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Formulation, Development Of Ibuprofen-Olive Oil Nanoemulsion: 3^2 Design Approches

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Abstract:

Ibuprofen, a phenyl propionic acid derivative, has been chosen as model drug in this study. It is postulated that nanoemulsion may serve as a promising drug delivery strategy to improve the oral absorption. The results indicated that compared to Ibuprofen suspension, nanoemulsion preparation significantly improved anti-inflammatory properties of the drug. Nanoemulsion system in improves the solubility and oral bioavailability of ibuprofen. A pseudoternary phase diagram was constructed in order to assess and identify a nanoemulsion region from oil (olive oil), surfactant (glycerol) and different sucrose esters (co-surfactant). Upon identifying such nanoemulsion region, the colloidal system was then developed as a carrier system for ibuprofen. The selected nanoemulsion (NE) region was characterised by measuring droplet size, polydispersity index, zeta potential and morphology. In addition, in vitro study were conducted to evaluate the ability of ibuprofen loaded nanoemulsion in enhancing the oral bioavailability of the drug upon administration.

Key Words: Silicone nanoemulsion, Oil-in-Water emulsion, Olive oil, Ibuprofen, pseudoternary phase diagram

Introduction-

Nanotechnology in pharmaceutical sciences absolutely has emerged at a great extent from the last couple of years. Nanoemulsions are systems that are thermodynamically stable, and have a defined combination of surfactant/cosurfactant, oils, and water [1]. When lipophilic drugs are applied topically, they are retained in the superficial skin layers. Local or systemic delivery of an active ingredient enhances its content by topical delivery of NEs using different types of mechanisms. NEs allow the entrapment of a higher quantity of drug in comparison to conventional topical preparations (e.g. creams, ointments, lotions, and gels). The solubility of poorly water-soluble drugs can be increased by NEs, in which drugs are dispersed in an oil droplet phase [2]. Different compositions of the NE enhance penetration of drug through the diffusional barrier of the skin. [3].

Examples of nonsteroidal anti-inflammatory drugs (NSAIDs) which have been formulated in nanoemulsion forms to enhance their skin permeations include ketoprofen, ibuprofen, indomethacin, aceclofenac and celecoxib etc [4].

Ibuprofen, a phenyl propionic acid derivative, plays a significant role in the treatment of rheumatoid arthritis, osteoarthritis and related conditions. This molecule has been chosen as model dug in this study. This is because ibuprofen suffers poor solubility and gastrointestinal absorption upon oral administration. Thus, it is postulated that nanoemulsion may serve as a promising drug delivery strategy to improve the oral absorption. Currently, ibuprofen is commercially available in the market as tablets, gel and oral suspensions [5].

The results indicated that compared to Ibuprofen suspension, nanoemulsion preparation significantly improved anti-inflammatory properties of the drug.

In this study, the ability of nanoemulsion was investigated in improving the solubility and oral bioavailability of ibuprofen. A pseudoternary phase diagram was constructed in order to assess and identify a nanoemulsion region from oil (olive oil), surfactant (glycerol) and different sucrose esters (co-surfactant) [7]. Upon identifying such nanoemulsion region, the colloidal system was then developed as a carrier system for ibuprofen. The selected nanoemulsion (NE) region was characterised by measuring droplet size, polydispersity index, zeta potential and morphology. In addition, in vitro study was conducted to evaluate the ability of ibuprofen loaded nanoemulsion in enhancing the oral bioavailability of the drug upon administration.

Materials and Methods

The active API ibuprofen, Pluronic 17 R4, sodium chloride and all other chemicals were of analytical grade and used as provided.

Preparation of nanoemulsion

Titration method was used to prepare using oil (olive acid), % NaCl concentration, surfactant (Pluronic 17 R4) and purified water (continuous phase). Briefly, oil phase mixed with Smix, that is, oil: Smix (0–3:3–0), was taken in various ratios (1–9:9–1), titrated with purified water and added dropwise to internal phase (drug loaded) with constant stirring. Pseudo ternary-phase diagrams were constructed to evaluate the clear nanoemulsion [8].

