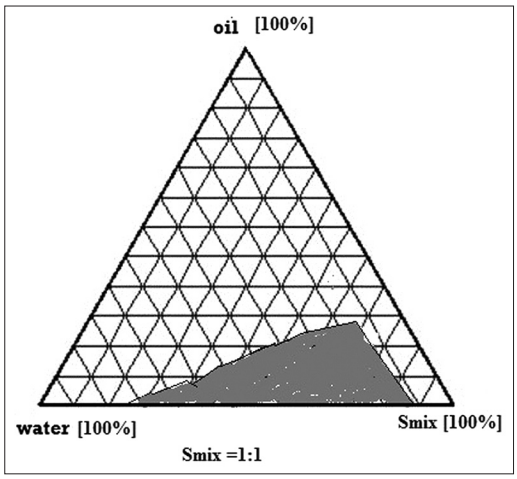

Figure 4: Screened of the surfactant–cosurfactant mixture

carboxyl and hydroxyl stretching.[28] IR graphs obtained clearly shows that there was no significant change in the nature of the peaks. This suggests that there was no chemical incompatibility between the drug and the excipients. Further study was done with DSC to examine chemical changes due to increasing in temperature. Figure 6 clearly shows the study. From the DSC graphs, which clearly depicts a sharp peak at a temperature of about 117-118°C formed due to the melting

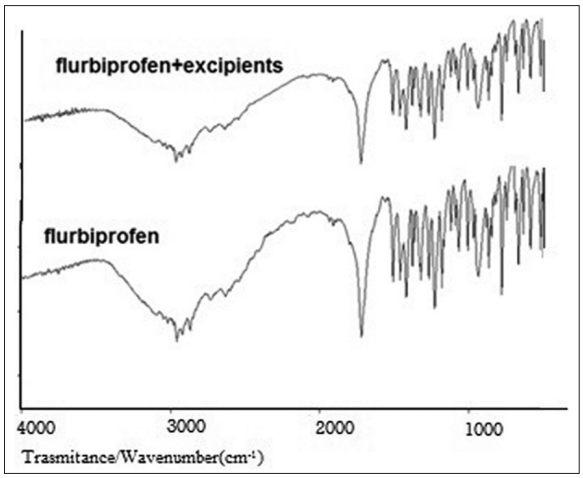

Figure 5: Infrared study for the drug excipients compatibility

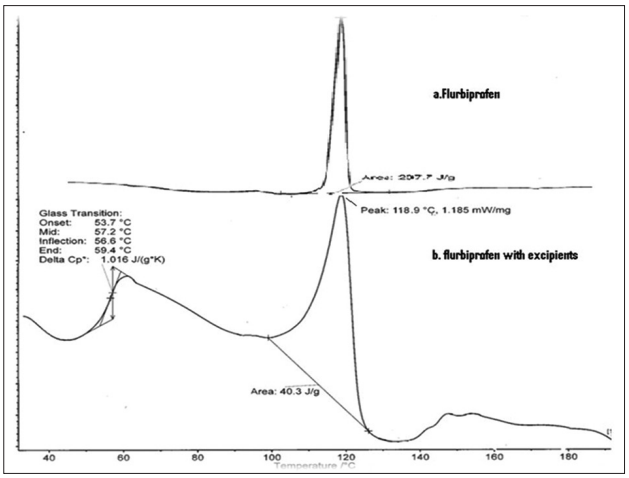

Figure 6: Differential scanning calorimeter study for the drug excipients compatibility

of the drug. Both graphs show the peak to be same with no significant changes.

Thus, from the above studies it can be concluded that there is no drug excipients interaction and can proceed for further formulation studies.

Viscosity

It is clear that lowering of viscosity enhances flow property. The flow property is characteristics of nanoemulsions. Nanoemulsions are categorized with high-flow property. Total nine formulation designed for optimization was subjected to the measurement of viscosity. Out of various data obtained, F_7 formulation showed lest viscosity and high-flow property than other formulations.

