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Design, Optimization, Preparation and Evaluation of Solid Dispersions of Albendazole using Factorial Design

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ABSTRACT

*To enhance the solubility and dissolution rate of albendazole, a poorly water soluble anthelmintic drug, by preparation of solid dispersions. The dispersion granules were prepared using a hot melting technique which involved preparation of a homogenous dispersion of albendazole in gelucire 44/14 and PEG 8000. A two-factor, four-level (4*2) statistical design was implemented to quantitate the influence of gelucire 44/14 and PEG 8000 on the dissolution profile, where gelucire 44/14 and PEG 8000 were chosen as independent variables, while T10 min (cumulative drug release in 10 minutes) and T60 min (cumulative drug release in 60 minutes) were chosen as dependent variables. The solid dispersions were characterized for their in-vitro dissolution rate. The optimized formulation was further characterized by DSC, XRD and SEM analysis. An appropriate statistical model was arrived at and a significantly enhanced dissolution rate was exhibited with the optimized formulation. DSC and XRD data indicated the amorphous nature of albendazole in solid dispersion. SEM revealed partial loss of drug crystallinity which can bring about significant changes in the drug dissolution rate. In conclusion the statistical model enabled us to understand the effects of formulation variables on the dispersion.*

Key Words: albendazole, Solid dispersion, dissolution enhancement, factorial design, Gelucire 44/14, PEG 8000.

INTRODUCTION

ABZ is a benzimidazole carbamate with a broad anti-parasitic spectrum [1]. ABZ was first approved for treatment of helminth infections in sheep in 1977, and subsequently approved for human use in 1983 [2]. In general, most ascariasis, trichuriasis, enterobiasis and hookworm infections can be successfully treated with single dose ABZ and strongyloidiasis with multiple doses of ABZ. ABZ has also used in the treatment of capillariasis, gnathostomiasis, and trichostrongyliasis, the cestode infections hydatidosis, taeniasis and neurocysticercosis, and the

tissue nematodes cutaneous larval migrans, toxicariasis, trichinosis and filariasis (in combination with other anthelmintics). ABZ has also been used successfully against mixed infections [2-4]. The biggest problem of albendazole is its low and erratic availability as a result of its low aqueous solubility.

Enhancement of bioavailability of hydrophobic drugs is one of the major challenges in drug development. Of the plethora of pharmaceutical technologies available to address this issue *viz*. micronisation, the use of surfactants and the formation of solid dispersions [5], solid dispersion is one of the useful methods for the dispersion of the drug into an inert, hydrophilic polymer matrix [6,7]. Solid dispersions display an enhanced solubility of drug because of the conversion of the drug's crystal lattice into an amorphous form, particle size reduction and increased wettability by the hydrophilic polymer. Therefore, the same pharmacological results can be obtained from a reduced amount of drug given to the patient.

Solid dispersions in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and hence possibly bioavailability of a range of hydrophobic drugs. Although a large number of studies have been published but the mechanisms underpinning the observed enhancement of the rate of drug release are not yet understood.

The use of solid dispersions as an effective source of improving the dissolution rate of poorly soluble drugs has been well studied and demonstrated [6,8,9].

PEG and gelucire are among the several carriers which have been employed in preparing solid dispersions [10-14]. PEG polymers are widely used for their low melting point, low toxicity, wide drug compatibility and hydrophilicity. Gelucire is a family of vehicles derived from the mixtures of mono- di- and triglycerides with polyethylene glycol (PEG) esters of fatty acids. These are available with a range of properties depending on their hydrophilic-lipophilic balance (HLB) and melting point range (33–65 °C). They have a wide variety of applications in pharmaceutical formulations as the preparation of fast release and sustained release formulations [10-17].

The aim of the present study was to improve the solubility and dissolution rate of ABZ by formulating a solid dispersion with PEG 8000 and Gelucire 44/14 at different ratios.

Full factorial experimental design is one of the best tools to study the effect of different variables on the quality determinant parameters of any formulation. A statistical model was therefore developed to optimize the solid dispersions. An appropriate statistical model was arrived at and a significant enhanced dissolution rate and flow properties were exhibited with the optimized dispersion granules.

MATERIALS AND METHODS

Materials

Albendazole was purchased from Cipla Pharma Co. (Mumbai, India). PEG 8000 & Gelucire 44/14 were procured from Sigma-Aldrich (St. Louis, MO, USA) and Gattefosse, France respectively. All the chemicals and solvents used in the study were of analytical grade and procured from Himedia Laboratories Ltd. (Mumbai, India) and SD Fine Chem (Mumbai, India).

