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SOLUBILITY ENHANCEMENT OF CARBAMAZEPINE USING LYOPHILIZED SOLID DISPERSION TECHNIQUE FOR ORAL ADMINISTRATION OF CAPSULES

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ABSTRACT

Carbamazepine (CBZ) belongs to BCS Class-II used to control some types of seizures in the treatment of epilepsy and neuropathic pain by blocking use-dependent sodium channels. The main objective of research work was to the suitable enhancing solubility of carbamazepine for oral administration of capsules. The lyophilized of CBZ were prepared employing different concentrations of polaxamer 407 and skimmed milk powder in different combinations as a carriers by lyophilization solid dispersion technique using 3^2 factorial design. CBZ was compatible with both the polymers and it was confirmed by FTIR and DSC study. Total nine formulations were designed and are evaluated for physicochemical properties, differential scanning calorimetry, X-Ray powder diffractometry, water content, saturation solubility, compressibility and fluidity, assay, and *in-vitro* drug release. All of lyophilized batches showed 1.84 to 4.94 times increase in saturation solubility. Its amorphous nature was confirmed from reducing melting point with low ΔH in DSC and minimizing of RDC in XRD. Polynomial equations were developed for saturation solubility, in-vitro drug release and validity were verified by designing 2 check point formulations. The maximum drug release was observed for batch F_9 (93.98±1.90%) than pure CBZ (28.38±0.10%) after 80 min. This might be due increase in wettability and decrease in crystallinity of CBZ particles with hydrophilic polymers. Formulated solid dispersions showed improvement in flow properties and compressibility of CBZ. All the batches were stable in normal temperature and humidity conditions in the form of drug contents. There were no any color changes which revealed stability of CBZ.

KEYWORDS: Carbamazepine; amorphous solid dispersions; oral administration; lyophilization technique.

INTRODUCTION

Epilepsy is a disease characterized by recurrent seizures, which are nothing but episodes of paroxysmal neuronal discharges. When compared to the general community, epilepsy patients had a mortality rate was observed twice or three times higher.[1, 2] Because of their altered physicochemical features, the majority of recently produced compounds for the treatment of epilepsy suffer from variable absorption that reduces their therapeutic efficacy. Moreover 40% new chemical entities developed in pharmaceutical industry are practically insoluble in water.[3] These slowly absorbed, weakly water-soluble medications cause gastrointestinal mucosal damage and insufficient and unpredictable bioavailability. For oral administration of drugs, solubility parameter is the most important rate limiting to achieve their desired concentration in systemic circulation for pharmacological response. Problem of solubility is a major challenge for formulation scientist, because of the decreased solubility, the majority of medication transport to the brain has been restricted.[4] Low solubility and high permeability for the therapeutic molecules are indicated by the Biopharmaceutical Classification System (BCS) class II. As the drug is having low solubility, it dissolute very poorly so it delays the absorption that indicates the rate of dissolution is the controlling step for absorption.[5, 6]

Carbamazepine (CBZ) has an anticonvulsant effect. The primary mechanisms of action for CBZ involve decreasing the excitation of neurons by blocking sodium and/or calcium channels. It also increases the inhibition of neurons by increasing or enhancing γ-amino butyric acid. CBZ comes under class of BCS II. Also protein binding of CBZ is very high and bioavailability is very low.CBZ is antiepileptic drug used in the treatment of trigeminal neuralgia and bipolar depression. The CBZ has poor micromeritics properties & solubility. It has included in BCS Class II drug. The bioavailability of CBZ is low and variable, so the aim of research work is to solve the problem associated with drug. CBZ, a dibenzapine derivative with a structure resembling the tricyclic antidepressants, is used to control different types of seizures in the treatment of epilepsy. One of the major issues with this drug is its very low solubility in biological fluids. The plasma half-life ranges from 18 to 60 h following a single dose and from 10 to 35 h during chronic therapy, which results into poor bioavailability.[7,