Construction of Phase Diagrams

Mixtures (4 g each) of lipid/surfactant, in the following ratios, were prepared in separate 100-mL volumetric flasks: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Water was then added to each flask at 5% w/w, intervals.

Experimental design for optimization

DESIGN-EXPERT software (Stat- Ease Inc., Minneapolis) was used for this statistical experimental study. To detect the influence of various independent variables on responses, 3^2 (three level-two factor) response surface methodology was employed for formulation optimization. For this study independent variables were selected the % Pluronic 17 R4 concentration (X_1) and % NaCl concentration (X_2), which were varied at three levels (−1, 0, +1). The dependent factors were select Globules size (nm) (Y_1) and Polydispersity index (Y_2). The statistical design shows by software for dependent and independent variables is given in table 1[9]. For optimization, the effect of independent variables (X_1, X_2) on dependent variables (Y_1, Y_2) was modelled by using the following equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_1 x_1 + \beta_3 x_1 x_2 + \beta_4 x_1^2 + \beta_5 x_2^2 \qquad (1)$$

Where,
Y is the response, β0 is the intercept and β1–β5 is regression coefficients. x_1, x_2 are individual effects. x_1x_2 is the interaction effect and x_1^2, $x_2^2$are the quadratic effects. The significance of the model was evaluated at P <0.05 level using One-way ANOVA [10].

Table no .1. Formulations batches for nanoemulsion

Batch no	Pluronic 17 R4 surfactant (%)	Sodium chloride (%)	Mean globules size (nm)	Polydispersity index
1	4	5	312	0.184
2	6	8	225.8	0.015
3	6	2	544	0.21
4	4	2	380	0.195
5	2	8	275	0.031
6	6	5	515	0.238
7	4	8	369	0.124
8	4	5	412	0.194
9	2	5	268	0.044
10	4	5	533.5	0.211
11	4	5	526	0.199
12	4	5	517	0.187
13	2	2	230	0.166

Characterizations

Diffusion study
The ex-vivo diffusion of the ibuprofen from the nano-formulations was studied as following way. A method similar to membrane release studies was utilized during the ex vivo skin diffusion studies, except that the skin circles were used.

Partition coefficient
The estimation of partition coefficient of nanoemulsion was carried out in octanol/water at 37^0 C. The graduated tubes was filled with water and octanol (50 ml each). This was followed with the addition of 30 mg equivalent weight of ibuprofen nanoemulsion in each flask and mechanical shaking of mixtures for 24 hours

Zeta potential
The zeta- potential, droplet size and size distribution of the ibuprofen nanoemulsion were investigated using Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK).

Models of membranes used for the pharmacokinetic study

Human skin biopsies
Human skin biopsies were obtained from cosmetic surgery in women. Before the experiment skin stored at -20^0C and frozen skin thawed at room temperature immediately before experiment

Treatment for inflammation

To investigate the anti-inflammatory activity of ibuprofen nanoemulsion, NO and PGE2 productions in LPS-stimulated RAW 264.7 cells were examined. For NO determination, RAW 246.7 cells were seeded in 96-well plates at a density of 2 · 105 cells/well and grown for 2 h for adherence.

RESULTS & Discussion-

Phase Diagrams with Individual Lipids

As the primary objective of this study was to compare different medium chain glycerides relevant to dosage form development, all phase diagrams were constructed using a common surfactant, pluronic, olive acid. Since the lipids and surfactant were nonionic in nature and preliminary studies showed that there was no effect of a change in pH on the phase diagrams, distilled water was used as the aqueous medium. A preliminary experiment showed that there was no change in phase diagram if 0.01 M HCl was used as the dilution medium instead of water. The lipid/surfactant/water phase diagrams of four medium chain lipids are shown in **Figure 1.**

