PREPARATION AND CHARACTERIZATION OF ETODOLAC BEARING EMULSOMES

VIVEK GILL, ARUN NANDA
Department of Pharmaceutical Sciences, Maharashtra Dayanand University, Rohtak-124001, Haryana, India
Email: gillvivek@gmail.com

ABSTRACT

Objective: Emulsomes are novel vesicular drug delivery system with an internal solid lipid core surrounded by one or more bilayers of phospholipids. Etodolac is a potent anti-inflammatory drug and is a drug of choice for the treatment of various diseases. The present study is focused on the development of emulsomes using etodolac as drug candidates having improved drug loading with sustained-release effect for patient compliance.

Methods: Emulsomes formulation composed of solid lipids (tristearin), phospholipids, cholesterol, stearylamine, and drug (etodolac) were prepared by lipid film hydration method followed by sonication to produce emulsomes of the nanometric size range. All the formulations were optimized by using box-behnken design of experiment considering 3 factors viz. drug to phospholipid ratio (A), tristearin to phospholipid ratio (B), stearylamine to phospholipid ratio (C) at 3 levels lower (-1), middle (0) and upper (+1). The response of the independent variables (A, R, C) was studied on the dependent variable viz. particle size (Y1), zeta potential (Y2), and entrapment efficiency (Y3). The responses were analyzed by design expert software to find out the optimized values within the design space.

Results: Compatibility with excipients was established by FTIR studies. The developed emulsomes were spherical shape vesicles as analyzed by TEM. The optimized batch (OB) was evaluated for particle size, zeta potential, and entrapment efficiency with experimental values 383.1 ± 11.7 nm, 47.2 ± 1.3 mV and 80.1 ± 3.2% and predicted values 390.394 nm, 45.000 mV and 81.642 %, respectively. The experimental values were found in reasonable agreement with predicted values by the design of the experiment. In vitro drug release study showed sustained release of the drug (88.69 % after 24 h).

Conclusion: Etodolac loaded emulsomes is a novel drug delivery system and found to reliable in terms of various characteristic parameters like particle size, zeta potential, entrapment efficiency, and drug release. 3-factors 3-levels Box-behnken design of the experiment is a suitable design for the optimization of emulsomes.

Keywords: Emulsomes, Etodolac, Box-Behnken design, Phospholipid, Tristearin

INTRODUCTION

Lipid-based drug delivery systems have gained the attention of researchers in the recent few decades due to their certain advantages over other drug delivery systems. Out of various lipid-based drug delivery system viz. liposomes, solid lipid nanoparticles, niosomes, ethosomes, emulsomes, glycerosomes, etc [1, 2]. Emulsomes have been proven to be advantageous for the delivery of lipophilic drugs. Emulsomes is a lipid-based, vesicular drug delivery carrier having structurally similarity with liposomes but differs only in core component as emulsomes unlike liposomes is composed of solid core [3]. Solid core is composed up of solid lipid which remains solid at 25 °C and having solid to liquid phase transition temperature near to physiological temperature [4, 5]. Due to lipid core, this system becomes advantageous as a carrier system for lipophilic drugs having high drug entrapped by encapsulating lipophilic drugs in lipid core as well as between phospholipid bilayers [6]. Drug entrapped in solid lipid core also exhibits sustained release [7]. Charge inducers are also added in emulsomes to induce surface charge to the vesicles to prevent aggregation of vesicles having a similar charge and stabilize the formulation by providing mono-dispersed vesicles [8]. Emulsomes can be used for the delivery of drugs via oral, parenteral, rectal, topical, intranasal, or ocular [9]. Therefore emulsomes may be considered as an efficient drug delivery system because of biocompatibility, biodegradability, stability in the gastrointestinal tract, high entrapment efficiency, and sustained drug release [10, 11].

