Original Article

Unsatisfied processing conditions in making ifosfamide nanostructured lipid carriers: Effects of various formulation parameters on particle size, entrapment efficiency, and drug loading capacity

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Abstract

Introduction: The objectives of the present investigation were to develop an optimized formulation of ifosfamide (IF) nanostructured lipid carrier (NLC) to sustain the release, to overcome the instability of the drug in an acidic environment during oral administration, drug leakage during storage, drug expulsion, and low drug loading (DL). **Materials and Methods:** The IF NLC was prepared by solvent diffusion technique. Response surface methodology was applied to optimize the formulation. Drug/lipid ratio, organic/aqueous phase ratio, and concentration of surfactant were considered as the formulation variables that mainly affects the particle size (PS), entrapment efficiency (EE) and DL of the NLC. A total of 20 sets of formulations were performed with different ratios of drug/lipid, organic/aqueous phase volume and various concentration of the surfactant. The formulation was evaluated for PS, EE, and DL, differential scanning calorimetry, Fourier transform infrared and *in vitro* dissolution. **Results:** Increasing the aqueous phase volume results in an increase of EE and a decrease in loading capacity. PS also decreases to extent. Increasing the lipid concentration, EE increases and as well the PS. An increase in the concentration of the surfactant resulted in a decrease in PS and a slight increase in encapsulation efficiency and loading capacity. **Conclusion:** The positive impact on the response variables (PS, EE and DL capacity) of the formulation of IF NLC would be obtained only if the processing conditions could be followed.

Key words: Effects of variables, ifosfamide, nanostructured lipid carrier, oral delivery, processing conditions

INTRODUCTION

Oral route is that the most outstanding and well-liked route for administration of medicine for systemic use due to simple administration and patient compliance compared with alternative routes.[1] However for many medicines, it can be an inefficient route of administration, especially for acid labile drugs. In this study, ifosfamide (IF) an alkylating agent is taken as a model drug to be designed for the oral delivery. Chitosan cross-linked sodium alginate nanostructured lipid carriers (NLC) is one of the approaches to overcome the instability of the drug in an acidic environment during oral administration. Solvent diffusion technique recommended to be the best method for the preparation of NLCs by the authors in their previous works[2,3] has been used to prepare IF NLC.

Excipients are enclosed in dosage forms to help manufacture, administration, or absorption. Different reasons for inclusion concern product differentiation, appearance enhancement or retention of quality.[4] However,

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excipients will initiate, propagate or participate in chemical or physical interactions with an energetic, presumably resulting in compromised quality or performance of the medication. Chemical interaction will cause degradation of the active ingredient, thereby reducing the number accessible for therapeutic effect; reaction merchandise could compromise safety or tolerance. Physical interactions will have an effect on rate of dissolution, uniformity of dose or easy administration. Understanding the chemical and physical nature of excipients, the impurities or residues related to them and the way they will interact with different materials, or with one another, forewarns the pharmaceutical technologist of potentialities for undesirable developments.

Excipients might have functional groups that move directly with active pharmaceutical ingredients. As an alternative, they will contain impurities or residues, or form degradation product that successively causes decomposition of the drug substance. They will conjointly cause unwanted effects like irritation of the skin or mucosal surfaces, sensitization reactions or adversely have an effect on appearance of the ultimate formulation. Not solely ought to the proper excipients, the proper quantity of the excipients conjointly to be run down within the formulation of a pharmaceutical failing of which can lead in to sure undesirable effects.

MATERIALS AND METHODS

Materials

Ifosfamide (99.0% purity) was purchased from Sigma Aldrich Co., Ltd. The excipients utilized in the preparation of the nanoparticles, were the following: Glycerol monooleate (GMO) and oleic acid were purchased from metro labs Ltd., Chennai. Lutrol® F 68 (poloxamer 188) was kindly given by BASF India Ltd. Ethanol, acetone and other chemicals were of analytical reagent grade. Water was double distilled.