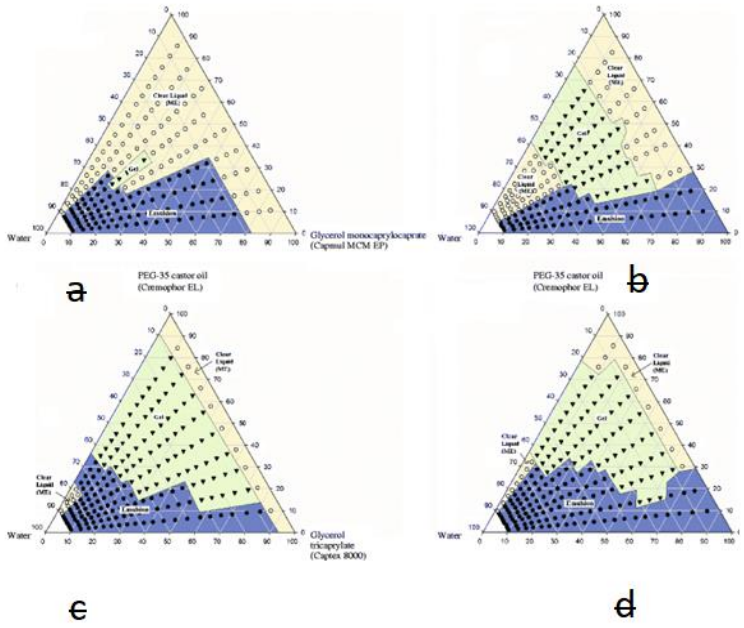

Figure 1: Phase Diagrams

Diffusion study

Data from the skin permeation study is skewed, it will be better discussed in terms of median values as presented in **Figure 2**. Comparing the sixteen nano-formulations, it was found that ibuprofen nanoemulsion obtained the highest median amount per area diffused, followed by all
13 batches

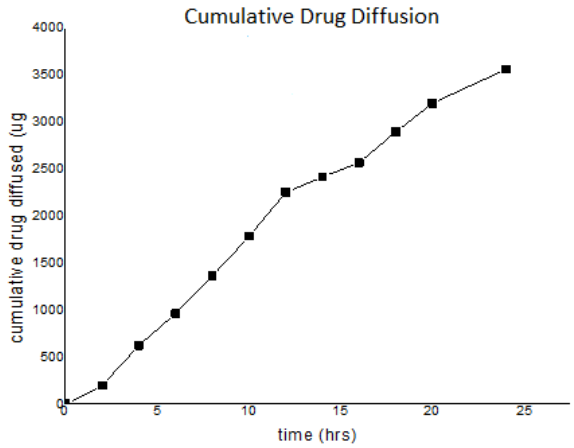

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Figure 2: diffusion studies of optimised batch

Partition coefficient

The estimated partition coefficient of ibuprofen nanoemulsion sample at 37° C was 3.35 which was in close proximity to the reported value of 3.2.

Zeta potential

Zeta-potential values in this study were used to relate the potential short- and long-term stability of the nano- formulations. Literature states that the zeta-potential of −20 mV or +20 mV signify short-term stability, while −30 mV (and lower) or +30 mV (and higher) is regarded as stable. It can be concluded that all the ibuprofen nanoemulsion zeta-potentials are within the range that is regarded as stable emulsions.

In vitro skin permeation study

On human skin, the cumulative permeation of the ibuprofen was 20.36 ± 3.78%, while that for optimized ibuprofen nano-emulsions' was 79.64 ± 5.34% after 24.0 h. Also in artificial/ synthetic membrane was 22.65 ± 1.45 %. In the optimized nano-emulsions', Pluronic 17 R4 is a surfactant by nature; therefore, it possesses the capability to enhance drug permeation by penetrating into the intracellular regions of the stratum corneum and eventually solubilizing the lipid components (Figure 3).