Turbidity

The measurement of light dispersion is an important criterion to be evaluated for colloidal or dispersed system. This phenomenon of scattering of light is termed as Tyndall effect. The measurement of the effect can be done either by refractive index or turbidity. In general turbidity is influenced by the particle size of droplet size of the dispersed system. As it was concluded turbidity as the function of wavelength of incident light and particle volume.[29] On the other hand, Rayleigh's equation shows the turbidity as directly proportional to particle size. The turbidity was determined for the nine selected formulation. Out of the formulations, F_2 showed the highest turbidity whereas F_7 showed the lowest. Thus, it can be expected that the droplet size for F_7 is the least among all nine formulations.

Drug release study

The drug release study was done using Franz's diffusion cell. The study was done for 24 h with an optimum interval of sampling. The rat skin was used as the diffusion membrane for the permeation studies. From the study of the release, pattern showed F_4 and F_7 showed a better release than others with the percentage cumulative drug release of 82.92 and 86.57 after 24 h. Thus, it was observed that F_7 showed a better drug release. After the optimization of the nine formulations F7 was found to be the best formulation as it showed less skin entrapment and maximum drug diffusion across the skin. A comparative study of the diffusion was also done using conventional gel and nanoemulsions incorporated gel. The study shows that nanoemulsions as shown in Figure 7 showed higher drug release than nanoemulsions incorporated gel. But the gel form is used as the formulation remains intact and adhered to show a better release.

Optimization of the formulation

The DOE was done by Design Expert™ version 8.0.1.4 and minor correlations of the data were done by use of other software like DOE++™. Design and optimization within design space is a sequential process which involves the initial transformation of raw data such that it fits the model. This is followed by determination of effect of individual variables and the interaction of these variables to the response. This process is followed by the study of the polynomial equation and co-relation of its value with the surface response plots. The model considered for the 3^2 factorial design is either linear or quadratic.

$$Y = b_0 + b_1.X_1 + b_2.X_2 + b_3.X_1.X_2 + b_4.X_1^2.X_2 + b_5.X_1.X_2^2 + b_6.X_1^2.X_2^2 + b_7.X_1^2 + b_8.X_2^2$$

Y represents measured response variable whereas X_1 and X_2 represents independent variables. The b_0 is termed as the population intercept and b_n (n = 1-8) represents the population slope. For general consideration of the effect quadratic equation the coefficients for error were removed by the software itself. The study of the design generally begins with the determination of the transformation of the given variables to improve fit between the response and independent variables. The transformation was determined by using the Box Cox plot that gives the optimum lambda value (λ) depending on which suitable transformation were made. For

the study of the transformation, the difference between the maximum and minimum responses should exceed beyond three. For the given experiment, only the turbidity showed the tendency of transformation of Inverse square root. The other responses (viscosity and drug release) were found to be in limits and did not require any transformation.

After transformation, the next step involves the selection of the model. For all the responses, it was concluded that quadratic models favored the design. Among the various responses the *F* test, *P* value, R^2 (regression) values were determined for responses. It was suggested that *F* value with low *P* value shows high significance. The goodness of the fit was determined by adjusted determination coefficient (Adj. R^2). By removing of the insignificant terms, an Adj. R^2 value near to one can be achieved. The various correlation data for fitting of the model of all three responses are enlisted in Table 3.

Form the table it can be concluded that drug release was found to be highly fitting the model (quadratic) used for the DOE. Thus, as per the ICH guidelines *in-vitro* drug release study can be considered to have a greater impact on the main CQA of the formulation. So its values were given a priority for evaluation of formulations.

Thereafter ANOVA for the factors involved in the formulation was determined to show the effect of the independent variables upon the response study. The significant effect of each factor with their effects describes in Table 4 shows the ANOVA for the drug release.

As we could be inferred from Table 4, it was clear that X_2 (Smix), X_1 (oil) and there interacted value along with quadratic value are significant, from the effect it is clear that the oil produces a negative and Smix produces a positive effect respectively upon response.