Preparation of solid dispersions

Solid dispersions (SDs) at various weight ratios were prepared by melting method. ABZ was added to the molten base comprising PEG 8000/Gelucire 44/14 or mixture. The blend was heated 10 ºC above the melting point of each carrier for 5 minutes with continuous stirring. The systems were placed in a freezer at -20 ºC for 24 h. The mass was crushed, ground gently with a mortar and pestle and passed through 500-µm sieve. The samples were kept in desiccators until the next experiments.

Formulation Design

This study investigated utility of a 2-factor, 4-level General factorial design and optimization process for solid dispersions of Albendazole. Amount of Gelucire 44/14 (A) and amount of PEG 8000 (B) were selected as the independent variables whereas T10 min (cumulative drug release in 10 minutes) and T60 min (cumulative drug release in 60 minutes) were selected as dependent variables. The prepared solid dispersions of Albendazole were evaluated for dissolution study. The responses were analyzed using ANOVA and the individual response parameters were evaluated using F test and polynomial equation was generated for each response using MLRA.

Statistical design

From the preliminary studies, it was observed that the concentration of drug and polymers affect the formulations of solid dispersion. Based upon this, two factors were selected:

X_1- amount of Gelucire 44/14

X_2- amount of PEG 8000

Four levels for two factors are presented in Table 1. As a result, 16 batches of solid dispersion were prepared using 4^2 factorial design (table 2).

Evaluation of Solid Dispersions

In vitro Dissolution studies

Drug dissolution studies was carried out using USP dissolution apparatus 2 using a paddle at a speed of 100 rpm with 900 mL of 0.1 M HCl as dissolution medium at 37^0C. Solid dispersion powders containing 100 mg of albendazole were dispersed on the surface of the dissolution medium and the time was recorded. At intervals, 5 mL samples were withdrawn through a filter. The amount of released drug was determined by UV spectrophotometer at 291 nm.

Data Analysis

The response surface methodology is a collection of mathematical and statistical techniques used for modeling and analysis of problems in which a response of interest is influenced by several variable and the objectives is to optimize this response. The run or formulation, which are designed based on factorial design are evaluated for the response. The response values are subjected to multiple regression analysis to find out the relationship between the factor used and the response value obtained. The response values subjected for this analysis are T10 min (cumulative drug release in 10 minutes) & T60 min (cumulative drug release in 60 minutes). The multiple regression analysis was done using DESIGN EXPERT 8.0.1 demo version software, which specially meant for this optimization process. Analysis of data was carried out using ANOVA and the individual parameter was evaluated with F-test. Using the regression coefficient of factor, the polynomial equation for the each response is generated

Formulations Optimization

The computation for optimized formulation was carried using software, DESIGN EXPERT 8.0.1. The optimized formulation was obtained by applying constraints (goals) on dependent

(response) and independent variables (factors). Constraints for responses and factors are shown in Table 3.

By utilizing the software, we got one solution for optimized formulation**.** The optimized formulation is prepared and evaluated for T10min & T60 min. Observe response value of the optimized formulation is compared with predicted value. The optimised batch(s) was further investigated by DSC, XRD, SEM and content uniformity.

Differential Scanning Calorimetry

DSC measurements were performed on a DSC Q10 V 9.9 differential scanning calorimeter with a thermal analyzer. Samples (about 1.675 mg Of ABZ or SDs and PMs containing an equivalent amount of the drug) were placed in sealed aluminum pans and heated under nitrogen flow (20 ml/min) at a scanning rate of 10 ^{o}C min^{-1} from 0 to 300 ^{o}C. An empty aluminum pan was used as a reference.

Powder X-ray diffraction studies

Powder X-ray diffraction pattern were traced employing X-ray diffractometer (XPERT-PRO) for the samples, using Ni filtered CuK (α) radiation, a voltage of 45 kV, a current of 20 mA. The sample was analyzed over 2θ range of 0-50^{o} with scan step size of 0.0170^{o} (2θ) and scan step time 20 s.

Scanning Electron Microscopy

Sample of pure drug, carrier and the solid dispersion formulation were mounted onto the stubs using double-sided adhesive tape and then coated with gold palladium alloy (150-200 A^{o}) using fine coat ion sputter (Joel, fine coat ion sputter, JPC-1100). The samples were subsequently analyzed under the scanning electron microscopy (SEM) for external morphology.

Content Uniformity

Solid dispersion containing an equivalent amount of 4mg of albendazole was added to a volumetric flask containing acidified methanol. The flask was shaken for 10 min and final volume was made up using buffer pH 6.8 & filtered through 0.45 μm membrane filters. The sample was diluted and analyzed spectrophotometrically at 291 nm.

RESULTS AND DISCUSSION

In vitro Dissolution studies

To explore the influence of the solid dispersion techniques on drug dissolution profile, pure drug, solid dispersions and physical mixtures were evaluated for dissolution studies in 0.1 N HCl. The dissolution profiles of albendazole from the physical mixtures and solid dispersions of all formulation were shown in Figure 1 and 2.