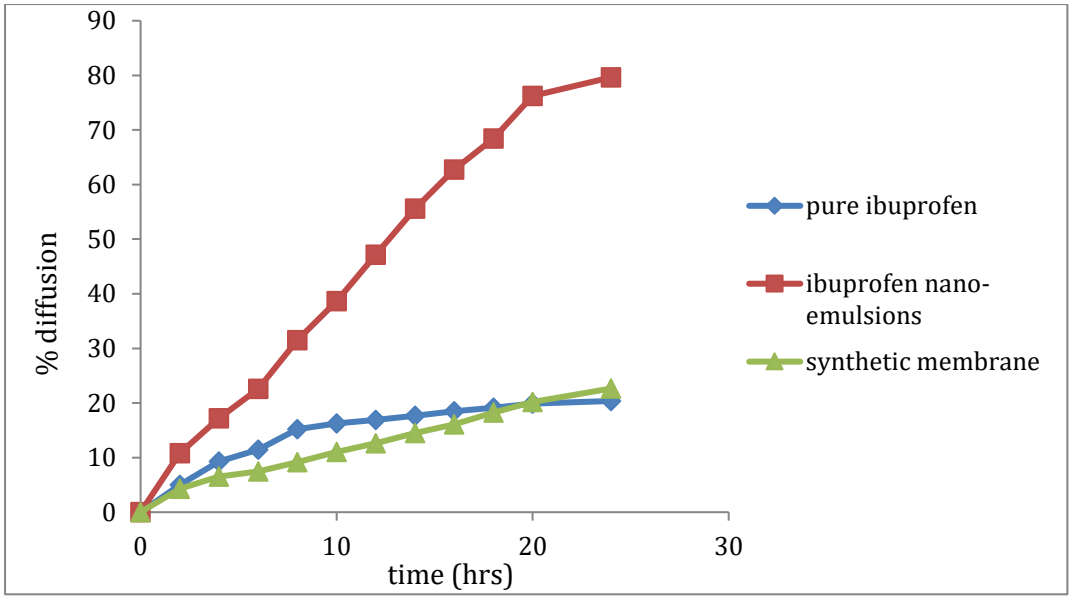


Figure 3: Skin permeation study

Anti-inflammatory activity

Ibuprofen nanoemulsion showed excellent inhibitory effects on NO and PGE2 production. It indicates (Figure 4) that prepared formulation shows anti-inflammatory action.

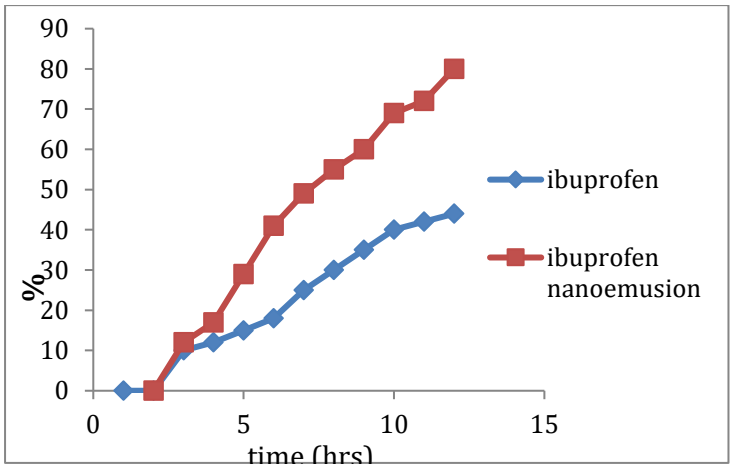
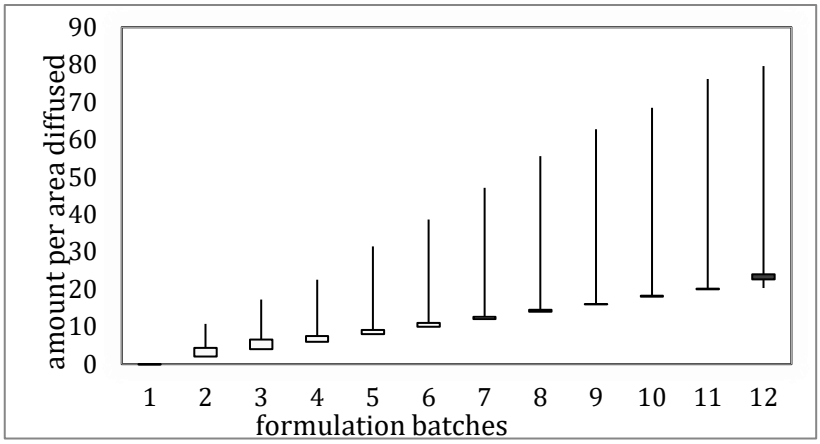


