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Quality By Design Approach For Development Of Amorphous Solid Dispersions Of Efavirenz By Melt-Quench Technique

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ABSTRACT

Melt-quench method is a novel and an evolving technique to develop amorphous solid dispersions (ASDs) of crystalline compounds to improve their solubility. Efavirenz is a crystalline compound and is practically insoluble in water so that it suffers dissolution limited bioavailability. The major objective of this work was to optimize carrier, plasticizer and cooling temperature as the independent factors used in melt-quench method for preparing Efavirenz ASDs (EASDs) to have maximum solubility and stability which were taken as the responses. Box-Behnken design (BBD) under response surface methodology was employed as the experimental design to develop EASDs. The prepared EASDs were characterized by differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) analysis to investigate the physical state of EFV and the stability of the EASDs upon storage. Solubility was also checked for all the EASDs. The effect of the factors on the responses were analysed found to be significant by analysis of variance (ANOVA) at $p < 0.05$. The DSC and X-RD results indicated that EFV was successfully converted into amorphous form by the applied method. Further, DSC and solubility studies also inferred that slow cooling with high concentrations of the carrier and plasticizer provided maximum stability of these ASDs upon storage by reducing recrystallization. The graphical optimization was performed and inferred that the EASDs prepared at 50% w/w of Soluplus as the carrier, 15% w/w of Poloxamer 188 as the plasticizer with -1.92^{o}C as the cooling temperature in melt-quench method provided EASDs with maximum solubility and stability.

Keywords: Melt-quench method, Amorphous solid dispersions, Efavirenz, Box-Behnken design, Optimization

INTRODUCTION

Human immunodeficiency virus attacks the CD4- T cells responsible for body immunity, thus makes them susceptible to diseases and infections. Antiretroviral drugs (ARVDs) are therapeutic agents for the treatment of retroviral infections, primarily the human immunodeficiency virus type 1 (HIV-1). Antiretroviral treatment therapy against HIV-1 does not eliminate the virus but inhibits its rapid replication and increases the life expectancy of infected people[1]. Efavirenz (EFV) is one of the most widely used anti-retroviral drugs used for the treatment of HIV-1. Efavirenz comes under the class of non-

nucleoside reverse transcriptase inhibitor (NNRTI). WHO latest guidelines also suggest that Efavirenz can be used as an alternate treatment for high dose dolutegravir and reportedly have non-inferior efficacy[2]. But, EFV exhibits extremely low aqueous solubility (9.0µg/ml) and high permeability which comes under the Class-II of BCS classification makes its formulation development into a challenging task[3]. EFV exhibits dissolution limited bioavailability around 40% when taken by oral route due to its intrinsic dissolution rate which is less than 0.1mg/min/cm[2,4]. EFV is currently marketed as tablets and capsules containing the drug in the crystalline form with controlled particle size. Efavirenz is a hydrophobic drug with low density and high flow resistance. Since the particle size and morphology are the critical parameters in the development of formulations for effective GI drug delivery, there is a need to develop the amorphous state of EFV with enhanced solubility related oral bioavailability.[5]

To enhance bioavailability many various formulation strategies were developed by researchers. Inclusion complexes ofefavirenzprepared with ß-cyclodextrin (ß-CD), hydroxypropyl ß-CD(HPßCD), and randomly methylated ß-CD (RMßCD) by Sathigari S et al reportedly enhanced dissolution[5]. High molecular weight of cyclodextrins may source a new problem by enhancing bulkiness of dosage form. Liquid crystal nanoparticles were prepared using sonication and spray drying method to enhance the bioavailability by Avachat et al.[6] for which costly equipment may be process limitation for these two methods. Polymeric micelles of EFV were prepared by Chiappetta et al.[7], also suffered with the previous process related problems. Solid solution of EFV prepared with Eudragit EPO and Plasdone-S-630 by hot-melt extrusion by Sathigari et al.[8] proved that the dissolution rate of drug extrudes was higher than the crystalline drug. In this technique critical parameters to be maintained should be optimized carefully in order to achieve dispersion of drug in the polymer matrix. EFV nanosuspension by using the media milling method reported by Patel et al.[9] enhanced oral bioavailability of EFV 2.49 times higher than marketed formulation. Instability of nanosuspension due to agglomeration and Ostwald's ripening may limit the use of these techniques. Alves LD et al. prepared amorphous solid dispersions (ASDs) of EFV in Povidone K-30 by conventional solvent evaporation and kneading methods to enhance the solubility of efavirenz[10]. Among various methods ASDs were found to be simple and economical methods to enhance the solubility of poorly soluble drugs as they do not require sophisticated instruments and support easy scale-up.

ASDs is defined as a group of solid products consisting of a hydrophobic drug dispersed in at least one hydrophilic carrier, resulting basically in enhanced surface area, leading to higher drug solubility and dissolution rate. Improving wettability, dispersbility and reducing aggregation and agglomeration of drug particles result in enhanced drug bioavilability. Further, the ASDs convert the crystalline drug into amorphous form which naturally is more soluble than the parent crystalline form. The amorphous nature of the ASDs has high entropy and Gibbs free energy, easily soluble than their corresponding crystalline solids. But, these properties of the ASDs make them thermodynamically unstable and eventually try to convert into crystalline form upon storage[11]. So, development of successful ASDs demands use of employing such process conditions and such materials which can reduce their thermodynamic instability. Conventional methods for preparation of solid dispersion like spray drying, co-precipitation and solvent evaporation[12] are including an organic solvent and not environmental friendly. Presence of residual solvents may also be observed in these methods which may lead to decrease in shelf life[13]. Applications of quench cooling or melt-quench method[11] are rarely reported technique for developing pharmaceutical

ASDs for enhanced bioavailability. This technique does not require any volatile organic solvent and sophisticated equipment and just careful control and monitoring of temperature is sufficient to produce ASDs.