Etodolac is a non-steroidal anti-inflammatory drug that is used in the treatment of rheumatoid arthritis that belongs to pyranocarbosylic acid group [12]. Etodolac is a selective COX-2 inhibitor, which is an enzyme responsible for the regulation of prostaglandins (inflammatory mediators) [13]. Etodolac is a white crystalline powder insoluble in water and soluble in alcohols, chloroform, dimethylsulfoxide, and polyethylene glycol. It is a very lipophilic drug and exists as a racemic mixture of (+) S and (-) R enantiomer. Despite its various applications, etodolac causes gastrointestinal disturbances, including peptic ulcers and gastrointestinal bleeding, due to these problems oral use of etodolac is avoided [14]. Response surface type designs of experiments have been widely used for the optimization of various process parameters for vesicular drug delivery systems [15]. The advantage of response surface type designs of experiments is that despite using a single factor, multiple factors can be studied at a time, including their interaction effect [16]. Various types of design using response surface methodology are doehlert matrix (DM), box-behnken design (BBD), and central composite design (CCD) [17]. Out of this box-behnken design has been mainly used to study the effect of 3 factors at 3 levels [18, 19]. In this design quadratic equation is generated for evaluating the mathematical relationship between independent and dependent variables to study the effect of the independent variable on the independent variable [20]. 3-dimensional response curves and 2-dimensional contour plots help in studying the response of 2 factors, including their interaction effect keeping other factors constant [17, 21].

MATERIALS AND METHODS

Material

Etodolac was procured from Balaji chemicals Surat, tristearin, and lecithin were procured from HiMedia, cholesterol was procured from LOBA chemie Mumbai, sephadex G-50 was procured from Sigma aldrich USA, stearylamine was procured from Ottolens, Mumbai. All the other solvents and chemicals used were of analytical grade.

Method of preparation of emulsomes

Emulsomes encapsulating etodolac were prepared by the lipid film hydration method as described by Gupta and Vyas [10] with slight modifications as per laboratory set up. To a 500 ml round bottom flask
(RBF) accurately weighed amount of lecithin, tristearin, cholesterol and stearylamine were dissolved in a small amount of chloroform. In a separate beaker, etodolac was dissolved in methanol. Drug solution in methanol was transferred into RBF having other ingredients dissolved in chloroform. Both the solutions were mixed and the organic solvent was evaporated until complete dryness under reduced pressure using a rotary evaporator. Thin dry film was formed over the inner wall of HPLC injection vial. The drug solution in RBF was sonicated by probe sonicator to obtain nano-sized emulsiomes vesicles. The free un-entrapped drug was removed by passing through sephadex G-50 column [22].

Optimization of emulsiomes formulation

The formulation was optimized using a box-behnken design of experiment which is a response surface type design of experiment wherein responses of 3 factors were studied at 3 levels. Three factors considered were phospholipid to etodolac ratio (A); phospholipid to tristearin ratio (B) and phospholipid to stearyl amine ratio (C) at three levels upper, middle and lower level (+1, 0, -1). A total no. of 17 experiments were designed with 5 center points and 12 points at edges of design space for estimation of pure error sum of squares to choose the best model among linear, two-factor interaction and quadratic model due to the analysis of variance (ANOVA), F-value [23]. The effect of independent variables (A, B, C) were studied on dependent variables i.e. particle size (Y1), zeta potential (Y2) and entrapment efficiency (Y3) by constructing their response surface models along with quadratic equation using design expert software [Design-expert software, version 11, State-Ease Inc., Minneapolis, MN]. The designed quadratic polynomial equation generated is as follows:

\[ Y = b + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X1^2 + b22X2^2 + b33X3^2 \]

Where \( Y \) is a response for each dependent variable; \( b \) is an intercept; \( b1, b2, b3, b12, b13, b23, b11, b22, b33 \) are regressed coefficients from experimental response values of \( Y; X1, X2, X3 \) and their combinations \( (X1X2, X1X3, X2X3) \) and square values \( (X1^2, X2^2, X3^2) \). Each variable was maintained at levels ±1, ±0, ±1 in triplicate in aqueous medium.