Figure 4: Anti-inflammatory activity

Tape stripping analysis

The tape striping technique is usually utilized to provide more specific data on whether topical delivery of the API within either the SCE or ED had occurred. Hence, the tape stripping was performed after the completion of the ex vivo (skin diffusion) permeation study of the nano-emulsions and nano-emulgels loaded with the ibuprofen to determine the average and median values of statins present in the SCE and ED after 12 h. The all batches of nano-emulgel containing ibuprofen had the good median concentration in the SCE and also delivered the highest median amount per area diffused transdermally, suggesting that ibuprofen encapsulated in the nano- emulgel (although ideal for SCE delivery) remained in the lipophilic stratum corneum. (see **Figure 5)**



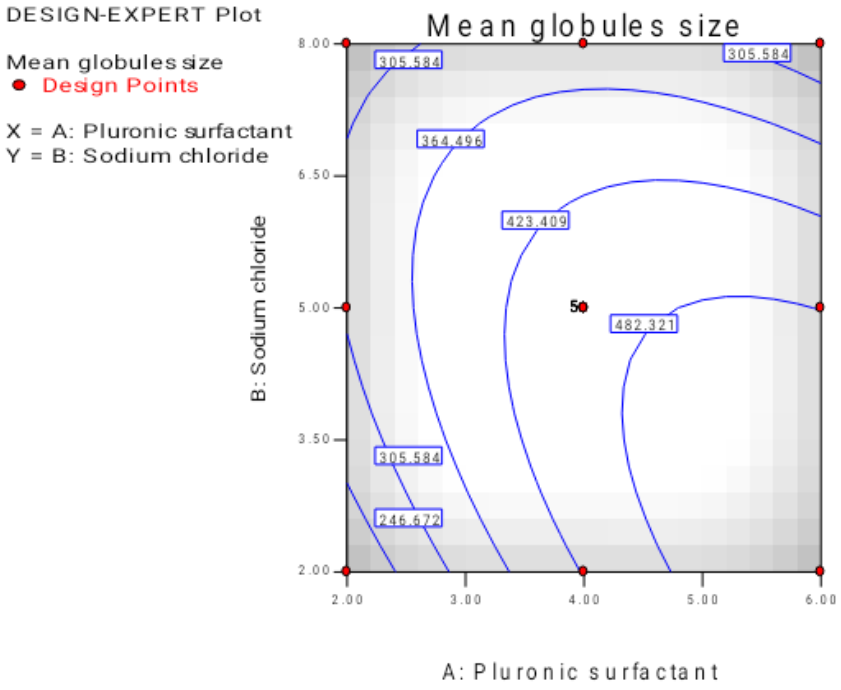


Figure 5: Tape stripping analysis

Optimization

(a)