The present research work was aimed to explore the suitability of melt-quench technique to develop ASDs by taking EFV as a model drug for its bioavailability enhancement. Alongside the hydrophilic polymer carrier, a plasticizer (such as polyethylene glycols and their co-polymeric derivatives like poloxamers) was also incorporated to check its ability in providing stability to the ASDs. The work was carried out by adopting highly acceptable statistical tool quality by design (QbD) using Design Expert software[14]. So, the experiment was designed to study the influence of carrier, plasticizer and processing temperature on solubility and stability of the prepared Efavirenz ASDs (EASDs). Further, optimization was performed to identify the best combination of the factors to achieve EASDs with maximum solubility and stability.

MATERIALS AND METHODS

Materials

Efavirenz was obtained as generous gift from M/s EASAI Pharma Technology Pvt. Ltd Visakhapatnam. Soluplus, and Poloxamer 188 were obtained as gift samples from NATCO Pharma ltd, Hyderabad. Remaining materials and reagents were obtained from Sigma Aldrich, Mumbai. All the materials used in the study are of pharmacopoeia grade

Preparation of EASDs by Melt-Quench Method

QbD aspects

The object quality of the EASDs development as mentioned as Quality target product profile (QTPP) was achieving stable ASDs of EFV so as to improve its solubility and so its dissolution limited bioavailability. The critical process parameters (CPPs) or independent factors chosen corresponding to the set objective were Factor A: Soluplus concentration (in the range of 20 – 40% w/w with respect to the EFV weight); Factor B: Poloxamer-188 concentration (in the range of 5 – 15% w/w with respect to the EFV weight); and Factor C: Cooling temperature (in the range of -30 to 30^{o}C). The critical quality attributes (CQAs) or responses are the quality characteristics which indicate and serve as measures of the desired quality of the products. In case of the present work, solubility (as Response 1, R1) and solubility change ratio (SCR) as an indicative of stability (as Response 2, R2) of the ASDs were chosen as the CQAs. Here, stability of the ASDs was represented in terms of solubility change ratio (SCR) which is defined as

$$\mathrm{SCR} = \frac{\mathrm{S}_0 - \mathrm{S}_6}{\mathrm{S}_0}$$

where, S_0 is solubility of the ASDs' immediately after preparation; and S_6 is the solubility of the ASDs' after 6 months of storage at accelerated stability testing conditions. The experimental design selected to study the influence of the factors on the responses was Box-Behnken design (BBD) under response surface methodology[15]. According to the selected BBD, the formulation combinations to develop the EASDs were shown in Table 1.

Preparation of EASDs

Melt-quench technique[11,16] was adopted for preparing the EASDs. One gram of Efavirenz and the corresponding weights of the Soluplus and P-188 (for example, 350 mg of Soluplus and 50 mg of P-188 for EASD1) according to their concentrations as mentioned in Table 1 were taken in a polythene bag. The bag was thoroughly shaken to achieve maximum possible homogenous mixing of the materials. Then this physical mixture was taken in a china dish and subjected to heating with occasional stirring on electric hot plate until complete melting of all the materials in the mixture. Then the molten mixture was immediately cooled down at corresponding temperatures viz. 30°C (air cooling) or 0°C (at the set temperature in freezer) or -30°C (at the set temperature in freezer) as per the respective formulation. After complete solidification (the time was varied based on the cooling temperature and composition), the obtained dispersions were collected and stored in moisture resistant containers.

Table 1: Combination of the factors with their levels for developing ASDs of Efavirenz

Standard order	Run order	Levels of the factors			Formulation code assigned
		A: Soluplus conc. (% w/w)	B: P-188 conc. (% w/w)	C: Cooling Temp (Cel)	
9	5	35.00	5.00	-30.00	EASD1
5	1	20.00	10.00	-30.00	EASD2
6	3	50.00	10.00	-30.00	EASD3
10	9	35.00	15.00	-30.00	EASD4
1	8	20.00	5.00	0.00	EASD5
2	12	50.00	5.00	0.00	EASD6
13	11	35.00	10.00	0.00	EASD7
3	4	20.00	15.00	0.00	EASD8
4	7	50.00	15.00	0.00	EASD9
11	6	35.00	5.00	30.00	EASD10
7	13	20.00	10.00	30.00	EASD11
8	2	50.00	10.00	30.00	EASD12
12	10	35.00	15.00	30.00	EASD13

Characterization of the EASDs

Percentage Yield

Percentage yield[17] of the developed EASD's prepared by waere calculated by using following formula

$$\% \text{ yield } = \frac{\text{Weight of EASD obtained}}{\text{Weight of the solids taken}} \text{ X } 100$$

Differential Scanning Calorimetry (DSC) Analysis

The prepared EASDs were subjected to DSC analysis to investigate the physical state of the EFV in its pure form and in the prepared EASDs. Each preparation sample was carefully weighed and sealed in aluminum pans with lids using empty pans as reference. Samples were heated at a rate 10^{o}C from -20^{o}C to 200^{o}C[18] and the spectra were recorded.

X-Ray Diffraction (X-RD) analysis

Alongside the DSC, X-RD is another technique to study the degree of crystallinity in powder samples. Pure EFV and its EASDs were subjected to this X-RD analysis. Samples were dried, grounded and passed through 80 mesh before subjected to X-RD. Samples were scanned at range of 0^{o} to 90^{o} (2θ angle) with scanning rate 2^{o}/min[19] and the spectra were recorded.