Albendazole is practically insoluble in water, and yielded the slowest dissolution rate with only 16.3% of the drug dissolved in 60 min. The result showed that blending of drug with PEG8000 or Gelucire 44/14 or both in form of PMs or SDs could enhance the release of ABZ. The value of %age cumulative drug release for pure drug (16.3%) was increased to 36.9% in PMs and up to 97.2% in SDs. The release values revealed more dissolution improvement of ABZ in SD with Gelucire 44/14 than SD with PEG 8000 at same carrier concentration among the batches containing individual polymer. However when both the polymers were used in combination, there were higher dissolution (up to 97.2%) as compared to batch containing single polymers(up to 79%). The faster dissolution rate of PMs compared to pure drug was observed for both of

polymers and could be attributed to the improvement of wettability of ABZ particles due to the presence of highly hydrophilic polymers. Dissolution rates for SDs were greater than those for PMs and Drug alone. The enhanced dissolution rates of SDs may be due to many factors such as decreased particle size of drug, specific form of drug in these SDs, in addition to other factors *viz.* the increase in drug wettability and preventing of drug aggregation by each polymer. Furthermore, both PEG and Gelucire 44/14 affected the crystallinity of the drug could be considered as an important factor in enhancement the dissolution rate. It is known that amorphous drug represents the most ideal case for fast dissolution.

Data Analysis

The responses were recorded and analysis of data was carried out using ANOVA. The individual parameter was evaluated using F-test and a polynomial equation for each response was generated using MLRA. The design and response summary data are represented in Table 4 and 5.

Response: T10 min (Y1)

In ANOVA Table 6, values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB are significant model terms.

Final Equation in Terms of Coded Factors:

$T10 = +61.26 +19.44 * A +16.29 * B -2.51 * A * B -8.79 * A^2 -13.04 * B^2$

Response: T60 min (Y2)

In ANOVA table 7, Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.

Final Equation in Terms of Coded Factors:

$T 60 = +90.72 +16.27 * A +13.32 * B -8.45 * A * B -10.39 * A^2 -11.38 * B^2$

On the basis of dissolution studies of formulations and constraints applied, the results of factorial design suggested only one optimized combination of polymers (Table 8) by which maximum desirability can be achieved. Three dimensional response surface plots and two dimensional contour plots were drawn from the data with DESIGN EXPERT 8.0.1 demo version software to estimate the effects of the independent variables on each response, as depicted in Figures (3, 4 and 5).The check out batch was prepared and responses were evaluated. The responses value observed in checkpoint batch was very near to optimized batch.

The check out batch (SD 17) and its corresponding Physical Mixture batch (PM17) were selected for further studies.

Differential Scanning Calorimetric

The DSC curve of pure albendazole exhibited a single endothermic response corresponding to the melting of the drug. Onset of melting was observed at 210 °C (Figure 6) while thermograms of PM and SD (Figure 7 and 8) showed absence of a albendazole melting peak but one exothermic peak was present at 199.77°C and 92.58 °C respectively. The PMs also showed no melting peak of albendazole (Figure 7), though peaks derived from ABZ were observed. The absence of a ABZ melting peak and the presence of one exothermic peak in SD suggest that ABZ was completely soluble in the liquid phase of the polymer or the absence of a crystalline form of ABZ. The exothermic peak might be due to crystallisation above Tg (glass transition temperature). The molecular motion of amorphous solids depends on temperature. The kinetic energy of amorphous solids increases significantly as the temperature gets close to Tg. Due to

the thermodynamic instability of amorphous solids, compared to the crystalline state, spontaneous crystallisation is always possible as soon as molecular mobility is above the threshold of nucleation.

Powder X-ray diffraction studies

The diffraction spectrum of pure albendazole showed that the drug was crystalline in nature as demonstrated by numerous peaks. Numerous diffraction peaks of albendazole were observed at 7.01º, 11.22º, 13.83º, 17.87º, 19.50º, 20.71º, 22.10º, 27.10º, and 28.16º (2 θ) (Figure 9) indicating crystalline ABZ. Some changes in the peak positions of ABZ were observed in PMs as well as SDs. The prominent peaks for pure ABZ were clearly seen at the same positions in PMs and SDs but with decreased intensities.