The polynomial equation for the responses for drug release was as follows:

$$Y_{\text{Drug release}} = 55.39 - 12.99 \times {}_1 + 11.41B_2 \times {}_2 - 0.86 \times {}_1. X_2 - 7.88 \times {}_1{}^2 + 9.18 \times {}_2{}^2$$

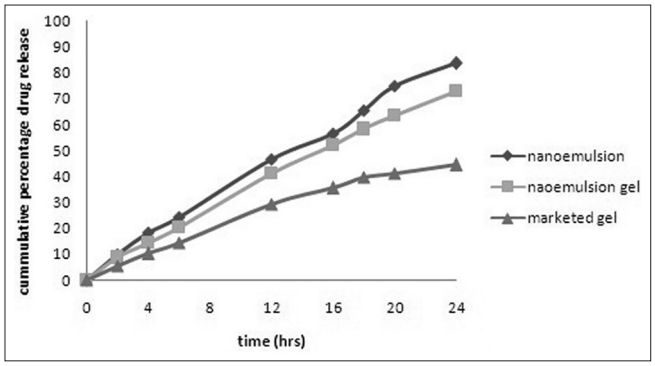

Figure 7: Comparative drug release study of the formulations

Finally, the data obtained from ANOVA was correlated with surface three-dimensional plot and contour plots. It was clear that turbidity and viscosity shows significant effect of the oil phase whereas drug release was affected by the Smix. Figure 8 shows a contour plot and the three-dimensional plot for drug release. From the contour plot, it can be predicted that there was a linear increase of Smix at higher concentration, but there is a gradual decrease in drug release with increase in oil concentration.

Optimization was done by grid search method within the desired design space. It can be assumed that formulation with low viscosity and turbidity with high diffusion was said to have the desired property of good formulation. Thus, ranges were selected such that the above characteristics were fulfilled. Study of the data showed that optimized formulation selected was said to have Smix = 49.61 and oil = 5.5 with the values close to that of F_7. Thus, F_7 was selected as optimized formulation.

Table 3: ANOVA for the various responses fitting in the selected model for design

Response	*F* value	Probe>*F*	R^2	Adjusted R^2	SD
Turbidity	10.46	0.0079	0.9641	0.9469	0.026
Viscosity	11.11	0.0055	0.9711	0.9509	0.55
Drug release	19.19	0.0013	0.9759	0.9587	2.72

SD: Standard deviation, ANOVA: Analysis of variance

Table 4: ANOVA for the variables affecting the response (drug release)

Source	Sum of square	Mean sum of square	*F* value	*P* value	Effects
Model	2091.38	418.28	56.72	0.0001	55.39
X_1 (oil)	1012.75	1012.75	137.34	0.0001	−12.99
X_2 (Smix)	780.85	780.85	105.89	0.0001	11.41
$X_1.X_2$	2.99	2.99	0.41	0.5446	−0.86
X_1^2	171.62	171.62	23.27	0.0019	−7.88
X_2^2	232.62	232.62	31.54	0.0008	9.18

ANOVA: Analysis of variance

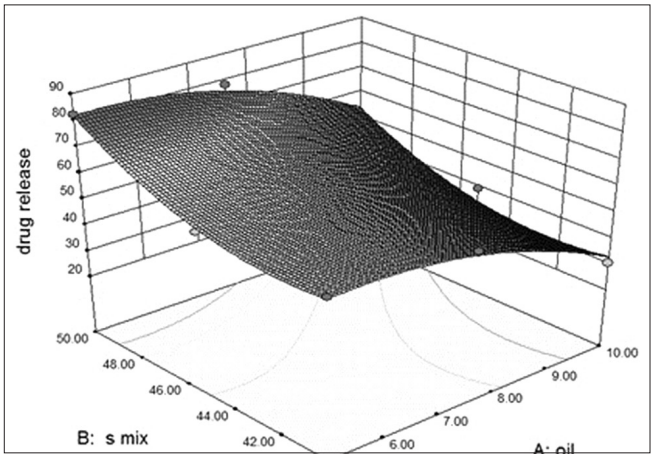

Figure 8: The three-dimensional response surface plot for drug release

Droplet size analysis

Droplet size analysis was performed by using Zetasizer clearly depicts the size of the droplet to be in the range of nanometer. The data obtained in which the particle size distribution was evaluated depending on weight basis was found to be 300 nm.

Stability studies

Stability of the drug substance generally refers to the physical and chemical existence of the dosage form unit. A drug must be evaluated physically, chemically and microbiologically as a part of the stability study.[23] The stability study gives a general idea about the shelf life of the product. Hence, optimized nanoemulsion was evaluated for: (a) Thermodynamic stability study and (b) accelerated stability study.