Drug content

EASDs equivalent to 100 mg of EFV was taken in a 50 ml volumetric flask to it added 40ml of methanol and mixed thoroughly. The contents were repeatedly warmed in a hot bath while mixing to dissolve the drug in the solvent. The solution was made up to volume with methanol and assayed for drug content after suitable dilution[20].

Solubility

Solubility of pure EFV and the prepared EASDs were determined by taking excess amount of sample in 10ml of water, and stirred at 100rpm for 24hrs at 37^{o}C in an orbital shaker. The samples were filtered and analyzed for Efavirenz content in UV spectrophotometer at 245nm.

Stability

The EASDs prepared from different rapid cooling temperatures, were kept in stability chambers for 6 months at 40^{o}C and 75% RH. After six months samples were withdrawn and investigated for changes in crystalline properties by estimating amount of drug content, XRD analysis, and solubility studies[21]. The difference between solubilities of the EASDs before (S_0) and after (S_6) stability studies was calculated and this difference relative to the initial solubility (S_0) was taken as the solubility change ratio (SCR). Higher difference in solubility result in higher SCR values which indicate lesser stability of the EASDs.

Design validation and Optimization

The results of the responses, solubility and SCR for all the developed EASDs according to the selected BBD were subjected to statistical analysis. This was performed to check whether the selected factors had significant influence on the responses or not and also whether the selected experimental design was suitable for final optimization. For this, analysis of variance (ANOVA) testing was done and normal plot of residuals were constructed for the both responses using the Design Expert software[22]. Finally, optimization by desirability functions approach was done by setting the desirability criteria of maximizing the solubility and minimizing the SCR (so that maximizing the stability) with the help of the software.

RESULTS AND DISCUSSION

Yield

The EASDs of all the formulations exhibited good yields of more than 85% as shown in Table 2. This result indicated that the selected experimental conditions were appropriate to obtain considerable amounts of product with minimum loss.

Assay

The drug content values were found to be ranging from 97.1 – 102.7% and shown in Table 2. This result signified that the drug was miscible with the carrier and the plasticizer during melting as well as the homogeneity was maintained even after quenching. Thus the EASDs exhibited acceptable drug content uniformity.

Table 2: Results of characterization studies of the ASDs of Efavirenz

Formulation	Observed results*				
	Yield (%)	Assay (%)	Initial Solubility (S_0, mg/mL)	Solubility after 6 months (S_6, mg/mL)	Solubility change ratio (SCR)
EASD1	91.7 ± 3.5	96.4 ± 1.9	0.94 ± 0.05	0.39 ± 0.04	0.59 ± 0.02
EASD2	93.4 ± 2.8	98.1 ± 3.2	0.99 ± 0.08	0.43 ± 0.06	0.57 ± 0.03
EASD3	90.2 ± 4.7	97.9 ± 2.6	1.21 ± 0.14	0.72 ± 0.10	0.41 ± 0.01
EASD4	89.6 ± 3.2	99.5 ± 1.4	0.97 ± 0.10	0.65 ± 0.04	0.33 ± 0.03
EASD5	92.8 ± 1.7	101.3 ± 2.5	0.48 ± 0.03	0.29 ± 0.05	0.40 ± 0.07
EASD6	88.5 ± 2.5	100.6 ± 3.7	0.76 ± 0.06	0.58 ± 0.07	0.24 ± 0.03
EASD7	86.9 ± 2.2	97.1 ± 2.6	0.92 ± 0.11	0.79 ± 0.09	0.14 ± 0.01
EASD8	90.4 ± 4.1	102.7 ± 3.2	0.85 ± 0.04	0.76 ± 0.08	0.11 ± 0.05
EASD9	89.7 ± 1.3	98.4 ± 4.5	1.19 ± 0.09	1.12 ± 0.11	0.06 ± 0.02
EASD10	85.3 ± 3.4	99.1 ± 1.9	0.42 ± 0.06	0.33 ± 0.06	0.22 ± 0.03
EASD11	89.2 ± 3.9	96.8 ± 2.4	0.37 ± 0.05	0.32 ± 0.04	0.13 ± 0.01
EASD12	88.5 ± 1.6	100.9 ± 3.1	0.63 ± 0.05	0.56 ± 0.07	0.11 ± 0.04
EASD13	86.9 ± 2.3	98.6 ± 2.9	0.52 ± 0.07	0.49 ± 0.06	0.06 ± 0.01
* Expressed in Mean ± Standard deviation for n = 3					

DSC analysis

The DSC spectra of the pure EFV, the plasticizer P-188 and the prepared EASDs at different cooling temperatures were shown in Fig 1(a) – 1(e). The spectrum of pure EFV exhibited a sharp endotherm at 139^{o}C. This could be corresponding to the melting point temperature of the crystalline EFV[23]. The spectrum of pure P-188 was shown in Fig 1(b) which indicated a sharp endotherm at 53^{o}C which could be attributed to either glass transition temperature (T_g) or melting point of the P-188[24]. These endotherms of the pure EFV and P-188 were not observed in the spectra of the EASDs. This designated that the EFV might possible be converted into amorphous form from the crystalline form due to the applied melt-quench procedure[11,25]. Irrespective of the quenching temperature, the drug was converted into amorphous form. This might help in increasing the solubility of EFV[25].