Preparation of nanostructured lipid carrier

Ifosfamide NLC was prepared by the solvent diffusion methodology.[5] GMO and oleic acid were used as the solid- and liquid-lipid material, respectively. Briefly, weighed GMO, oleic acid and the drug were dissolved into 5 ml of mixed organic solvent of ethanol and acetone (1:1 v/v) in a water bath at 35°C. The resultant organic solution was quickly dispersed into 50 ml of aqueous solution of poloxamer 188 (1% w/v) at room temperature (25°C) beneath sonication at 36 W for 2 min. To this primary emulsion, 2 ml poly vinyl alcohol solution (0.5% w/v) and 10 ml of sodium alginate (1.5% w/v) were added one after the other and emulsified at 30 W for 2 min. 0.5 ml $CaCl_2$ solution was added under stirring condition. The sodium alginate was then ionically cross-linked by adding 6.25 ml of chitosan solution under magnetic stirring for 30 min until NLC suspension were obtained. Prepared NLCs were placed in vacuum desiccator for 24 h at room temperature to evaporate the residual organic solvent that was then used for further experiments.

Experimental design

Preliminary experiments indicated that the variables, such as ratio between the drug and lipid concentration, ratio of organic/aqueous phase volume and the concentration of surfactant were the main factors that affected the particle size (PS), drug loading (DL) efficiency and entrapment efficiency (EE) of the IF NLC. Thus, a central composite rotatable design- response surface methodology was used to systemically investigate the influence of these three critical formulation variables on PS, DL (% w/w) and EE (% w/w) of the prepared NLC. The details of the design are listed in Table 1. For each factor, the experimental range was selected on the basis of the results of preliminary experiments and the feasibility of preparing the NLC at the extreme values. The value range of the variables was drug/lipid ratio (X_1) of 1:3-1:7, organic/aqueous phase ratio (X_2) of 1:5-1:15 and surfactant concentration (X_3) of 0.25-1%. A total of 20 tests were conducted. All the formulations in these experiments were prepared in duplicate.

Determination of drug entrapment efficiency

Drug loading and EE were very resolute by assessing the amount of encapsulated drug inside the nanoparticles.[6] Succinctly, unencapsulated insoluble drug (if any) were first filtered out by filtering the nanoparticle dispersion through 3 μm nitrocellulose membrane filter. Following this, ethanol (9.5 ml) was added in the filtered formulation (0.5 ml) and mixed well with the help of a cyclomixer. This helped extraction of drug from lipid to ethanol. The mixture was then centrifuged for 15 min at 5000 rpm and the supernatant was assembled. The drug engrossment in the supernatant was measured by high performance liquid chromatography (HPLC) assay. In addition to unencapsulated undissolved drug, some portion of the unencapsulated drug might be dissolved in the aqueous phase. Therefore, allowance of dissolvable unencapsulated drug was assessed by ultrafiltration

Table 1: Independent variables and their corresponding levels of NLC preparation for CCRD

Variables	Levels				
	−1.682	−1	0	+1	+1.682
Drug/lipid ratio	1:1.64	1:3	1:5	1:7	1:8.36
Organic/aqueous phase ratio	1:1.59	1:5	1:10	1:15	1:18.41
Concentration of surfactant %	−0.14	0.25	0.57	1	1.29

NLC: Nanostructured lipid carriers, CCRD: Central composite rotatable design

procedure using centrifugal filter tubes 10 kDa molecular weight cut-off. Succinctly, nanoparticle dispersion was put into the centrifugal filter tube and centrifuged for 30 min at 5000 rpm. Then, the allowance of drug in the aqueous phase was very resolute by HPLC assay. EE and DL were calculated using the following equations 1 and 2.[7]

$$EE(\%) = \frac{w_{total} - w_{free}}{w_{total}} \times 100\% \quad (1)$$

$$DL(\%) = \frac{w_{total} - w_{free}}{w_{lipid}} \times 100\% \quad (2)$$

Particle size and zeta potential measurement by dynamic light scattering (DLS)

Particle size analysis and PI of IF NLC were performed using DLS (Zetasizer ZEN 3600, Malvern, UK) with a scattering angle of 90° at 25°C. The IF NLC sample was diluted in distilled water prior to measurement. Zeta potential measurements were also made using an additional electrode in the same instrument.