Drug and excipients compatibility studies

Etodolac and other ingredients i.e. lecithin, tristearin, stearyl amine, and cholesterol were mixed separately in the ratio of 1:1 and all ingredients, including drug, were also mixed in equal proportions to form a physical mixture. The mixtures were placed in properly sealed glass vials and vials were kept at room temperature. The FTIR spectra of drug, excipients, and their physical mixtures were recorded and analyzed for any deviation in principle peaks of etodolac. The spectra of all the samples were analyzed at 4000-600 cm⁻¹.

Characterization of optimized emulsiomes

Transmission electron microscopy

The emulsiomes were characterized for their shape and surface morphology by using a transmission electron microscope (TEM)(Hitachi 7500, Japan). Phosphotungstic acid (1 %) was used as a negative stain. Carbon coated samples were placed over a copper grid and subjected to TEM analysis.

Particle size and zeta potential measurement

Emulsiomes samples were analyzed in triplicate in an aqueous medium. Average particle size and zeta potential were measured at 25 °C by zeta sizer (PCS, Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK). All the measurements were carried out with an angle of 90 ° at 25 °C [24].

Determination of drug entrapment efficiency

Emulsiomes dispersion was drop-wise filtered through sephadex G-50 column. Filterate was treated with a few drops of triton X-100. Triton X-100 breaks the phospholipid bilayer of emulsiomes vesicles and the entrapped drug comes out in solution, which was analyzed by HPLC technique to determine the area under the curve for evaluation of entrapped drug [24]. Drug entrapment efficiency was calculated using the formula:

\[ \text{Entrapment efficiency (%) = \frac{\text{Amount of drug encapsulated in vesicles}}{\text{Initial amount of drug taken}} \times 100} \]

Drug excipients compatibility studies

The drug-excipients compatibility studies were performed by analyzing FTIR spectrum of samples for any deviation in principle peaks of the drug in the spectrum. As shown in fig. 1 no deviation of principle peaks was observed in spectrum of etodolac when compared with the various spectrum of physical mixtures. Results confirm the physical compatibility of the drug with the excipients used in the formulation.

Preparation and optimization by the box-behnken design of experiment

Etodolac loaded emulsiomes were prepared by the lipid film hydration method. Based on preliminary studies and experimental trial runs, three levels of each independent variable were decided for studies. Box-behnken design of the experiment was considered to be the best suitable design of the experiment for studying the effect of three variables at three levels. On applying the design of the experiment using design expert software total of 17 runs with 5 center points and 12 edge points within the design space were obtained [28]. All the 17 batches were prepared and their responses were recorded as shown in table 2. It was observed from the responses obtained that independent variables (A, B, C) have a

---

**Table 1: Variables used in the box-behnken design of experiment**

| Independent variables | Levels |
|-----------------------|--------|
| \( A = \text{Etodolac to Phospholipid ratio (% w/w of Phospholipid)} \) | -1, 0, +1 |
| \( B = \text{Tristearin to Phospholipid ratio (% w/w of Phospholipid)} \) | 1%, 3%, 5% |
| \( C = \text{Stearylamine to Phospholipid ratio (% w/w of Phospholipid)} \) | 50%, 100%, 150% |
| \( Y1 = \text{Particle Size (nm)} \) | Minimum, Mean, Maximum |
| \( Y2 = \text{Zeta Potential (mV)} \) | Minimum, Maximum |
| \( Y3 = \text{Entrapment efficiency (%)} \) | - |
significant effect on the dependent variables (particle size, zeta potential, entrapment efficiency). Responses obtained from 17 experimental runs were put in design expert software to obtain results fitted to first-order, second-order, and quadratic models along with predicted values and conclusion. Responses were analyzed for the best fit model with significant quadratic (p<0.0001) and insignificant lack of fit (p>0.0525) as shown in table 3. Three dimensional (3-D) response curves were generated as shown in fig. 2. Response curves showed the interaction effect independent variables on the dependent variable and useful in studying the effect of two factors on one response at a time [15]. Polynomial equations were also generated for all three responses (Y1, Y2, Y3). Equations helped evaluate the effect of an individual as well as the interaction of variables on the responses [17]. A positive sign in the equation for a factor represents a synergistic effect on the response, while a negative sign represents the antagonistic effect.