[8] It shows an erratic dissolution profile in gastric and intestinal fluid due to its poor water solubility. The peak plasma concentration and the time taken to reach depend on the extent and the rate of dissolution of the drug, respectively. The rate of dissolution can be increased by increasing the surface area of the available drug by various methods viz; micronization, complexation and solid dispersion.[9] The paradigm of solubility challenges faced by formulation scientists is still largely unchanged. The poor aqueous solubility and subsequent dissolution rate of any drug are one of the most considerable challenges during formulation design and development.[10] Low aqueous solubility is the major problem encountered with formulation development of new chemical entities. Any drug required to be absorbed being exist in the form of an aqueous solution at the site of absorption. The major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability relies upon on several factors together with aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre systemic metabolism, and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.[11]The enchantment of drug solubility thereby its oral bioavailability remains one of the most difficult aspects of drug development system in particular for drug delivery system. There are numerous techniques available and reported in literature to increase the solubility of poorly water soluble drugs. The techniques are chosen on the basis of certain aspects such as properties of drug under consideration, nature of excipients to be selected, and nature of intended dosage form. Solubility improvement techniques can be categorized into physical and chemical modifications of the drug, and other techniques. In order to get a porous, amorphous powder with high degree of interaction between drug and cyclodextrin, lyophilization/freeze drying technique is considered suitable. In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug and polymer at reduced pressure. Thermolabile substances can be suitable for made into complex form by this method. The limitation of this technique is the use of specialized equipment, time consuming process, and yield poor flowing powdered product. Lyophilization/freeze drying technique is considered as an alternative to solvent evaporation and involves molecular mixing of drug and carrier in a common solvent.[12] It is an important issue is to design an optimized formulation with an appropriate dissolution rate in a short time period and minimum trials.

Many statistical experimental designs have been recognized as useful techniques to optimize the process variables. For this purpose, response surface methodology (RSM) utilizing a polynomial equation has been widely used. The different types of response surface methodology designs include 3-level factorial design, central composite design, Box-Behnken design and D-optimal design. Response surface methodology (RSM) is used when only a few significant factors are involved in experimental optimization.[13, 14] The technique requires less experimentation and time, thus proving to be far more effective and cost effective than the conventional methods of formulating dosage forms. Hence an attempt is made in this research work to formulate solid dispersion by lyophilization/freeze drying technique of Carbamazepine using polaxamer 407 (POLAX) and skimmed milk powder (SMP). Instead of normal and trial method, a standard statistical tool design of experiments is employed to study the effect of formulation variables on the release properties and solubility. Large scale production needs more simplicity in the formulation with economic and cheapest dosage form.

MATERIALS AND METHODS

Carbamazepine (CBZ) and polaxamer 407(POLAX) were received as gift sample from CTZ Pharma. Pvt. Ltd, Mumbai, India. Skimmed Milk Powder (SMP) is obtained by removing water from pasteurized skim milk and purchased from Alpha Milk Foods Pvt. Ltd, Mumbai. Distilled water was used for all dissolution experiments and all other chemicals and reagents were of analytical grade.

Preparation of physical mixtures and phase solubility study

The drug and carriers were passed through a 40-mesh screen and mixed thoroughly in a mortar and pestle. For solubility studies, ratio of drug and carrier is used as shown in Table 1. Dissolve one part of drug and respective amount of POLAX and SMP in 10 ml distilled water in saturation tubes. The solution was sonicated for 15 min and transfer to mechanical shaker rotating at speed 100 rpm for 24h. These solutions were filtered by using whatmann's filter paper number 45. Finally saturation solubility was determined. It was observed that solubility was increased with POLAX and SMP concentration rang 0.2 to 1 and 0.5 to 2 respectively.

Table 1: Phase solubility study of drug, polaxamer 407 and skimmed milk powder.

Drug-polymer ratio			Solubility
CBZ	**POLAX**	**SMP**	**(mg/ml)**
1	-	-	**0.098**
1	1	1	0.184
1	1	0.5	0.167
1	1	2	0.337
1	2	0.5	0.233

Statistical analysis by factorial design

$A3^2$ randomized full factorial design is used to study the effect of the different processes and polymers on the solubility of the drug. In the design 2 factors each at 3 levels and experimental formulation batches were performed for 9 possible combinations. The concentration of POLAX and SMP were determined from initial trial batches and used as independent variables. The saturation solubility and % drug release of

the lyophilized formulation was used as dependent variables. The formulation using 3^2 randomized full factorial design as shown in Table 2. All the statistical and regression analysis procedures in the response parameters were performed using the Minitab® software package. Statistical analysis was carried out which includes the analysis of variance (ANOVA) to determine the significance of each independent variable (process, polymer), two-way interactions (process-polymer). The general linear model used for the experimental design equation (1) was:

$Y=\beta_1+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2$ …….. (Eq. 1)

The student-Newman-Keuls test with SAS software was performed to determine the best polymer process combination that would give the best possible results to enhance the solubility.