RESULTS

Formation of ifosfamide loaded nanostructured lipid carrier

The IF NLC was prepared by solvent diffusion technique with three different ratios of lipid viz. 1:3, 1:5, and 1:7. However, based on the nanoparticle recovery and EE, among the three different ratios, 1:3 ratio providing an EE of 77% and DL capacity of 6.14% is selected as the best ratio than the other two. The other two ratios produced low DL, which causes high drug wastage during the preparation procedure itself and larger particles also. These have been repeatedly tried for 3 times, for reproducibility and for consistency.

Particle size and zeta potential measurement by dynamic light scattering

The PS analysis by DLS study showed that the IF NLC have average PS of 223 nm with a minimum PI of 0.194. The particles were having unimodal PS distribution and narrow range. The ZP of the IF NLC was found to be -25 mV and it is sufficiently high to form stable colloidal nanosuspension.

DISCUSSIONS

Preliminary experiments indicated that the variables, such as ratio between the drug and lipid concentration, ratio of organic/aqueous phase volume and the concentration of surfactant were the main factors that affected the PS, DL, and EE of the IF NLC. The formulation was optimized and the PS, DL, and EE of the optimized IF NLC were 223 nm, 6.14%, and 77%, respectively. Although the results of the optimized formulation obtained were found to be satisfactory, certain trails made to optimize the formulation let down in unsatisfied responses.

Influence of aqueous phase volume

The ratio of the oil phase and the aqueous phase showed great impact on the EE of NLC. Aqueous phase volume of 5, 10, and 15 ml were made used in the formulation. Figure 1a clearly shows that increasing aqueous phase volume results in an increase in EE. This could be due to lesser aggregation of the particles in a larger space. Different studies have shown that the aqueous phase volume has paramount effect on the formation of nanoparticles. In a recent study, with increased volume of aqueous phase, increase in drug content of particles prepared by homogenization and sonication was observed.[8] Aqueous phase volume beyond 10 ml was not advantageous, because of lower concentration of nanodispersion without an increase in EE.

Effect of surfactant concentration

The type of compound employed for stabilization has a pronounced effect on PS. The mean PS was found to decrease sharply with an increase in concentration of Lutrol F 68 up to 1% w/v. Higher surfactant concentration reduces the surface tension and facilitate particle partition. The decrease in the PS is accompanied by a rapid tremendous increase in the surface area. Thus, the process of primary coverage of the newer surfaces competes with the agglomeration of the uncovered surfaces. Hence, an increase in the surfactant concentration in the primary dispersion results in rapid coverage of the newly formed particle surfaces. There was an optimum concentration, above which an increase in surfactant concentration did not result in a decrease in PS due to saturation point.[9]

The broadness of the size distribution observed at higher surfactant concentrations could be due to the higher viscosity of the continuous phase, which disperses the stirring energy.[10] Thus, the PI value increased with increasing surfactant concentrations. Stability (high ZP either positive or negative) increased with increasing concentrations of surfactant. Thus, higher stability was observed with formulations having 1% w/v surfactant. An increase in concentration of surfactant resulted in a slight increase in EE and DL, which is shown in Figure 1a and c.[11]

Influence of drug/lipid matrix ratio

Increase in matrix content is expected to raise the EE by providing more space to incorporate the drug. Increment of the lipid content also reduces the escaping of drug