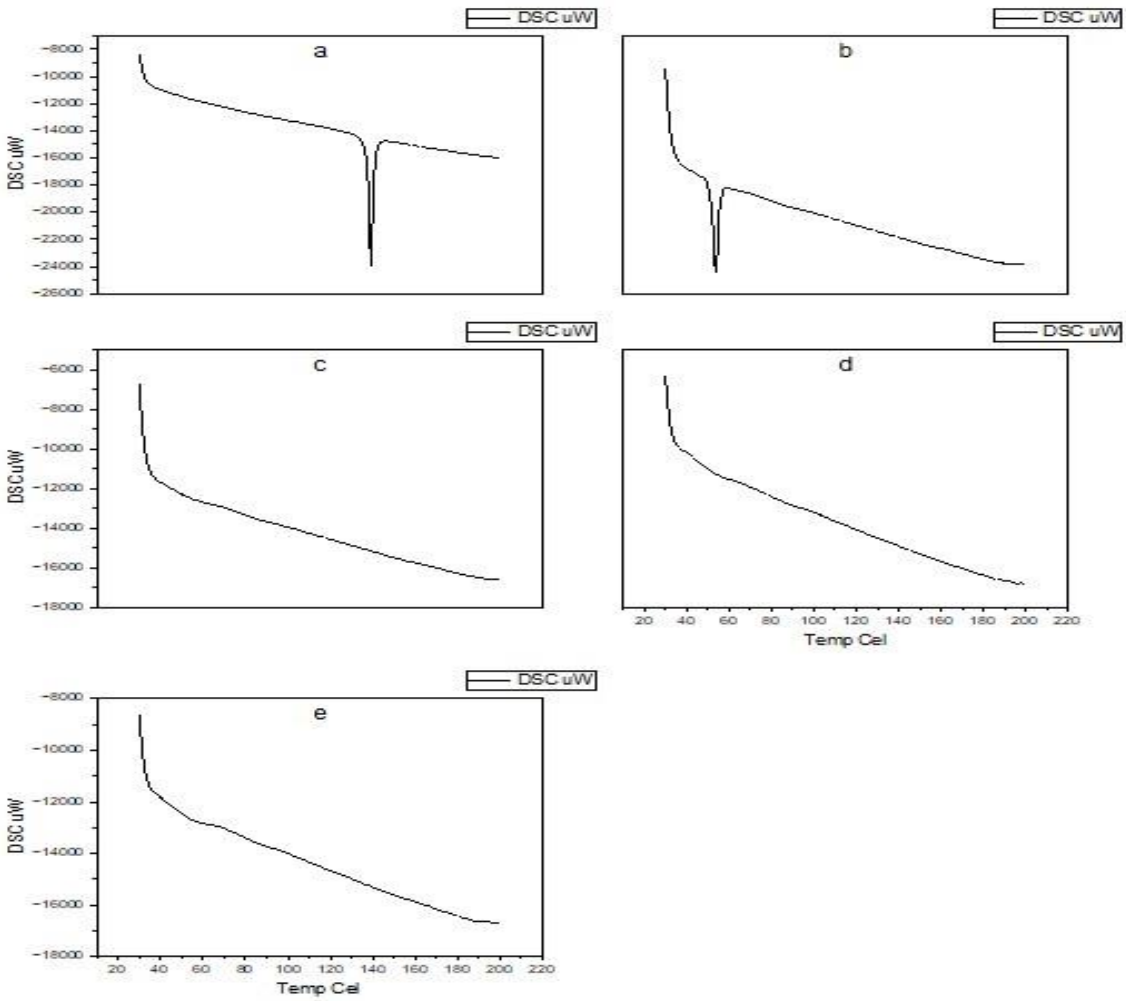

Fig 1: DSC spectra of (a) Pure EFV; (b) Pure Poloxamer 188; (c) EASDs prepared -30°C; (d) EASDs prepared 0°C; and (c) EASDs prepared 30°C

X-RD analysis

The X-RD spectra of pure EFV and the prepared EASDs were shown in Fig 2(a) – 2(d). The spectrum of pure EFV had many sharp and high intense peaks thus indicating the crystalline nature of the pure EFV. Whereas, the spectra of the EASDs did not show such multiple peaks and this might be because of the possible conversion of the crystalline EFV into its amorphous form[25] which was also indicated by the DSC analysis. These together designated that the adopted melt-quench technique and the process and formulation aspects employed in this work were suitable enough to develop ASDs.