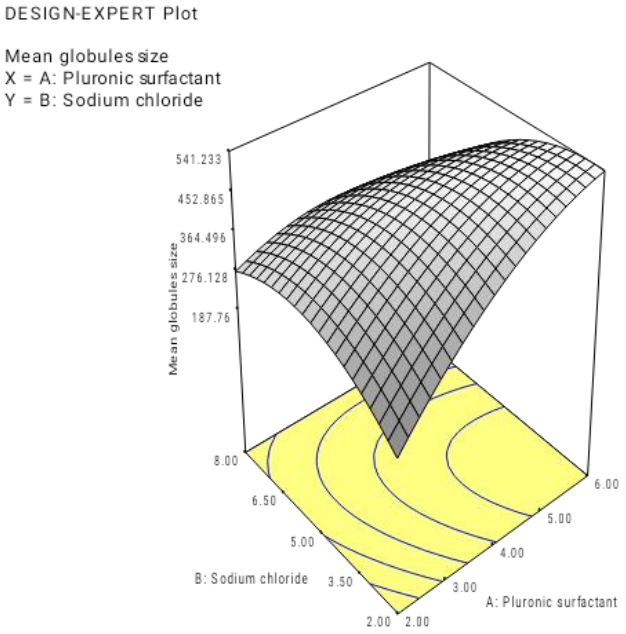
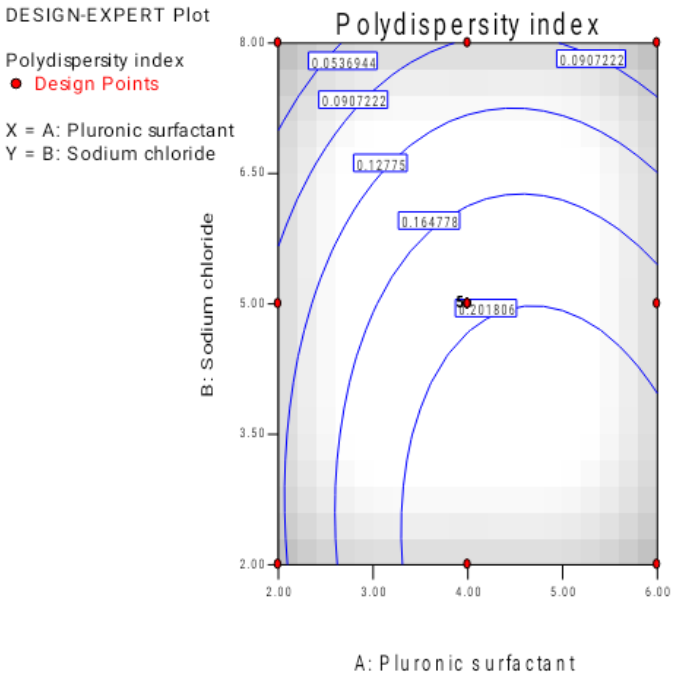
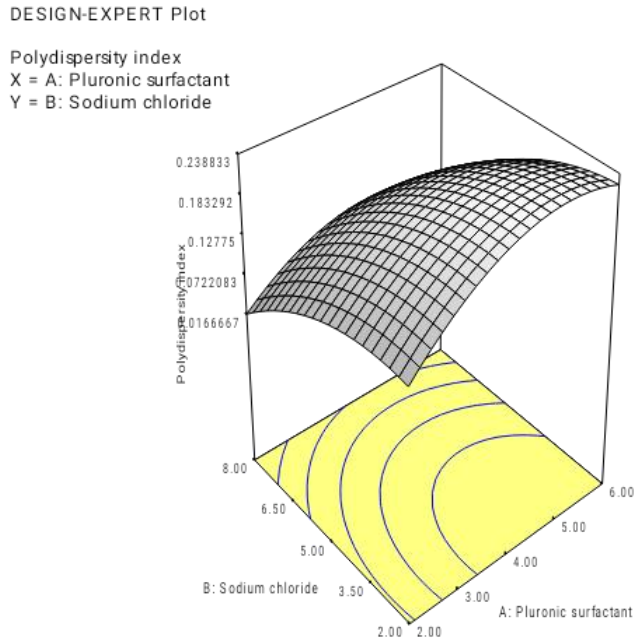


Fig.6. 2D graph and 3D graph for mean globules size



(a)



(b)

Fig.7. 2D graph and 3D graph for mean polydispersity index

A 3^2 response surface methodology was employed to diagnose the effect of independent variables (X_1, X_2) on dependent variables (Y_1, Y_2) with a minimum number of experimental runs. To analyze the effect of these independent variables, 2D Figure 6a, 7a and 3D Figure 6b, 7b counter plots were constructed. The selected independent factors for study were % Pluronic concentration (X_1) and NaCl concentration (X_2), whereas dependent variables for this study were selected as Globules size (nm) and Polydispersity index. The Globules size in the range 225.8 to 544 nm and Polydispersity index in the range of 0.015 to 0.238 found for all 13 experimental runs at three levels, which are enumerated in table 1. The mathematic relation between dependent variable and independent variables are investigated by deriving polynomial equations and counterplots. For the quadratic model of Y_1 response, correlation coefficient (R^2) value was 0.742281 and for the linear model of Y_2 response R^2 value is 0.792132, signifying good fit (shown in table 3). For Globules size (Y_1) and Polydispersity index (Y_2) response following equations was obtained.

$Y1=+458.33+85.30\ X1-47.37X2-62.42\ X1^2-79.42\ X2^2-90.8\ X1\ X2$….(2)

$Y2= +0.20+0.037\ X1-0.067\ X2-0.054\ X1^2-0.035\ \ X2^2-0.015X1\ X2$…(3)

Table No.2. Summary of results of regression analysis for responses Y1 and Y2.