Table 2: 3^2 randomized full factorial design and levels of two independent variables.

Formulation	CBZ (mg)	POLAX (mg)	SMP (mg)
F1	100	20	50
F2	100	60	50
F3	100	100	50
F4	100	20	125
F5	100	60	125
F6	100	100	125
F7	100	20	200
F8	100	60	200
F9	100	100	200

Independent variables	Levels		
	-1	0	+1
Polaxamer	20 mg	60 mg	100 mg
Skimmed milk powder	50 mg	125 mg	200 mg

Preparation of CBZ-POLAX-SMP solid dispersion by lyophilization technique

Preparations of CBZ-POLAX-SMP containing the model drug and different excipients were prepared by using mini delvac lyophilizing process. Briefly, according to the compositions listed in Table 2, CBZ and the excipients were weighed accurately and dissolved in a certain volume of deionized water for polymer and organic phase, ethanol for drug and then mixed both solutions slowly to form a uniform solution by magnetic stirring. The solutions were applied onto a cryogenic solid substrate of the apparatus, whereby the solutions were frozen and solidified rapidly. The obtained frozen solids were collected and quickly transferred to bench top tray freeze dryer for lyophilization, and the temperature was gradually increased from -50 to 25°C over 48 h. The resultant dry powders were stored in transparent vacuum desiccators at room temperature.

Preparation of CBZ-POLAX-SMPSD capsule formulations

In this study, a dry granulation method was used to prepare the CBZ-POLAX-SMP SD capsule formulations. The desired CBZ-POLAX-SMP SD was mixed with microcrystalline cellulose, lactose, magnesium stearate and talc according to the compositions in Table 3. The well-dispersed mixtures were compressed into slug tablets with a hydraulic press. The tablets were ground into granules and then sieved with 40-mesh sieves to obtain the capsule content.

Table 3: Compositions of CBZ-POLAX-SMPSD capsules.

Sr. No.	Ingredients	Capsules (2) content for formulation F_1 to F_9
1	CBZ-POLAX-SMP SD	Equivalent to 100 mg
2	Microcrystalline Cellulose	25
3	Lactose	50
4	Magnesium Stearate	3
5	Talc	2

The content equivalent of 100 mg CBZ was filled into hard gelatin capsules of applicable size for further investigation.

Characterization of amorphous solid dispersions

Physicochemical properties of CBZ-POLAX-SMPSD and related capsule formulations

The physicochemical properties of CBZ-POLAX-SMPSD produced by solid dispersion technique and the related capsule formulations were investigated and compared to pure CBZ.

Fourier Transform Infrared Spectroscopy (FTIR)

The pellets were prepared at high compaction pressure by using potassium bromide. These were examined using Bruker FT-IR spectrometer model. The spectra of drug and formulation were compared with that of the original spectra.[13]

Differential Scanning Calorimetry (DSC)

Thermal analysis (DSC) of the samples was performed on a Shimadzu DSC 60 which was calibrated for temperature and enthalpy using pure Indium. Approximately 3–5 mg of CBZ-POLAX-SMP SD or the related capsule formulation was loaded into aluminum pans and subsequently compressed (PerkinElmer, Waltham, MA, USA). The samples were heated at a ramp rate of 10°C/minute from 0 to 220°C with nitrogen as the sample purge gas at a flow rate of 40 ml/min. The instrument was equipped with a refrigerated cooling system. Data were treated mathematically using DSC TA universal analysis program.[13]

X-Ray Powder diffractometry (XRPD)

CBZ-POLAX-SMP SD and the related capsule formulation were examined by wide angle X-ray powder diffraction (XRPD) using a Philips 1710 X-ray

diffractometer with a copper target and nickel filter (Philips Electronic Instruments Inc., Mahwah, NJ, USA). The voltage was 40 kV, and the current was 25 mA. The samples were measured in the 2-theta range from 3 to 50° using a step size of 0.05 2-theta degree with a dwell time of 2 s.