The extent of crystallinity of the phases will influence the dissolution of the dosage forms. An amorphous or metastable form will dissolve at the fastest rate because of its higher internal energy and greater molecular motion which enhance thermodynamic properties relative to crystalline materials. XRD patterns of solid dispersions lacked the intense diffraction peaks associated with crystalline ABZ implying the solid dispersions contain amorphous drug

Scanning Electron Microscopy

Figure 10 displayed SEM photographs for ABZ, PEG8000, Gelucire 44/14, SD 17 and its corresponding physical mixture PM 17. The drug crystals seemed to be smooth-surfaced, irregular in shape and size. Drug crystals were much smaller than PEG or Gelucire particles. The physical mixture of the drug and carrier showed the presence of drug in the crystalline form. It was easy to recognize the polymer particles from that of drug despite the reduction in size of particles of polymers during mixing and its presence in high amount. In case of SDs, it was difficult to distinguish the presence of drug crystals. The drug surface in Solid dispersion seems to be more porous in nature. Solid dispersion appeared as uniform and homogeneously mixed mass with wrinkled surface. Drug crystals appeared to be incorporated into the particles of the polymers. The solid dispersion looked like a matrix particle. The results could be attributed to dispersion of the drug in the molten mass of the polymer.

Solid state characterization studies revealed partial loss of drug crystallinity which can bring about significant changes in the drug dissolution rate. However, other factors like reduced particle size, surface area and closer contact between the hydrophilic carrier and the drug may also be influential in enhancing drug solubility and dissolution rate observed with solid dispersion particles.

Content Uniformity

The content uniformity of solid dispersion batch SD17 and corresponding physical mixture batch PM17 was compared. Content uniformity of both batches was found to be within IP limit (96-99%).

Table 1: Level and Factors for factorial design

FACTORS	LEVELS			
	0	**1**	**2**	**3**
X_1- Amount of Gelucire 44/14(mg)	0 mg	100mg	200 mg	300mg
X_2- Amount of 50/13 (mg)	0 mg	100mg	200 mg	300mg

Table 2: Four Level Factorial design

Batch No. Physical Mixtures)	Batch No. solid dispersions)	Coded value		Actual value (in mg)		
		Gelucire 44/14	PEG 8000	Drug	Gelucire 44/14	PEG 8000
PM1	SD1	0	0	200	0	0
PM2	SD2	1	0	200	100	0
PM3	SD3	2	0	200	200	0
PM4	SD4	3	0	200	300	0
PM5	SD5	0	1	200	0	100
PM6	SD6	0	2	200	0	200
PM7	SD7	0	3	200	0	300
PM8	SD8	1	1	200	100	100
PM9	SD9	1	2	200	100	200
PM10	SD10	1	3	200	100	300
PM11	SD11	2	1	200	200	100
PM12	SD12	2	2	200	200	200
PM13	SD13	2	3	200	200	300
PM14	SD14	3	1	200	300	100
PM15	SD15	3	2	200	300	200
PM16	SD16	3	3	200	300	300

Table 3: Constraints for responses and factors

Name	Goal	Lower Limit	Upper Limit
Amt. of Gelucire 44/14	In range	0 mg	300 mg
Amt. of PEG 8000	In range	0 mg	300 mg
T10 min	Maximize	0	75
T60 min	Maximize	0	100

Table 4: Design Layout of Factorial Design and Summary of Experimental Results

Batch No. (solid dispersions)	Factors		Responses	
	Gelucire 44/14 (mg)	PEG 8000 (mg)	T10 min %age	T60 min %age
SD1	0.00	0.00	3.1	16.3
SD2	100.00	0.00	26.9	69.1
SD3	200.00	0.00	30.6	75
SD4	300.00	0.00	44.8	79.1
SD5	0.00	100.00	23.7	66.4
SD6	0.00	200.00	33.9	73.8
SD7	0.00	300.00	42.7	76
SD8	100.00	100.00	47.9	74.9
SD9	100.00	200.00	54.7	80.8
SD10	100.00	300.00	55.9	81.9
SD11	200.00	100.00	63.9	90.2
SD12	200.00	200.00	72.3	97.2
SD13	200.00	300.00	72.1	96.9
SD14	300.00	100.00	70.8	90
SD15	300.00	200.00	72.2	96.2
SD16	300.00	300.00	70.6	94.3

Table: 5: Observed & predicted values for percent of ABZ released after 10 min and 60 min from solid dispersions

Batch No. (solid dispersions)	Cumulative drug release			
	T10 min %age		T60 min %age	
	Observed value	Predicted value	Observed value	Predicted value
SD1	3.1	13.39375	16.3	49.03625
SD2	26.9	26.35625	69.1	59.88375
SD3	30.6	39.31875	75	70.73125
SD4	44.8	52.28125	79.1	81.57875
SD5	23.7	24.25625	66.4	57.91875
SD6	33.9	35.11875	73.8	66.80125
SD7	42.7	45.98125	76	75.68375
SD8	47.9	37.21875	74.9	68.76625
SD9	54.7	48.08125	80.8	77.64875
SD10	55.9	58.94375	81.9	86.53125
SD11	63.9	50.18125	90.2	79.61375
SD12	72.3	61.04375	97.2	88.49625
SD13	72.1	71.90625	96.9	97.37875
SD14	70.8	63.14375	90	90.46125
SD15	72.2	74.00625	96.2	99.34375
SD16	70.6	84.86875	94.3	108.22625