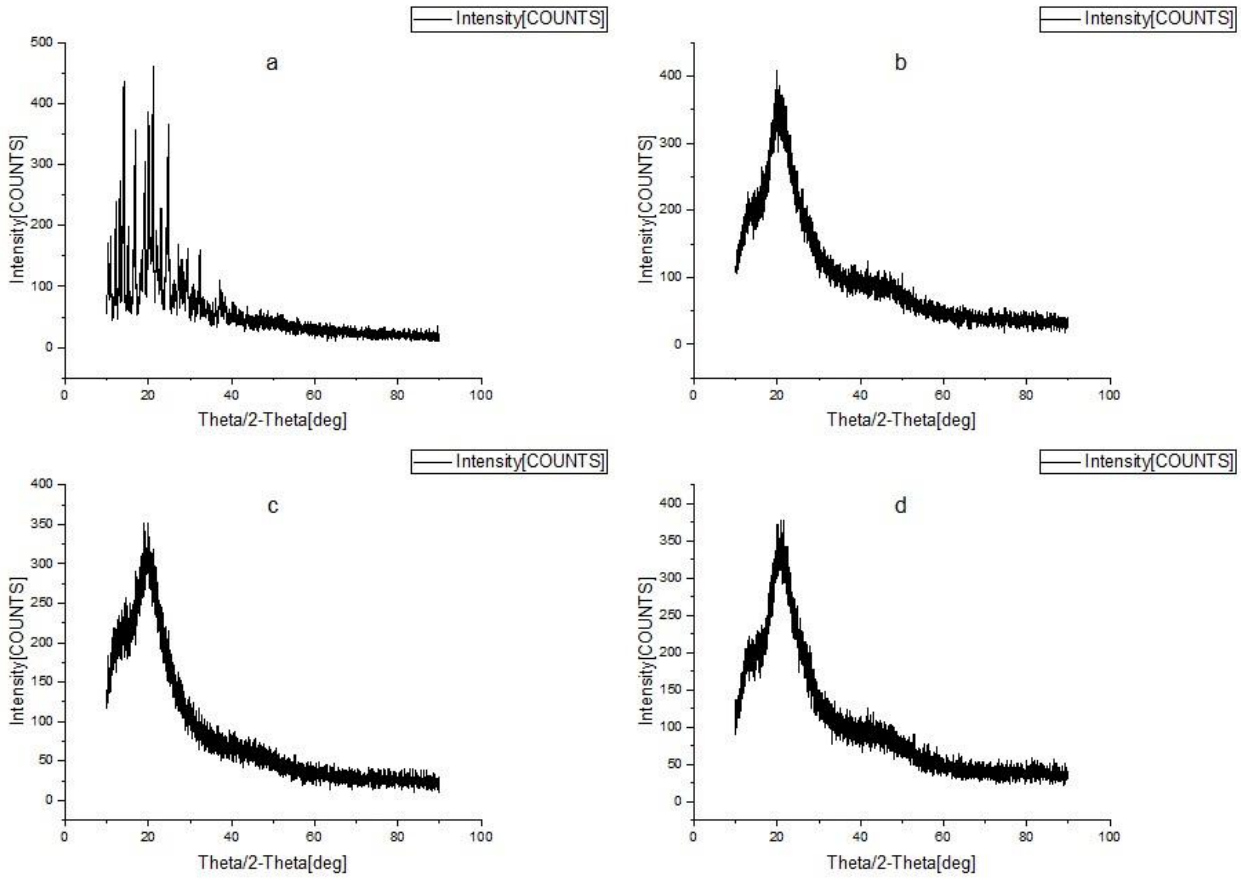

Fig 2: X-RD spectra of (a) Pure EFV; (b) EASDs prepared -30°C; (c) EASDs prepared 0°C; and (d) EASDs prepared 30°C

Solubility

All the EASDs were subjected to solubility testing immediately after preparation and the obtained results were shown as S_0 in Table 2. All the EASDs showed improved solubility than the solubility of the pure EFV which was found to be 0.013 mg/mL. As this solubility, S_0 was taken as one of the responses, the effect of the factors on it was analyzed using Design Expert software. The factors were found to have linear effect on the S_0 and the regression equation between this solubility and the factors was obtained as

$S_0 = +0.79 + 0.14* A + 0.12* B – 0.27* C$

Influences of all the factors were shown in Fig 3(a) and 3(b). Factor A had positive effect on the solubility that upon increase in the concentration of Soluplus, the solubility was found to be increased. This could be attributed to the hydrophilic nature of the carrier. Higher concentration of this carrier could impart more hydrophilicity and reduce the crystallinity of the drug to more extent and hence the solubility of Efavirenz was increased[26]. Factor B also had positive effect on the solubility. This could be attributed to the hydrophilic nature as well as the ability to improve wettability by decreasing interfacial tension between the drug and water of the Poloxamer 188[27]. Factor C was found to have negative effect on the solubility that upon decreasing the temperature provided for quenching the solubility was found to be increased. Rapid solidification of the molten drug might when exposed to -30°C lead to the formation of

more random amorphous form of the drug which could have higher solubility. Rather, solidification happened at +30^{o}C could take more time and allow for ordered solidification of the drug. This might result in less amorphous state with possible crystallinity if any[25]. So, quenching at higher cooling temperature resulted in ASDs with less solubility.

Solubility change ratio

Even though ASDs significantly improve the solubility of crystalline drugs, they suffer with poor thermodynamic stability. Because of the molecular flexibility in the glassy state of the ASDs, crystallinity may be developed upon ageing. The rate of recrystallization of the ASDs depends on several factors like the stabilization effect of the plasticizer, rate of cooling during solidification etc. So, this instability of the ASDs of recrystallization can result in decrease in solubility of the drug upon ageing. The composition of the ASDs and the processing conditions are critical in maintaining the stability of the ASDs during shelf-life[28]. This recrystallization of the ASDs represents their instability which eventually results in decrease in solubility of the drugs[29]. So, the stability of the prepared EASDs was represented in this work as the solubility change ration (SCR). If the ASDs exhibit more degree of recrystallization, the solubility will be decreased to greater extent. Hence higher SCR ratio indicates lesser the stability of the ASDs.

The obtained results of the SCR for all the EASDs were shown in Table 2. These results of the SCR were also subjected to DoE analysis to investigate the effect of the factors and it was observed that the factors had quadratic effect on the SCR. The regression equation depicting the quadratic regression between the factors and the SCR was obtained as

$$SCR = +0.14 - 0.050 * A - 0.11 * B - 0.16 * C + 0.028 * AB + 0.031 * AC + 0.029 * BC + 0.036 * A^2 + 0.022 * B^2 + 0.14 * C^2$$

All the three factors A, B and C had negative effect on the SCR that an increase in the level of these factors decreased SCR and hence increased the thermodynamic stability of the ASDs. The influence of all the factors on the SCR was shown as contour plots in Fig 3(c) and 3(d).