![Compatibility studies by analysing FTIR spectrum of various samples.](image)

**Fig. 1:** Compatibility studies by analysing FTIR spectrum of various samples. (A) Comparison of spectra of etodolac with physical mixture (lecithin+etodolac), (B) Comparison of spectra of etodolac with physical mixture (stearylamine+etodolac), (C) Comparison of spectra of etodolac with physical mixture (tristearin+etodolac)

| Batches | Variables | Responses |
|---------|-----------|-----------|
| A       | B         | C         | Drug: PHL (in mg) | TRI: PHL (in mg) | STR: PHL (in mg) | Size (nm) | Zeta potential (mV) | Entrapment efficiency (%) |
| F1      | 1         | 50        | 10          | 206       | 56.4       | 69.42 |
| F2      | 5         | 50        | 10          | 405       | 57.4       | 76.46 |
| F3      | 1         | 150       | 10          | 564       | 52.1       | 78.67 |
| F4      | 5         | 150       | 10          | 641       | 55.8       | 88.46 |
| F5      | 1         | 100       | 5           | 432       | 27.8       | 74.82 |
| F6      | 5         | 100       | 5           | 488       | 28.3       | 81.67 |
| F7      | 1         | 100       | 15          | 651       | 66.8       | 73.41 |
| F8      | 5         | 100       | 15          | 780       | 59.3       | 83.88 |
| F9      | 3         | 50        | 5           | 189       | 22.6       | 69.68 |
| F10     | 3         | 150       | 5           | 492       | 25.7       | 75.46 |
| F11     | 3         | 50        | 15          | 489       | 68.5       | 72.78 |
| F12     | 3         | 150       | 15          | 678       | 64.4       | 77.67 |
| F13     | 3         | 100       | 10          | 378       | 42.3       | 80.56 |
| F14     | 3         | 100       | 10          | 392       | 45.5       | 82.02 |
| F15     | 3         | 100       | 10          | 365       | 44.1       | 79.96 |
| F16     | 3         | 100       | 10          | 398       | 48.7       | 81.78 |
| F17     | 3         | 100       | 10          | 390       | 44.8       | 79.41 |
Table 3: Analysis of variance (ANOVA) of the calculated model for responses

| Result of the ANOVA | Particle size (nm) | Zeta potential (mV) | Entrapment efficiency (%) |
|---------------------|-------------------|---------------------|---------------------------|
| **Regression:**     |                   |                     |                           |
| Sum of Squares      | 4.013E+05         | 3339.59             | 358.02                    |
| Degree of freedom (df) | 9                 | 9                   |                           |
| Mean square         | 44583.44          | 371.07              | 39.78                     |
| F-value             | 106.99            | 37.75               | 26.16                     |
| p-value             | <0.0001           | <0.0001             | <0.0001                   |
| **Inference**       | Significant       | Significant         | Significant                |
| **Lack of fit tests:** |                   |                     |                           |
| Sum of squares      | 2413.75           | 46.76               | 5.51                      |
| Degree of freedom (df) | 3                 | 3                   |                           |
| Mean square         | 804.58            | 15.59               | 1.84                      |
| F-value             | 6.40              | 2.83                | 1.43                      |
| p-value             | 0.0525            | 0.1705              | 0.3575                    |
| **Inference**       | Not significant   | Not significant     | Not significant            |
| **Residual:**       |                   |                     |                           |
| Sum of Squares      | 2916.95           | 68.81               | 10.64                     |
| Degree of freedom (df) | 7                 | 7                   |                           |
| Mean square         | 416.71            | 9.83                | 1.52                      |

The polynomial equation of response \( Y_1 \) (particle size) is shown as:

\[
Y_1 = 382.60 + 57.63A + 135.75B + 124.63C - 30.50AB + 18.25AC - 28.50BC + 98.50A^2 - 27.18B^2 + 106.58C^2
\]

The model F value of 106.99 for particle size implied that the model was significant \((p<0.0001)\) with a lack of fit value of 6.40, which was not significant \((p = 0.0525)\) [29]. As shown in polynomial equation factors A, B, C and interaction of AC have a synergistic effect, while the interaction of AB and BC has an antagonistic effect on particle size. The predicted R^2 value of 0.9025 is justified with an adjusted R^2 value of 0.9835, which indicates the adequacy of the model to predict the response of particle size. Adeq. precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Adeq. precision value 39.289 indicated an adequate signal [30].