Table: 6: Analysis of Variance (ANOVA) of Dependent Variable (T10 min %age)

Source	Sum of Squares	df	Mean Square	F Value	p- value Prob > F
Model	6532.71	5	1306.54	70.21	< 0.0001
A-gelucire	3360.53	1	3360.53	180.59	< 0.0001
B-peg 8000	2359.88	1	2359.88	126.82	< 0.0001
AB	31.08	1	31.08	1.67	0.2253
A^2	244.14	1	244.14	13.12	0.0047
B^2	537.08	1	537.08	28.86	0.0003
Residual	186.09	10	18.61		
Cor Total	6718.79	15			

Table 7: Analysis of Variance (ANOVA) of Dependent Variable (T60 min %age)

Source	Sum of Squares	df	Mean Square	F Value	p- value Prob > F
Model	5034.59	5	1006.92	15.26	0.0002
A-gelucire	2353.37	1	2353.37	35.67	0.0001
B-peg 8000	1577.98	1	1577.98	23.92	0.0006
AB	352.88	1	352.88	5.35	0.0433
A^2	341.33	1	341.33	5.17	0.0462
B^2	409.05	1	409.05	6.20	0.0320
Residual	659.82	10	65.98		
Cor Total	5694.41	15			

Table 8: Composition of Checkout Batch

Gelucire (mg)	PEG 8000(mg)	T10 min (%age)	T 60 min (%age)	Desirability
272.93	214.51	75	97.7267	0.989

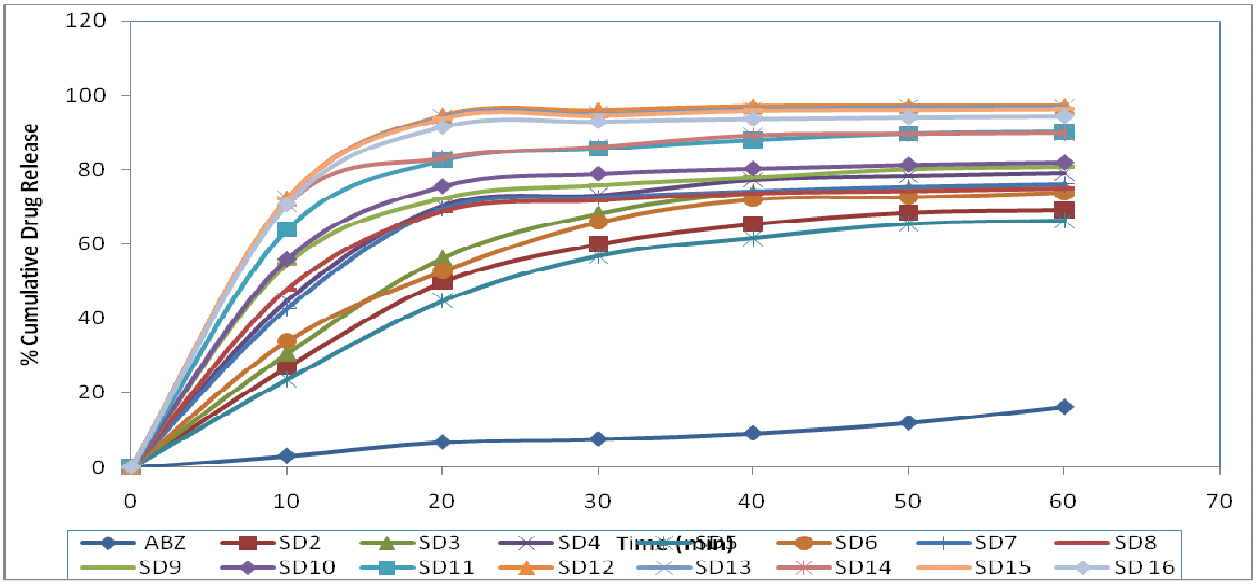

Figure 1a: Dissolution profiles for pure ABZ and Solid Dispersions.