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	REMARKS
RESPONSE Y1						
Mean	2006501	1	2006501			

Linear	57118.15	2	28559.07	2.282667	0.1525	
2FI	32978.56	1	32978.56	3.221466	0.1063	
Quadratic	45169.79	2	22584.9	3.36626	0.0946	Suggested
Cubic	9636.173	2	4818.087	0.645368	0.5632	
RESPONSE Y2						
Mean	0.307077	1	0.307077			
Linear	0.035014	2	0.017507	5.255078	0.0276	
2FI	0.0009	1	0.0009	0.249887	0.6291	
Quadratic	0.018211	2	0.009106	4.48764	0.0557	Suggested

Table No.3. ANOVA of models for Y1 and Y2

Source	**Std. Dev.**	**R-Squared**	**Adjusted R-Squared**	**Predicted R-Squared**	**PRESS**	**REMARK**
RESPONSE Y1						
Linear	111.8538	0.313438	0.176126	-0.37154	249937.7	
2FI	101.1787	0.49441	0.32588	-0.65218	301077.6	
Quadratic	81.90968	0.742281	0.558196	0.159208	153218.2	Suggested
Cubic	86.40393	0.79516	0.508384	0.629773	67466.73	
RESPONS Y2						
Linear	0.057719	0.512437	0.414924	-0.02035	0.069719	
2FI	0.060014	0.525608	0.367478	-1.1576	0.147426	
Quadratic	0.045045	0.792132	0.643656	-1.05796	0.140618	Suggested
Cubic	0.009571	0.993297	0.983913	0.990213	0.000669	

In above equations, positive and a negative value represent the synergistic and antagonistic effect, respectively. **Table 3** shows the ANOVA of the model for Y_1 and Y_2 response. For entrapment efficiency (Y_1) factor, quadratic equation predicts that it was affected by the independent variables likeX_1, X_2 and X_1^2 . Equation (3 and 4) indicates, the positive value has positive/ synergistic effect. While a negative values has antagonistic effect on response. [16]. Also, the effects of these independent variables on Globules size and Polydispersity index were significant at P <0.05. The p-values of both quadratic and Linear model were also less than 0.05. Both the models were significant at F values of 3.36 and 4.48 at P <0.05. the diagnostic case statistics for various response variables with actual, predicted and residual values

Conclusion

Nanoemulsion has the potential of enhancing the oral bioavailability of i792buprofen. A pseudoternary phase diagram was successfully constructed to optimise the concentration of oil, surfactant and co-surfactant mixture in order to determine the nanoemulsion region suitable for drug delivery. We have identified that the combination of olive oil, SE L-1695 and glycerol produced a large nanoemulsion region which could be utilised for drug delivery. In vitro study utilising gut epithelium lining showed that the P_{app} for the optimised nanoemulsion loaded with 3% (w/w) ibuprofen displayed

10.6 times higher drug transport than the control formulations. “Furthermore, the oral bioavailability for ibuprofen nanoemulsion was 2.2-folds higher relative to the control formulation when evaluated

in vivo”. "Collectively, this work demonstrates that through judicious selection of excipients, a stable nanoemulsion for ibuprofen was developed that provide enhanced oral bioavailability.

References

1. Yousaf A.M., Malik U.R., Shahzad Y., Mahmood T., Hussain T. Silymarin-laden PVP-PEG polymeric composite for enhanced aqueous solubility and dissolution rate: preparation and in vitro characterization. J. Pharm. Anal. 2019; 9:34–39

2. Devadasu V.R., Deb P.K., Maheshwari R., Sharma P., Tekade R.K. Elsevier Inc.; 2018. Physicochemical, Pharmaceutical, and Biological Considerations in GIT Absorption of Drugs

3. Groo A.C., De Pascale M., Voisin-Chiret A.S., Corvaisier S., Since M., Malzert-Fréon A. Comparison of 2 strategies to enhance pyridoclax solubility: nanoemulsion delivery system versus salt synthesis. Eur. J. Pharmaceut. Sci. 2017; 97:218–226.