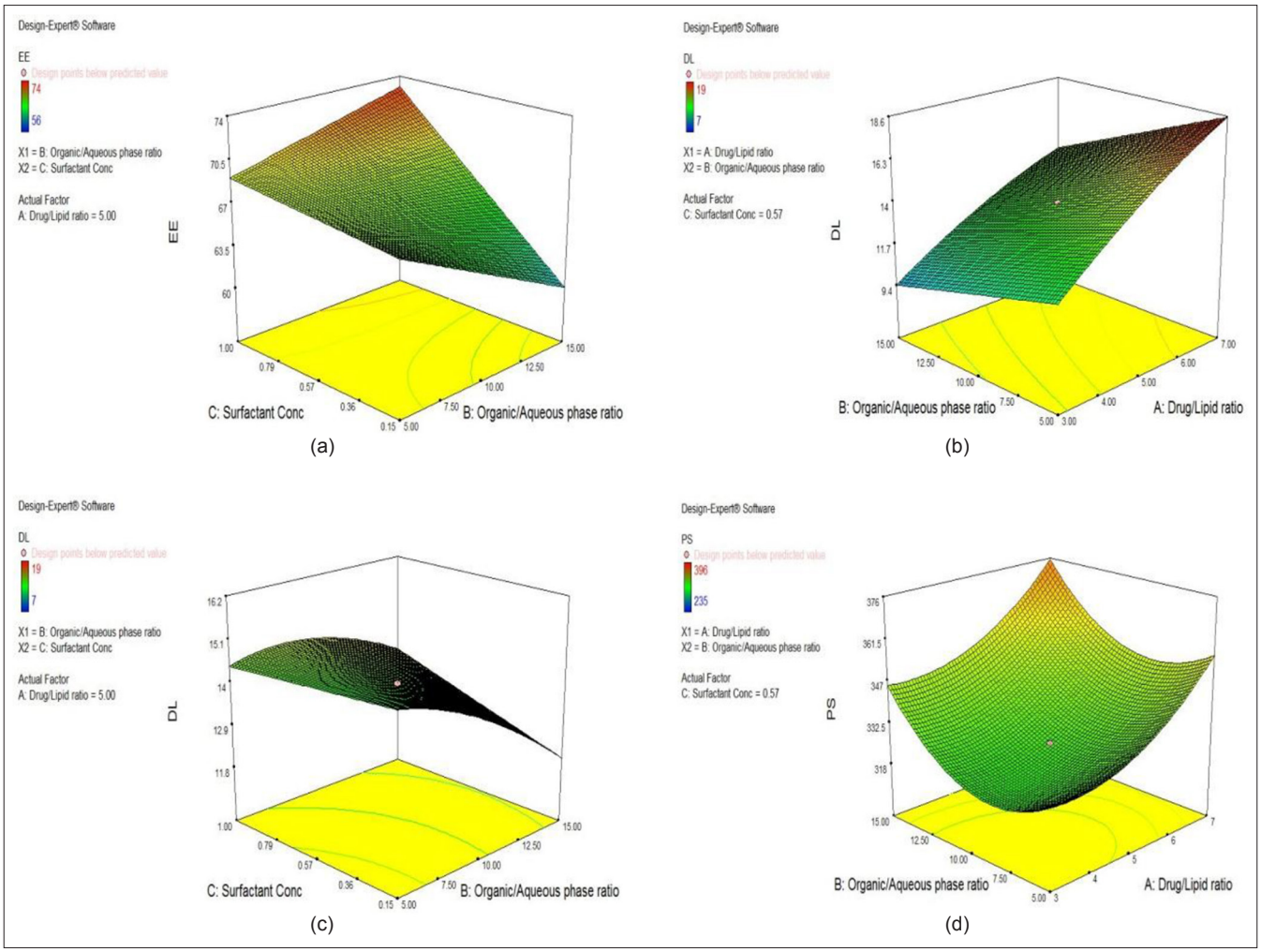

Figure 1: Three-dimensional response surface plots showing the effect of the variable on the response. (a) Effect of organic/aqueous phase ratio and surfactant concentration on the entrapment efficiency; (b) the effect of drug/lipid ratio and organic/aqueous phase ratio on the drug loading; (c) the effect of organic/aqueous phase ratio and surfactant concentration on the drug loading; (d) The effect of drug/lipid ratio and organic/aqueous phase ratio on the particle size

into the external phase, which accounts for an increase in EE.[12] EE significantly increased at drug: Lipid ratio increasing from 1:3 to 1:7, whereas with this increasing ratio an increase in PS was observed. Numerous studies have reported that increasing lipid content results in larger particles and broader PS distribution.[13-15] Larger PS with an increase in lipid content could be attributed to decrease in emulsifying efficiency and increase in particle agglomeration and increased DL as well, which is shown in Figure 1b and d.