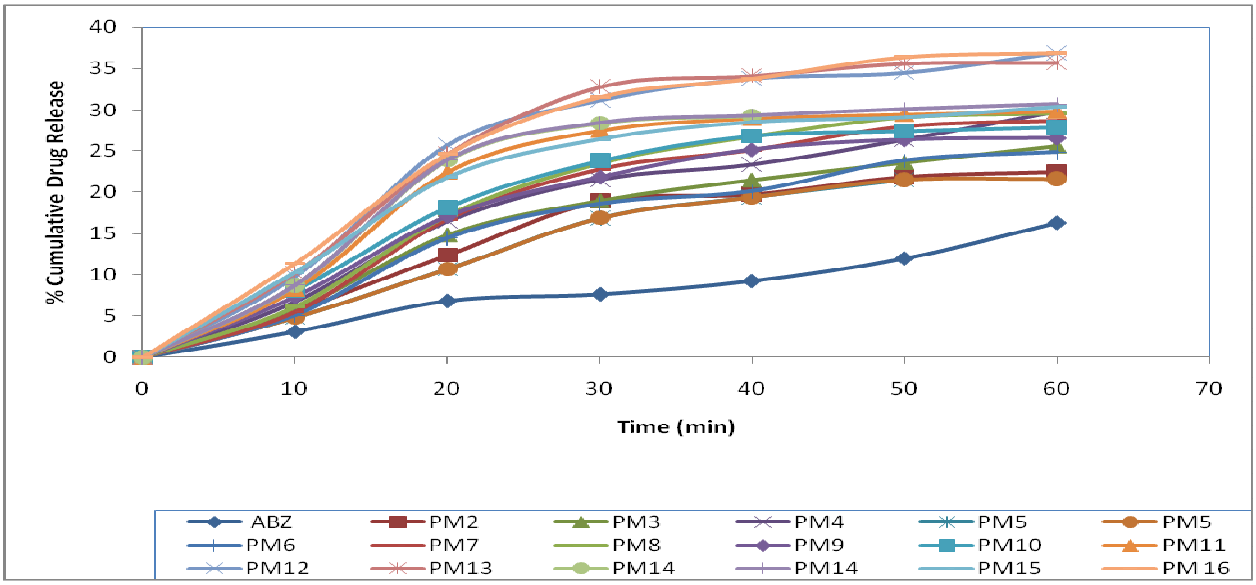

Figure 1b: Dissolution profiles for ABZ and PM's corresponds to amount of albendazole in solid dispersions.

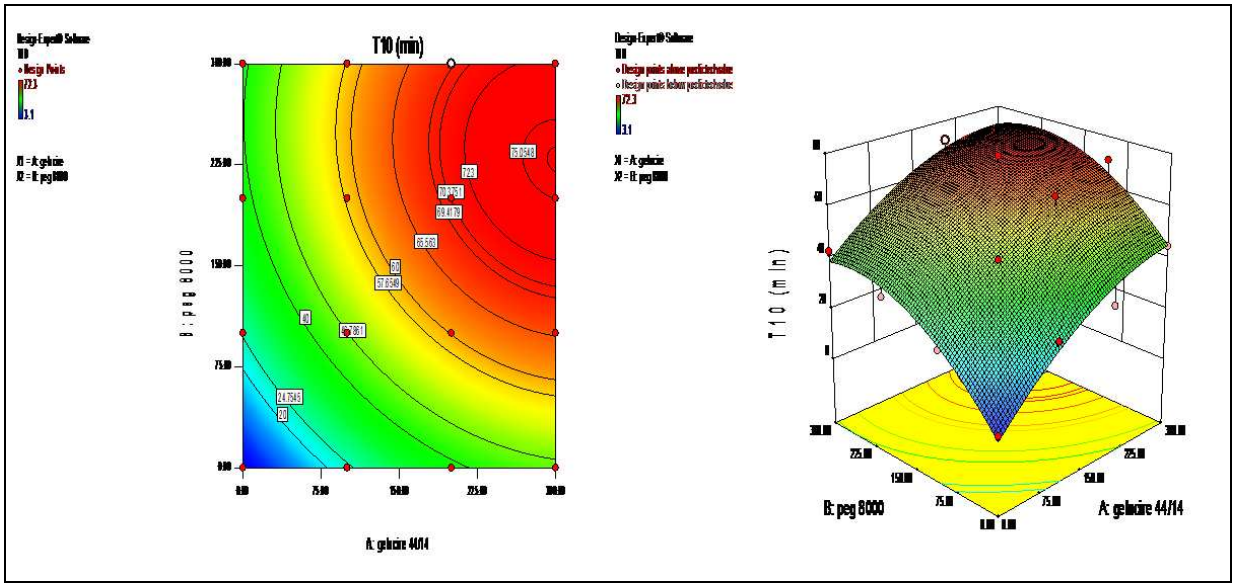

Figure 1c: Surface and contour plots showing cumulative % release in first 10 min. of solid dispersions as a function of gelucire-44/14 and PEG 8000