It was found from the thermodynamic stability study that the product was sufficiently stable as they did not show any change in the physical appearance or resulted in phase separation. The study was further aided with evaluation of turbidity and viscosity as shown in Table 5 that greatly affects the stability of the nanoemulsions. It was concluded that there was no significant change in parameters thus formulation was considered stable.

Table 5: Effect of parameters on thermodynamic studies

Temperature	Time (months)	Turbidity (NTU)	Viscosity (cps)
4.0±0.5°C	0	8.1	13.2
	1	8.4	13.5
	2	8.6	13.8
	3	8.8	13.98
25±0.5°C	0	8.1	13.2
	1	8.6	13.65
	2	8.9	13.97
	3	9.2	14.23

NTU: Nephelometric turbidity unit, cps: Centipoises

Further, the product was studied for three month's stability study; various parameters studied involve turbidity and viscosity at temperatures of 4°C and 40°C respectively. The change in the parameters was found to be insignificant that is, there was no significant change in the nature of the formulation.

For accelerated stability studies, samples were withdrawn at an interval of 0,1,2,3 months. The samples were analyzed by spectrophotometrically following the ICH guidelines Q1. The accelerated stability study was as follows in Table 6.

The order of degradation was determined by graphical method. The first order degradation and the zero order degradation process were evaluated depending upon the R^2. It was determined that zero order degradations shows greater R^2 value.

CONCLUSION

The studies proved peppermint oil as a novel, efficient, effective, and economic excipient in dispersed system for nanoemulsions delivery technology. An effective blend of surfactant and cosurfactant, Tween 20 and ethanol in 1:2 ratio, enhanced the stability of nanoemulsions. It was also observed that a high percent of surfactant and cosurfactant mixture was required for lower droplet size and easy flowability of the nanoemulsions. The various *in-vitro* studies carried out such as viscosity, turbidity, drug release, and correlation with the formulation variable gave the best optimized formulation. It was observed that the optimized formulation had a close resemblance with the F_7 formulation therefore; it was subjected to various physical evaluations and stability testing. The optimized formulation was further incorporated in gel and was subjected to the *in-vivo* analysis which ensured its effectiveness as an anti-inflammatory and analgesic formulation. Thus, from the various data analysis it was concluded that developed nanoemulsions had a great potential for effective transdermal drug delivery.

Table 6: Degradation of optimized nanoemulsions

Temperature (°C)	Time (month)	Concentration (mg)	Concentration degraded (mg)	Percentage of remaining	Log percentage of remaining
30±0.5°C	0	50.00	0.00	100.00	2.00
	1	49.93	0.07	99.65	1.9984
	2	49.90	0.10	99.50	1.9978
	3	49.85	0.15	99.25	1.9967
40±0.5°C	0	50.00	0.00	100.00	2.00
	1	49.88	0.12	99.40	1.9973
	2	49.82	0.18	99.10	1.9960
	3	49.78	0.22	98.90	1.9951
60±0.5°C	0	50.00	0.00	100.00	2.00
	1	49.69	0.31	98.45	1.9932
	2	49.38	0.62	96.90	1.9863
	3	49.24	0.76	96.20	1.9831