CONCLUSIONS

Many stability issues encountered throughout development and post commercialization is ascribed to inadequate matching of the ingredients and their limits in dosage forms, lack of awareness of the complexities of chemical and physical interactions resulting in undesirable effects. Several such problems concern low levels of novel entities formed by drug-excipient interactions and mismatching limits in dosage forms create queries regarding stability, safety or tolerance. Such incidents have in all probability been inflated by the growing sophistication of analytical techniques to notice, establish and quantitate low level impurities, poor knowledge on drug-excipient interaction and limits of excipients in dosage form.

Drug-excipient interactions could take a protracted time to be manifested in standard stability testing programmes, and aren't forever foretold by stress and preformulation studies. It's attainable to scale back the likelihood of such undesirable and costly scenarios by allying knowledge with awareness of excipient reactivity and of the residues that they will contain and also their limits to be utilized in the formulations. Such awareness could facilitate to anticipate undesirable interactions and avoid their incidence. An even handed selection of excipients and their usage limits can

exclude or limit residues promoting undesirable effects. Maybe it might be a topic for a future initiative.

In summary, information of drug-excipient interactions, right excipient and also the right amount of the excipient could be a necessary prerequisite to the development of dosage forms that are stable and of fine quality. It is hoped that this research provides some perspective of this important area of pharmaceutical technology.

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REFERENCES

1. Sachan NK, Bhattacharya A, Pushkar S, Mishra A. Biopharmaceutical classification system: A strategic tool for oral drug delivery technology. Asian J Pharm 2009;3:76-81.
2. Velmurugan R, Selvamuthukumar S, Manavalan R. Multi criteria decision making to select the suitable method for the preparation of nanoparticles using an analytical hierarchy process. Pharmazie 2011;66:836-42.
3. Velmurugan R, Selvamuthukumar S. The analytic network process for the pharmaceutical sector: Multi criteria decision making to select the suitable method for the preparation of nanoparticles. Daru 2012;20:59.
4. Crowley PJ, Martini LG. Excipients in Pharmaceutical Products: Encyclopedia of Pharmaceutical Technology. New York: Marcel Dekker Inc; 2002.
5. Hu FQ, Zhang Y, Du YZ, Yuan H. Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system. Int J Pharm 2008;348:146-52.
6. Das S, Ng WK, Kanaujia P, Kim S, Tan RB. Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: Effects of process variables. Colloids Surf B Biointerfaces 2011;88:483-9.
7. Selvamuthukumar S, Anandam S, Krishnamoorthy K, Rajappan M. Nanosponges: A novel class of drug delivery system - Review. J Pharm Pharm Sci 2012;15:103-11.
8. Budhian A, Siegel SJ, Winey KI. Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content. Int J Pharm 2007;336:367-75.
9. Reddy LH, Vivek K, Bakshi N, Murthy RS. Tamoxifen citrate loaded solid lipid nanoparticles: Preparation, characterization, *in vitro* drug release, and pharmacokinetic evaluation. Pharm Dev Technol 2006;11:167-77.
10. Song X, Zhao Y, Hou S, Xu F, Zhao R, He J, *et al*. Dual agents loaded PLGA nanoparticles: Systematic study of particle size and drug entrapment efficiency. Eur J Pharm Biopharm 2008;69:445-53.
11. Liu J, Gong T, Wang C, Zhong Z, Zhang Z. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: Preparation and characterization. Int J Pharm 2007;340:153-62.
12. Shah KA, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles of tretinoin: Potential in topical delivery. Int J Pharm 2007;345:163-71.
13. Trotta M, Debernardi F, Caputo O. Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. Int J Pharm 2003;257:153-60.
14. Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001;47:165-96.
15. Battaglia L, Trotta M, Gallarate M, Carlotti ME, Zara GP, Bargoni A. Solid lipid nanoparticles formed by solvent-in-water emulsion-diffusion technique: Development and influence on insulin stability. J Microencapsul 2007;24:660-72.

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