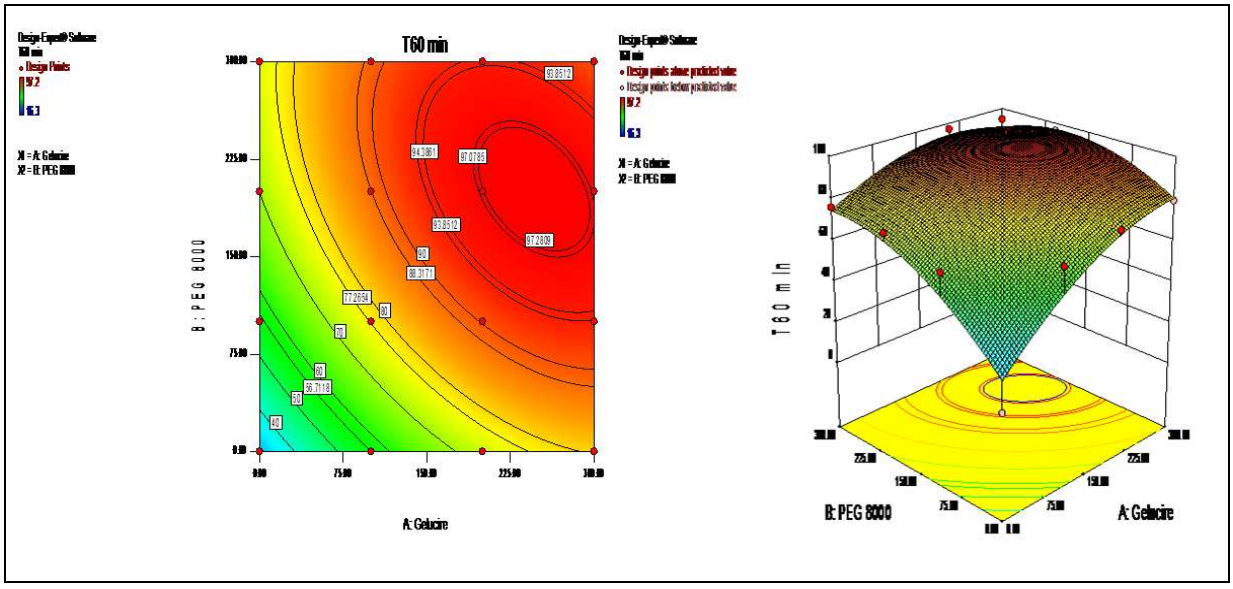

Figure 2: Surface and contour plots showing cumulative % release in first 60 min. of solid dispersions as a function of gelucire-44/14 and PEG 8000

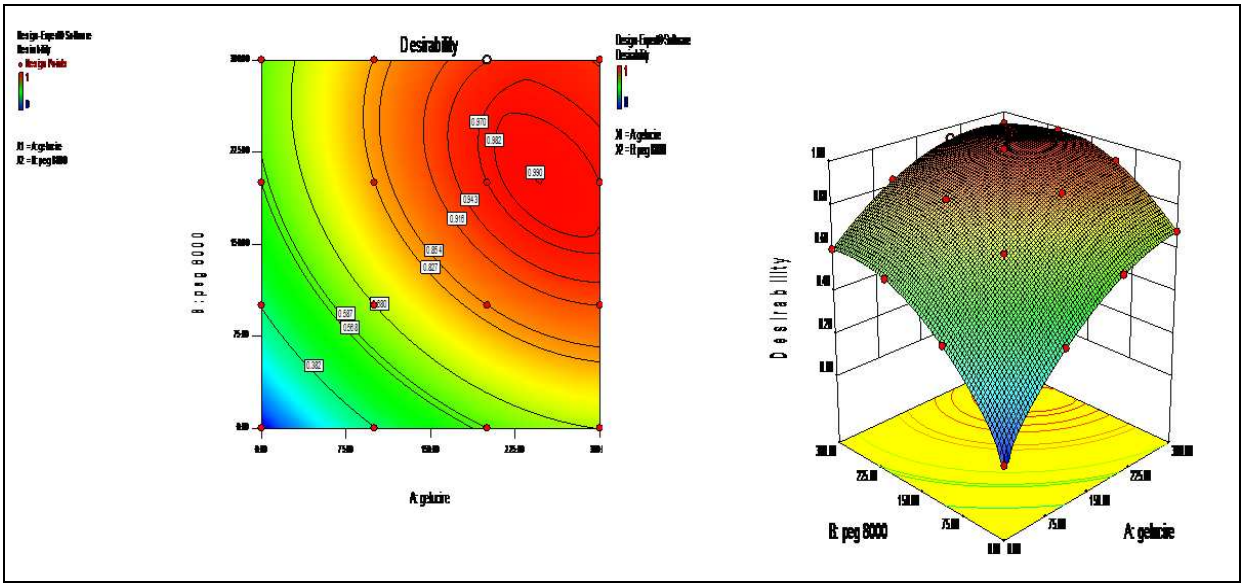

Figure 3: Surface and contour plots showing desirability of solid dispersions as a function of gelucire-44/14 and PEG 8000