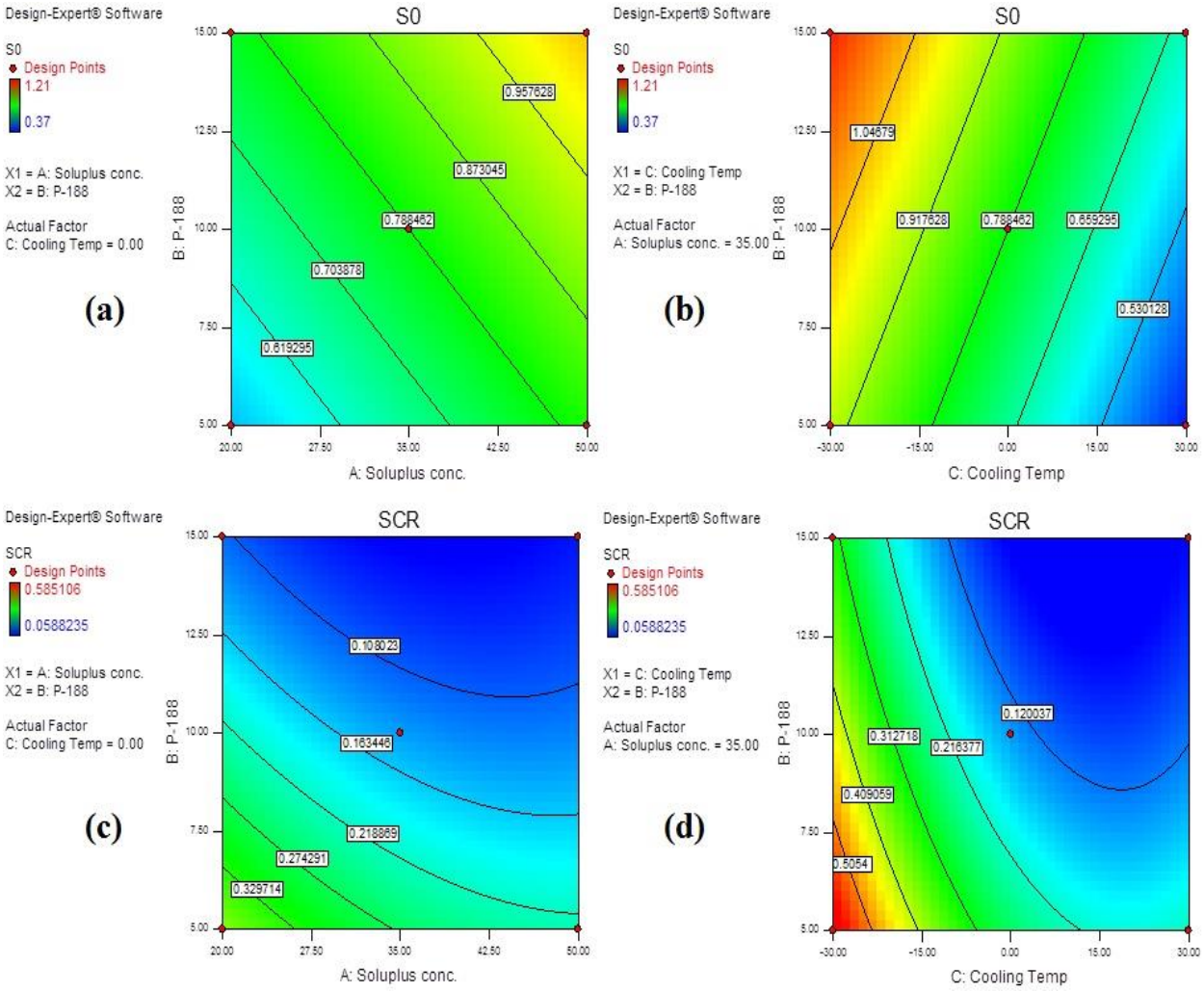

Fig 3: Contour plots showing the effects of (a) Factors A and B on the S_0; (b) Factors C and B on the S_0; (c) Factors A and B on the SCR; and (d) Factors A and B on the SCR

Any carrier present along with the drug in its amorphous form would decrease the free energy of recrystallization. So, presence of high concentration of Soluplus might decrease the free energy to greater extent[26]. This caused less degree of recrystallization during storage and hence lesser SCR values were observed which indicated more stability. The similar effect with much higher degree was observed with Factor B i.e. the concentration of Poloxamer 188. This could be due to the plasticizing nature of the Poloxamer that stabilizes the amorphous state of solids[30]. So, high concentration of Poloxamer 188 provided more stability that was indicated by the lesser SCR values.

The factor C, quenching temperature was found to reduce the SCR and thus increase the stability. Thermodynamic stability of the EASDs also depends on the cooling rate at which the molten mixture was subjected to quenching. Lesser quenching temperature causes rapid solidification which can result in poorly ordered solid state that can have higher free energy[25]. So, the EASDs solidified at -30^{o}C were found to have maximum SCR which indicated least stability. This could be attributed to the possible high free energy that might cause recrystallization of the drug in the ASDs thus resulting in decrease in solubility

upon storage. Whereas the EASDs solidified at 30°C might be more ordered with least free energy. So, even though the initial solubility of these ASDs was relatively lesser they maintained the solubility due to more stability thus exhibiting lesser SCR values.

These inferences form the SCR values were also supported by the DSC analysis performed on the EASDs after six months of storage at accelerated stability testing conditions. These spectra were shown in Fig 4(a) – 4(c). The spectrum in Fig 4(a) was representing the EASDs prepared at -30°C. In this spectrum, broad endotherms were observed at 53°C and at 133.5°C which might be corresponding to the P-188 and EFV respectively. The EASDs prepared at the quenching temperature of -30°C might rapidly solidify yielding ASDs with possible high free energy[25]. This high free energy might cause recrystallization of the EASDs to certain extent which was represented by the endotherms in the DSC. Whereas, in case of the EASDs prepared by quenching at 0°C indicated smaller endotherms in their DSC spectrum as shown in Fig 4(b). But, the spectrum of the EASDs obtained by quenching at 30°C (shown in Fig 4(c)) did not indicate any significant endotherm corresponding to EFV and hence no sign of considerable recrystallization. These results confirmed the SCR results indicated by solubility studies. Rapid quenching at lower temperatures could result ASDs with more entropy and free energy which caused higher initial solubility but they undergo higher degree of recrystallization upon storage which eventually reduce the solubility. Whereas, slow quenching at relatively higher temperature resulted in ASDs with relatively lesser entropy and lesser free energy. So, though the initial solubility was relatively lesser, they exhibited greater stability and thus could maintain the solubility with lesser SCR values.