The polynomial equation of response \( Y_2 \) (zeta potential) is shown as:

\[
Y_2 = 45.08 - 0.2875A - 0.0625B + 19.33C + 0.6750AB - 2.00AC - 1.80BC + 5.30A^2 + 5.05B^2 - 4.83C^2
\]

Inference on Response: 
- Significant
- Significant
- Significant

Lack of fit test: 
- Not significant
- Not significant
- Not significant

Residual: 
- Significant
- Significant
- Significant

Fig. 2: 3-dimensional response curves showing: (A1) Effect of factors A and B on particle size; (A2) Effect of factors A and C on particle size; (A3) Effect of factors B and C on particle size; (B1) Effect of factors A and B on zeta potential; (B2) Effect of factors A and C on zeta potential; (B3) Effect of factors B and C on zeta potential; (C1) Effect of factors A and B on entrapment efficiency; (C2) Effect of factors A and C on entrapment efficiency; (C3) Effect of factors B and C on entrapment efficiency.
The model F value of 37.75 for zeta potential implied that the model was significant (p=0.0001) with a lack of fit value of 2.83, which was not significant (p = 0.1705) [29]. As shown in polynomial equation factors C and interaction of AB have a synergistic effect, while factors A, B and interactions of AC and BC have an antagonistic effect on zeta potential. The predicted R² value of 0.7704 is justified with an adjusted R² value of 0.9539, which indicates the adequacy of the model to predict the response of zeta potential. Adeq. precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Adeq. precision value 17.7890 indicated an adequate signal [30].

The prepared mulsomes were examined under transmission electron microscope (TEM) for surface morphological studies. The surface morphology of optimized batch is shown in fig. 3. The results showed that the mulsomes were spherical [9]. All the mulsomes vesicles were observed to be mono-dispersed and no sign of aggregated vesicles was observed. The TEM image is shown in fig. 3.

### Table 4: Optimized values of variables

| Desired | Optimized value of variables | Predicted response |
|---------|------------------------------|--------------------|
|         | DRUG: PHL (% w/w of PHL)     | TRI: PHL (% w/w of PHL) | STR: PHL (% w/w of PHL) | Particle size (nm) | Zeta potential (mV) | Entrapment efficiency (%) |
| 0.797   | 3.834                        | 90.171             | 969                 | 390.394           | 45.000             | 81.642                |

### Particle size and zeta potential

The average particle size and zeta potential of various batches vary from 189 nm to 780 nm and 22.6 mV to 68.5 mV, respectively. The average particle size and zeta potential of the optimized batch were found to be 383.1±11.7 nm and 47.2±1.3 mV, respectively (fig. 4 and fig. 5).

### Drug entrapment efficiency

The effects of various variables were studies on the responses and the responses were analyzed for different parameters as discussed above. Finally, the optimized values of all three variables considered in the study were evaluated by the desirability criteria [31]. Desirability criteria in emulsomes formulation were minimum particle size with maximum zeta potential and entrapment efficiency. The maximum value of desirability is 1 and the values of variables having desirability fig. near to 1 are considered to be optimized [31] for formulating emulsomes. As evaluated by design expert software, the optimized values within design space having maximum desirability of 0.797 are selected for formulation shown in table 4.
In vitro drug release study

The in vitro drug release study of an optimized batch was carried out using a dialysis membrane. The dialysis membrane allows drug molecules to diffuse through but restricts the diffusion of emulsomes [8]. The cumulative drug release during 1st hour of 28.45% indicated the initial burst release, which may be due to the initial release of drug from the phospholipid bilayer. After 1 h the release pattern showed slow release of the drug over 24 h study up to 88.69%. The sustained release of drug is due to the slow release of drug entrapped in solid lipid core composed of tristearin [25]. The overall release study confirms the release of drug in a controlled manner up to 24 h with a cumulative drug release of 88.69%. The cumulative drug release profile of the optimized batch is shown in fig. 6.