4. Putra O.D., Umeda D., Fujita E., Haraguchi T., Uchida T., Yonemochi E., Uekusa H. Solubility improvement of benexate through salt formation using artificial sweetener. Pharmaceutics. 2018;10

5. Ma H., Chen G., Wang T., Li Q., Liu Y. Design, synthesis, and biological evaluation of a novel water-soluble prodrug of docetaxel with amino acid as a linker. Chem. Biol. Drug Des. 2016:363–369.

6. Wilson V., Lou X., Osterling D.J., Stolarik D.F., Jenkins G., Gao W., Zhang G.G.Z., Taylor L.S. Relationship between amorphous solid dispersion in Vivo absorption and in Vitro dissolution: phase behavior during dissolution, speciation, and membrane mass transport. J. Contr. Release. 2018; 292:172–182.

7. Schultz H.B., Thomas N., Rao S., Prestidge C.A. Supersaturated silica-lipid hybrids (super-SLH): an improved solid-state lipid-based oral drug delivery system with enhanced drug loading. Eur. J. Pharm. Biopharm. 2018; 125:13–20.

8. Vithani K., Jannin V., Pouton C.W., Boyd B.J. Colloidal aspects of dispersion and digestion of self-dispersing lipid-based formulations for poorly water-soluble drugs. Adv. Drug Deliv. Rev. 2019;142:16–34.

9. Sun C., Gui Y., Hu R., Chen J., Wang B., Guo Y., Lu W., Nie X., Shen Q., Gao S., Fang W. Preparation and pharmacokinetics evaluation of solid self-microemulsifying drug delivery system (S-SMEDDS) of Osthole. AAPS PharmSciTech. 2018; 19:2301–2310.

10. Sindi A.M., Hosny K.M. Preparation and evaluation of protective effect of pumpkin seed oil based self nanoemulsifying oral delivery system against ibuprofen-induced peptic ulcer. J. Drug Deliv. Sci. Technol. 2019;52:415–420.

11. Dizaj S.M., Vazifehasl Z., Salatin S., Adibkia K., Javadzadeh Y. Nanosizing of drugs: effect on dissolution rate. Res. Pharm. Sci. 2015;10:95–108

12. Ahmad J., Amin S., Kohli K., Mir S.R. Construction of pseudoternary phase diagram and its evaluation: development of self-dispersible oral formulation. Int. J. Drug Dev. Res. 2013;5:84–90

13. Mazaleuskaya L.L., Theken K.N., Gong L., Thorn C.F., Fitzgerald G.A., Altman R.B., Klein T.E. PharmGKB summary: ibuprofen pathways. Pharmacogenetics Genom. 2015;25:96–106.

14. Doucet O, Ferrero L, Garcia N, Zastrow L. O/W emulsion and W/O/W multiple emulsion: physical characterization and skin pharmacokinetic comparison in the delivery process of caffeine. International journal of cosmetic science. 1998 Oct;20(5):283-95.

15. Foe FM, Tchinang TF, Nyegue AM, Abdou JP, Yaya AJ, Tchinda AT, Essame JL, Etoa FX. Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. BMC complementary and alternative medicine. 2016 Dec;16(1):1-2.

16. Hou Y., Wang H., Zhang F., Sun F., Xin M., Li M., Li J., Wu X. Novel self-nanomicellizing solid dispersion based on rebaudioside A: a potential nanoplatform for oral delivery of curcumin. Int. J. Nanomed. 2019;14:557–571

17. Horoz B.B., Kiliçarslan M., Yüksel N., Baykara T. Influence of aluminum tristearate and sucrose stearate as the dispersing agents on physical properties and release characteristics of Eudragit RS microspheres. AAPS Pharm. Sci. Technol. 2006;7:1–7

18. Vithani K., Hawley A., Jannin V., Pouton C., Boyd B.J. Solubilisation behaviour of poorly water-soluble drugs during digestion of solid SMEDDS. Eur. J. Pharm. Biopharm. 2018;130:236–246.

19. Paul S., Heng P.W.S., Chan L.W. Improvement in dissolution rate and photodynamic efficacy of chlorin e6 by sucrose esters as drug carrier in nanosuspension formulation: optimisation and in vitro characterisation. J. Pharm. Pharmacol. 2018;70:1152–1163.