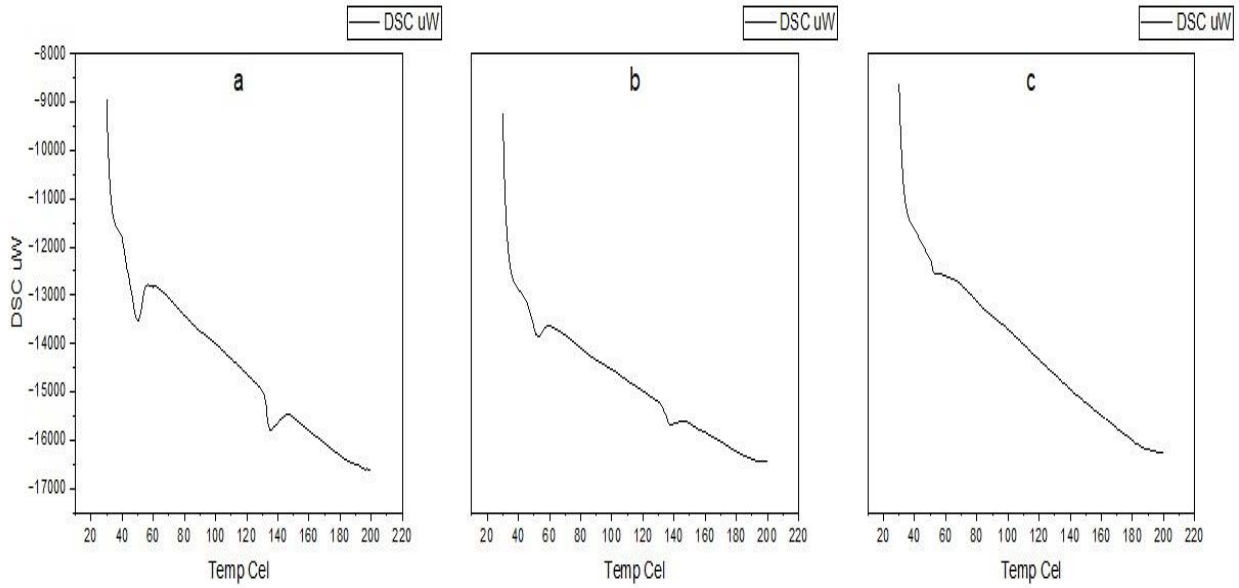

Fig 4: DSC spectra of the EASDs after six months of storage at accelerated stability testing conditions. (a) EASDs prepared at -30°C; (b) EASDs prepared at 0°C; and (c) EASDs prepared at 30°C.

Design validation and optimization

The results of ANOVA test for both the responses were shown in Table 3 and Table 4. The linear regression model in case of the response S_0 and the effects of all the factors on the S_0 were found to be significant at $p < 0.05$. Similarly, the ANOVA for quadratic model in case of the response SCR and all the three main

factor effects on the SCR were found to be significant at $p < 0.05$. Further, the normal plots of residuals of both the responses (Shown in Fig 5) yielded straight lines rather than sigmoid shape which indicated the selected models were significant enough to proceed for optimization[22].

Table 3: ANOVA test results of response surface linear model for initial solubility (R1)

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
Model	0.85	3	0.28	22.59	0.0002	**Significant**
A: Soluplus conc.	0.15	1	0.15	12.09	0.0070	**Significant**
B: P-188	0.11	1	0.11	8.64	0.0165	**Significant**
C: Cooling Temp	0.59	1	0.59	47.05	< 0.0001	**Significant**
Residual	0.11	9	0.013			
Cor Total	0.96	12				
Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-Mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant						

Table 4: ANOVA test results of response surface quadratic model for the SCR (R2)

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
Model	0.38	9	0.043	112.35	0.0012	**Significant**
A: Soluplus conc.	0.020	1	0.020	53.17	0.0053	**Significant**
B: P-188	0.093	1	0.093	243.74	0.0006	**Significant**
C: Cooling Temp	0.21	1	0.21	560.76	0.0002	**Significant**
AB	3.132×10^{-3}	1	3.132×10^{-3}	8.25	0.0640	
AC	3.939×10^{-3}	1	3.939×10^{-3}	10.37	0.0486	**Significant**
BC	3.472×10^{-3}	1	3.472×10^{-3}	9.14	0.0566	
A^2	2.915×10^{-3}	1	2.915×10^{-3}	7.68	0.0695	
B^2	1.139×10^{-3}	1	1.139×10^{-3}	3.00	0.1817	
C^2	0.043	1	0.043	114.49	0.0017	**Significant**
Residual	1.139×10^{-3}	3	3.798×10^{-4}			
Cor Total	0.39	12				
Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-Mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant						