CONCLUSION

Etodolac loaded emulsomes were successfully prepared by the lipid film hydration method. The formulation was optimized by using a box-behnken design of experiment and results revealed that box-behnken design is a suitable design of experiment for optimizing formulation by considering 3 factors at 3 levels. Results of various parameters i.e. particle size (383.1±11.7 nm), zeta potential (47.2±1.3 mV) and entrapment efficiency (80.1±3.2 %) showed reasonable agreement with predicted values by the design of experiment as particle size (390.394 nm), zeta potential (45.000 mV) and entrapment efficiency (81.642 %). The difference in
experimental values and predicted values are not significant, which proves the suitability of the box-behnken design of experiment with formulation for optimization. In vitro drug release study showed sustained release of the drug throughout for 24 h. Thus it can be concluded that emulsomes are promising drug delivery system for lipophilic drugs like etodolac with better entrapment efficiency and sustained drug release, which can be optimized by box-behnken design of experiment.

ACKNOWLEDGMENT

The authors are thankful to Director, Aryabhatta Central Instrumentation Laboratory, Maharishi Dayanand University, Rohtak, Haryana, India for allowing to use instrumentation facilities.

FINANCING

No funding was availed in the present work.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflict of interest.

REFERENCES

1. Jain KK. Drug delivery systems—an overview. In: Jain KK, (ed.) Drug Delivery Systems. Totowa: Humana Press; 2008: p. 1-50.
2. Lala RR, Shinde AS, Nandvikar NY. Solid lipid nanoparticles: a promising approach for combinational drug therapy in cancer. Int J Appl Pharm 2018;10:17-22.
3. Lowell GH, Kaminski RW, Van Cott TC, Slike B, Kersey K, Zawoznik E, et al. Proteosomes, emulsomes, and cholera toxin B improve nasal immunogenicity of human immunodeficiency virus gp160 in mice: induction of serum, intestinal, vaginal, and lung IgA and IgG. J Infect Dis 1997;175:292-301.
4. Anseem AS, Yogev A, Zawoznik E, Friedman D. In emulsomes, a novel drug delivery technology. International Symposium on Control and Release of Bioactive Materials; 1994: p. 1369.
5. Anseem AS, Friedman D. Solid lipid nanoemulsion, United States Patent no. 5,662,932(02-09-1997): 1997.
6. Kretschmar M, Anseem S, Zawoznik E, Moshbach K, Dietz A, Hof H, et al. Efficient treatment of murine systemic infection with Candida africans using amphotericin B incorporated in nanosize range particles (emulsomes). Mycoses 2001;44:281-6.
7. Paliwal R, Paliwal SR, Mishra N, Mehta A, Vyas SP. Engineered chylomicron mimicking carrier emulsomes for lymph targeted oral delivery of methotrexate. Int J Pharm 2009;380:181-8.
8. Vyas SP, Subedar R, Jain S. Development and characterization of emulsomes for sustained and targeted delivery of an antimicrobial agent to liver. J Pharm Pharmacol 2006;58:321-6.
9. Ucisik MH, Sleytr UB, Schuster B. Emulsomes meet S-antiviral agent to liver. J Pharm Pharmacol 2015;3:392-400.
10. Gupta S, Vyas SP. Development and characterization of Amphotericin B bearing emulsomes for passive and active macrophage targeting. J Drug Target 2007;15:206-17.
11. Ucisik MH, Kupcu S, Schuster B, Sleytr UB. Characterization of curcu emulsomes: nanoformulation for enhanced solubility and delivery of curcumin. J Nanobiotechnol 2013;11:37.
12. Jones RA. Etodolac: an overview of a selective COX-2 inhibitor. Inflammopharmacology 1999;7:269–75.