Water content measurements

The residual water contents were measured by an Aquapal III Karl–Fischer Titrator. CBZ-POLAX-SMP SD or the related capsule formulation was accurately weighed and dissolved in anhydrous methanol and then quickly transferred to a Karl–Fischer titration vessel for titration, and the water content was calculated. Three replicates of each sample were measured with the same procedure.

Saturation solubility studies

Solubility studies of Carbamazepine were performed in water and in 0.1NHCl solution, for a period of 48 h with sample analysis at nine time intervals according to the method of Connors and Higuchi.[15] The 48h time duration was selected since it allowed the drug to reach equilibrium solubility. An excess amount of the drug and lyophilized powder in the medium was ensured equilibrium during the 48 h period. The studies were conducted at room temperature (25°C). The solution was filtered through a 0.45 µ pore size filter. The solution concentration was measured using UV-Visible spectrophotometer at 285 nm.

Compressibility and fluidity analysis

The Carr index (CI) is an important indicator for evaluating the compressibility and fluidity of powder samples. The CI was calculated from bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) using the equation $CI= (\rho_{tapped}-\rho_{bulk}) \times 100/\rho_{tapped}$. Bulk and tapped densities were measured by the cylinder method using a 50–1200 tapped density meter. CBZ-POLAX-SMP SD or the related capsule formulation was sieved with 18-mesh sieves and accurately weighed, followed by pouring into a cylinder. The volume was measured to obtain the bulk density, and then, the cylinder was tapped until the sample volume became constant to determine the tapped density.

Drug content determination

An accurately weighed sample (CBZ-POLAX-SMP SD or the related capsule formulation, approximately 100 mg of CBZ) was dispersed in methanol (10 ml) and mixed thoroughly to extract CBZ. The solution was filtered through a membrane filter (0.45 µm, Gelman GHP Acrodisc, VWR, West Chester, PA, USA), and 50 µl filtrate was appropriately diluted with methanol, vortexed, and suitably diluted and absorbance was measured at 285 nm using double beam UV spectrophotometer (Shimadzu 1800, Japan). Three replicates of each sample were measured with the same procedure, and the drug contents were expressed as percentages compared to the theoretical amount.

***In-vitro* dissolution testing**

In-vitro dissolution testing was performed according to the USP II paddle method using a Lab India Disso 8000 apparatus (Mumbai, India). The paddle speed was 50 rpm, and the dissolution medium was maintained at 37±0.5°C. Accurately weighed amounts of the solid dispersions CBZ-POLAX-SMPSD or the related capsule sample was placed into the dissolution vessel containing 900 ml of 0.1N HCl. During testing, 1 ml for each samples were withdrawn from vessel at predefined intervals at 2, 5, 10, 30, 45 and 60 min and immediately filtered with 0.45 µm filter, suitably diluted and analyzed by UV-Visible spectrophotometrically at 285 nm. All experiments were carried out in triplicate.

Stability study of lyophilized capsule formulation

The stability studies of formulations were studied using the reported standard procedure at different temperatures. The CBZ-POLAX-SMPSD-related capsules were wrapped in aluminum foil and placed in petri dishes. These containers were stored at ambient humid conditions, at 25°C/60% RH for 3 months. The samples were analyzed for physical changes such as color, texture, drug content and drug release characteristics and compared with the initial values before storage. The F1 (dissimilarity value) and F2 (similarity value) were calculated for drug release parameter by using DD-solver tool while the value of correlation coefficient was calculated for drug content.[16]

RESULTS AND DISCUSSION

Lyophilization is freezing process in which a homogenous solution freezes into thin films on a cryogenic surface within typically 50 to 1000 ms, depending on the properties of the solvent. The degree of super cooling is so high that nucleation and growth of crystals may be minimized or prevented, especially when the polymer is contained in the liquid feed solution. Rapid freezing prevents phase separation during freezing, allowing the drug and the polymer to be molecularly dispersed within the final formulation, leading to the formation of amorphous material in the nanostructure.[17]