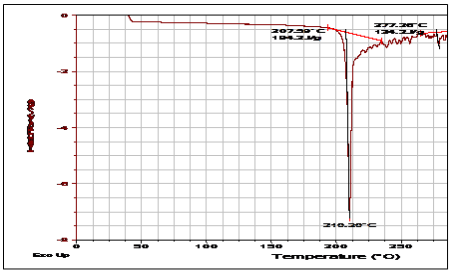

Figure 4: DSC Thermogram of drug (albendazole)

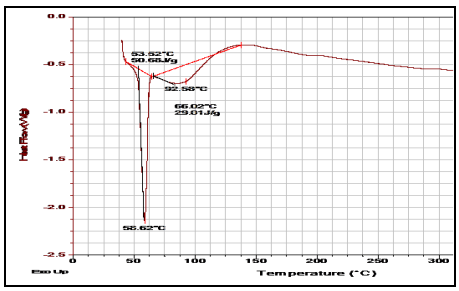

Figure 7: DSC Thermograms of batch SD17

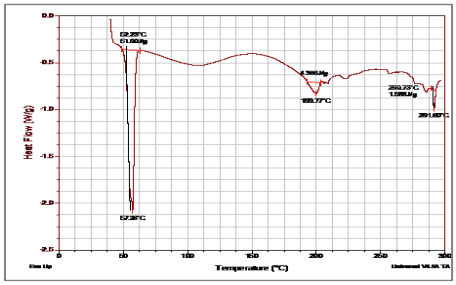

Figure 8: DSC Thermograms of batch PM17

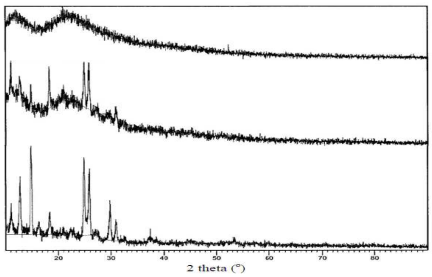

Figure 9: XRD patterns of ABZ, PM 17and optimized solid dispersion formulation SD 17.

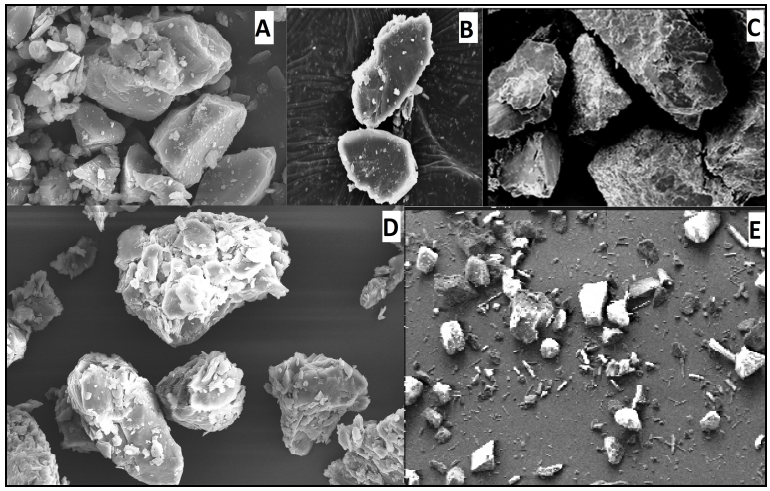

Figure 10: SEM photographs of A. Albendazole; B. PEG 8000; C. Gelucire 44/14; D. SD17; E. PM17.

CONCLUSION

The results of the experimental study showed that the factors amount of PEG 8000 and Gelucire 44/14 significantly influences the dependent variable T10 min (cumulative drug release in 10 minutes) & T60 min (cumulative drug release in 60 minutes). In present work it was shown that the use of solid dispersions can improve the drug properties, like solubility, which was mainly achieved by amorphization with polymeric carriers, PEG 8000 & Gelucire 44/14. The drug amorphization was accessed by DSC, XRD and SEM, where it was proved that ABZ particles lost their crystalline structure presenting an amorphous porous structure in solid dispersions as compared to their corresponding physical mixtures. The ratio of polymers and ABZ required to the maximum amorphization of ABZ was accessed by optimization using four level 2 factor full factorial design. On the basis of dissolution studies of formulations and constraints applied, the results of factorial design suggested only one optimized combination of polymers by which maximum desirability can be achieved. The check out batch was prepared and responses were evaluated. The responses value observed in checkpoint batch was found to be very near to optimized batch. The optimized batch was further studied by DSC, XRD and SEM, which indicated that ABZ particles lost their crystalline structure presenting an amorphous porous structure. It can be concluded that Solid dispersion of Albendazole was found to be useful technique for enhancement of bioavailability.

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