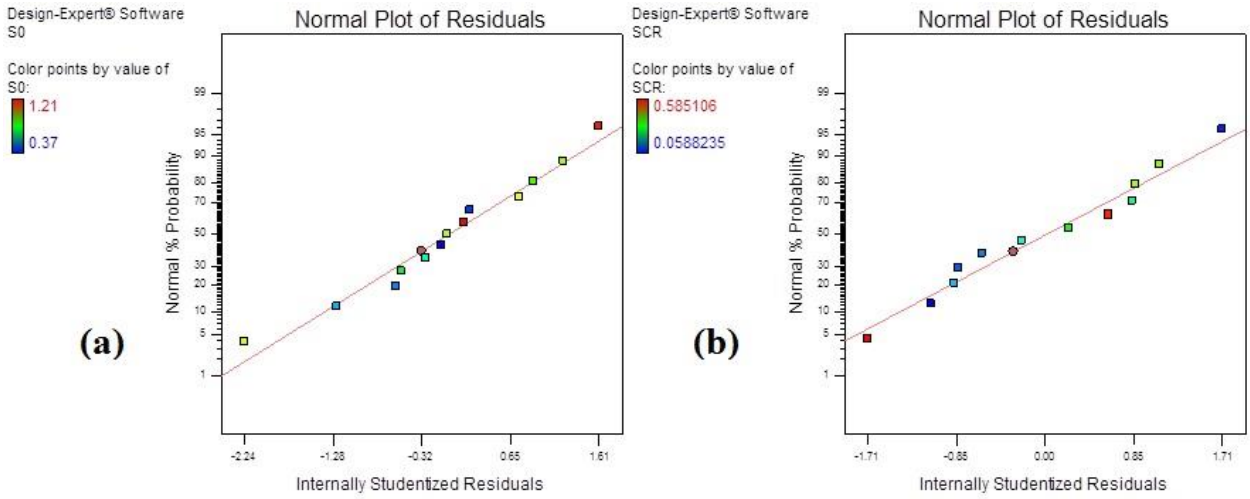

Fig 5: Normal plots of residuals for (a) THE response S_0; and (b) The response SCR

The optimization criterion was selected as per the set QTPP that the ASDs developed to have high solubility (maximizing S_0 with lower limit of 1 mg/mL) and high stability (minimizing SCR with upper limit of 0.08). The obtained overlay plot form the software was shown in Fig 5. The yellow color region in the plot indicates design space that any point of combination of the factor levels in this region would yield ASDs with desired quality characteristics. One such combination with maximum desirability was identified by the software and its predicted response values were shown in Table 5. A new ASD formulation at this combination of Soluplus at 50% w/w, Poloxamer at 15% w/w with quenching temperature of –1.92°C was developed and characterized for the responses S_0 and SCR. The obtained experimental results of the S_0 and SCR were found to be 1.03 mg/mL and 0.072. These experimental values were found to be correlated with the software predicted values as these were within the 95% confidence intervals region of the predicted values. Hence, the applied QbD approach was successful in yielding ASDs with maximum solubility yet having desired stability.

Table 5: Comparison of the predicted and observed values of the responses for the optimized ASDs of Efavirenz

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: Soluplus conc. (50% w/w) B: P-188 (15% w/w) C: Cooling Temp (-1.92°C)	R1: S_0 (mg/mL)	1.06	0.91	1.20	**1.03**
	R2: SCR	0.077	0.023	0.13	**0.072**

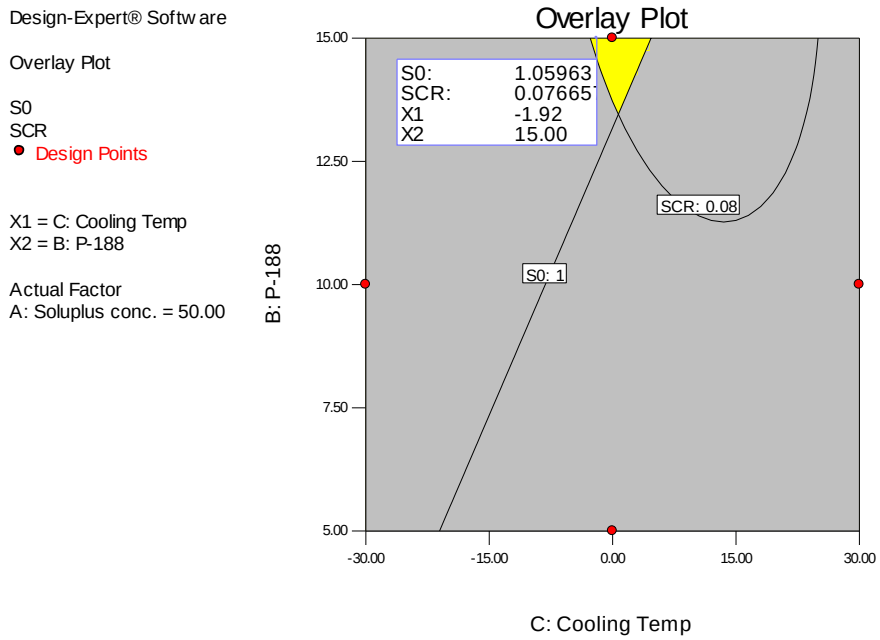

Fig 6: Overlay plot showing the design space (yellow region) with the predicted best optimized combination of the factors and their responses

CONCLUSION

Bioavailability enhancement of poorly water soluble drugs by developing ASDs is always a choice of techniques. But, the stability of the developed ASDs is always a major challenge as they are prone for recrystallization upon storage. In this work, the role of carrier, plasticizer and cooling temperature in increasing solubility and maintaining the stability of the ASDs of Efavirenz prepared by melt-quench method was studied. Quality by design approach was adopted using Design Expert software. Box-Behnken design was used to study the effects of the factors on the selected responses. All the selected three factors were found to have significant effect on both the solubility and stability of the ASDs. Particularly, plasticizer and cooling temperature used for quenching were found to have more significant effect on the stability of the ASDs. The ASDs prepared with higher concentration of the plasticizer and at slow rate of cooling were found to be more stable and retain the improved solubility even upon storage at accelerated stability testing conditions.

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