REFERENCES

1. Nelson AO, Patric OO, Ndidi CN. Nanotechnology and drug delivery. Trop J Pharm 2009;8:266-72.
2. Tadros TF, Vandamme A, Levecke B, Booten K, Stevens CV. Stabilization of emulsions using polymeric surfactants based on inulin. Adv Colloid Interface Sci 2004;108-109:207-26.
3. Shafiq S, Faiyaz S, Sushma T, Ahmad FJ, Khar RK, Ali M. Design and development of oral oil in water ramipril nanoemulsion formulation: *in vitro* and *in vivo* evaluation. J Biomed Nanotech 2007;3:28-44.
4. Ambade KW, Jadhav SL, Gambhire MN, Kurmi SD, Kadam VJ, Jadhav KR. Formulation and evaluation of flurbiprofen microemulsion. Curr Drug Deliv 2008;5:32-41.
5. Yaan JS, Ansari M, Samaan M, Acosta EJ. Linker based lecithin nanoemulsion for transdermal delivery of aceclofenac nanoemulsion. AAPS PharmSciTech 2007;8:E-104.
6. Fialho SL, da Silva-Cunha A. New vehicle based on a microemulsion for topical ocular administration of dexamethasone. Clin Experiment Ophthalmol 2004;32:626-32.
7. Sommerville ML, Cain JB, Johnson CS Jr, Hickey AJ. Lecithin inverse microemulsions for the pulmonary delivery of polar compounds utilizing dimethylether and propane as propellants. Pharm Dev Technol 2000;5:219-30.
8. Li L, Nandi I, Kim KH. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. Int J Pharm 2002;237:77-85.
9. D'Cruz OJ, Yiv SH, Uckun FM. GM-144 a novel lipophilic vaginal contraceptive gel-microemulsion. AAPS Pharm Sci Tech 2001;2:E5.
10. Zhao X, Chen D, Gao P, Ding P, Li K. Synthesis of ibuprofen eugenol ester and its microemulsion formulation for parenteral delivery. Chem Pharm Bull (Tokyo) 2005;53:1246-50.
11. Shah P. Nanoemulsion: a pharmaceutical review. Sys Rev Pharm 2010;1:24-32.
12. Walstra P. Emulsion stability. In: Becher P, editor. Encyclopedia of Emulsion Technology. New York: Marcel Dekker; 1966. p. 1-62.
13. Shinoda K, Sito H. Effect of temperature on phase equilibra and the type of dispersion of ternary phase system containing water, cyclohexane and water. J Colloid Interface Sci 1968;26:70-4.
14. Amani A, York P, Chrystyn H, Clark BJ, Do DQ. Determination of factors controlling the particle size in nanoemulsions using Artificial Neural Networks. Eur J Pharm Sci 2008;35:42-51.
15. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: Anti-inflammatory study of a selected formula. Eur J Pharm Sci 2003;19:203-10.
16. Bass J, Athreya B, Brandstrup N, Brewer E, Di Ianni M, Goldsmith D, *et al*. Flurbiprofen in the treatment of juvenile rheumatoid arthritis. J Rheumatol 1986;13:1081-3.
17. Lomen PL, Turner LF, Lamborn KR, Brinn EL. Flurbiprofen in the treatment of rheumatoid arthritis. A comparison with aspirin. Am J Med 1986;80:89-95.
18. Ozbay L, Unal DO, Cakici I, Fenercioglu A, Erol D. Clinical study on the bioequivalence of two tablet formulations of flurbiprofen. Eur J Drug Metab Pharmacokinet 2009;34:1-5.
19. Shakeel F, Baboota S, Ahuja A, Ali J, Aqil M, Shafiq S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS PharmSciTech 2007;8:E104.
20. Adnan A, Ahmad FJ, Khar RK, Ali M. Nanoemulsion components screening and selection: a techinal note. AAPS PharmSciTech 2009;10:69-77.
21. Tenjarla S. Microemulsions: An overview and pharmaceutical applications. Crit Rev Ther Drug Carrier Syst 1999;16:461-521.
22. Singh S, Gajra B, Rawat M, Muthu MS. Enhanced transdermal delivery of ketoprofen from bioadhesive gels. Pak J Pharm Sci 2009;22:193-8.
23. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 2007;66:227-43.
24. Akter S, Gaurav KJ, Farhan JA, Roop K. Investigation of nanoemulsion system for transdermal delivery of domperidone. Curr Nanosci 2008;4:381-90.
25. Wooster TJ, Golding M, Sanguansri P. Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir 2008;24:12758-65.
26. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: Formulation development and bioavailability assessment. Int J Pharm 2001;212:233-46.
27. Attwood D. Microemulsion. In: Kreuer J, editor. Colloidal Drug Delivery Systems. New York: Marcel Dekker; 1994. p. 31-71.
28. Paradkar A, Maheshwari M, Tyagi AK, Chauhan B, Kadam SS. Preparation and characterization of flurbiprofen beads by melt solidification technique. AAPS PharmSciTech 2003;4:E65.
29. Melik DH, Fogler HS. Turbidimetric determination of particle size distributions of colloidal systems. J Colloid Interface Sci 1983;92:161-89.

How to cite this article: Aiswarya G, Reza KH, Rajan RK. Development, evaluation, and optimization of flurbiprofen nanoemulsions gel using quality by design concept. Asian J Pharm 2015;9:35-43.

Source of Support: Nil. **Conflict of Interest:** None declared.