13. Colebatch AN, Marks JI, Edwards CJ. Safety of non-steroidal anti-inflammatory drugs, including aspirin and paracetamol (acetaminophen) in people receiving methotrexate for inflammatory arthritis (rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, other spondyloarthritids). Cochrane Database Syst Rev 201;11:CD008872.
14. James E, Reynolds F. Martindale: the extra pharmacopoeia. London: Royal Pharmaceutical Society; 1996.
15. Singh B, Dahya M, Saharan V, Ahuja N. Optimizing drug delivery system using systemic “Design of experiments” part-2 fundamental aspects. Crit Rev Ther Drug 2005;2:215-93.
16. El-Malah Y, Nazal S, Khanfar N. D-optimal mixture design: optimization of ternary matrix blends for controlled zero order drug release from oral dosage forms. Drug Dev Ind Pharm 2006;32:1207–18.
17. Singh B, Kumar R, Ahuja N. Optimizing drug delivery system using systemic “Design of experiments” part-I fundamental aspects. Crit Rev Ther Drug 2004;22:27-105.
18. Moghaddam SM, Ahad A, Aquil M, Imam SS, Sultana Y. Optimization of nanostructured lipid carriers for topical delivery of nimesulide using box-behnken design approach. Artif Cells Nanomed Biotechnol 2017;45:617-24.
19. Rane S, Prabhakar B. Optimization of paclitaxel containing pH sensitive liposomes by 3-factor, 3-level box-behnken design. Indian J Pharm Sci 2013;75:420-6.
20. Rathee S, Kamboj A. Optimization and development antidiabetic phytosomes by box-behnken design. J Liposome Res 2017;28:1-26.
21. Kraisit P, Sarisuta N. Development of triamcinolone acetonide loaded nanostructured lipid carriers (NLCs) for buccal drug delivery using box-behnken design. Molecules 2018;23:982.
22. New RRC. Introduction and preparation of liposomes. In: New RRC. ed. Liposomes: A practical approach. Oxford: IRL Press; 1990. p. 1-104.
23. Govinder S, Pillay V, Chetty DJ. Optimization and characterization of biodegradable controlled release tetracycline microspheres. Int J Pharm 2005;306:24–40.
24. Jones K, Mehnert W, Drechsel M, Bunjes H, Johann C, Mader K. Investigations on the structure of solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J Controlled Release 2004;95:217-27.
25. Pal A, Gupta S, Isivsal A, Dube A, Vyas SP. Development and evaluation of tripalmitin emulsomes for treatment of experimental visceral leishmaniasis. J Liposome Res 2012;22:62-71.
26. Singh N, Verma PK, Nanda S. Nanotechnology based oral formulations of tolbutamide by using biodegradable polymer. Int J Pharm Sci 2019;10:5599-605.
27. Shen J, Burgess DJ. In vitro dissolution testing strategies for nanoparticulate drug delivery systems: recent developments and challenges. Drug Delivery Transl Res 2013;3:409–15.
28. Yasir M, Sara IJS, Chauhan I, Gaur PK, Singh AP, Puri D. Solid lipid nanoparticles for nose to brain delivery of donepezil: formulation, optimization by box-behnken design, in vitro and in vivo evaluation. Artifi Cells Nanomed Biotechnol 2018;46:1838-51.
29. Dholakia M, Dave R, Thakkar V, Rana H, Gohel M, Patel N. Newer opioid analgesic in situ gel of mosfioxacin hydrochloride: optimization using box-behnken stasitical design. Int J Pharm 2019;19;1855-18.
30. Gopi G, Kannan K. Formulation development and optimization of nateglinide loaded ethyl cellulose nanoparticles by box-behnken design. Int J Pharm Pharm Sci 2015;7:310-5.
31. Ferreira SL, Trinca RE, Ferreira HS, Matys GD, David JM, Brando GC, et al. Box-Behnken design: an alternative for the optimization of analytical methods. Anal Chim Acta 2007;597:179–86.