Practical yield and solubility studies

Solubility study for the drug and formulations were carried out by using distilled water and 0.1N HCl solutions as a dissolution medium. In solubility determinations the concentration in solution depends on the drug's solubility. Excess drug accounts for any loss that may occur in solution, whereby more drugs is released from the suspended particles so that the amount of dissolved drug remains constant. This concentration is the drug's equilibrium solubility in a particular solvent at a particular temperature. [18] The initial rate of solubilization of the drug varies hyperbolically with time. During the initial time period, the drug goes into solution continuously, increasing the concentration of drug linearly. As the time increases, more and more drug goes into solution, until the solution is saturated with the

drug, and equilibrium solubility is observed. The solubility of the drug was found very low solubility in distilled water and 0.1N HCl. It was observed that formation of lyophilized CBZ by using polaxamer 407 and skimmed milk powder helps to improve the saturation solubility of CBZ. The physical mixtures of the drug and the two polymers were tested for any positive effect on the solubility of the drug in the presence of polymers.

Table 4: Solubility profile and practical yield of lyophilized of CBZ.

Sr. No.	Batch code	Practical Yield (%)	Solubility in distilled Water (mg/ml)	Solubility in 0.1 N HCl (mg/ml)
1	CBZ	-	0.098	0.333
2	F_1	83.3	0.180	0.602
3	F_2	79.36	0.200	0.819
4	F_3	80	0.224	0.902
5	F_4	91.83	0.300	0.882
6	F_5	87.71	0.484	1.084
7	F_6	82.05	0.291	1.459
8	F_7	88.55	0.311	1.105
9	F_8	90.00	0.315	1.416
10	F_9	89.85	0.386	1.616

A look at solubility profiles is shows (Table 4) a F_1 to F_4 increase in solubility for all the physical mixtures as compared to the solubility of carbamazepine alone at any given time point. However, this was not a significant increase in solubility and hence can be considered equivalent to untreated drug for all practical purposes. Polaxamer 407 and skimmed milk powder increase the solubility of Carbamazepine when they were incorporated as solid dispersions. Formulation of lyophilized CBZ enhances the saturation solubility than drug by 1.84 to 4.94 times in distilled water and 1.824 to 4.853 times in 0.1 N HCl solutions. Solubility of batch F_5 was increased by 4.94 times in distilled water and F_9 was increased by 4.853 times in 0.1N HCl. This might be because of polaxamer which acts a surfactant and produces viscous solution in water as well as 0.1N HCl solutions. Skimmed milk and polaxamer was also responsible for enhancing the wettability of lyophilized CBZ as compared to pure CBZ. Practical yield of freeze dried techniques were determined by previous given formula. It was observed that lyophilized batches gave practical yield in the range of 79.36 % to 90 % as shown in (Table 4). Concentration of both the polymers were not affecting on practical yield of lyophilized batches.

Preparation of CBZ-POLAX-SMPSD and related capsule formulations

Freezing process in which a homogenous solution freezes into thin films on a cryogenic surface within typically 50 to 1000 ms, depending on the properties of the solvent. The degree of super cooling is so high that nucleation and growth of crystals may be minimized or prevented, especially when the polymer is contained in the liquid feed solution. Rapid freezing prevents phase separation during freezing, allowing the drug and the polymer to be molecularly dispersed within the final formulation, leading to the formation of amorphous material in the nanostructure. [17] During the process, the solvent is rapidly frozen and solidified. Then the solvent is removed by sublimation during lyophilization to obtain a nano structured matrix-like composition. The polymers, including POLAX and SMP, which have different solubilities in the GI digestive fluid, were chosen as excipients. To obtain better physicochemical properties and increase patient compliance via an oral administration route, CBZ-POLAX-SMPSD related capsule formulations were prepared by granulation with microcrystalline cellulose, and magnesium stearate as supporting agents. A 3^2 randomized full factorial design is used to study the effect of the different processes and polymers on the solubility of the drug. In the design 2 factors each at 3 levels and experimental formulation batches design as shown in Table 2.

Amorphous state of CBZ-POLAX-SMPSD

Pure CBZ and all formulation of CBZ-POLAX-SMPSD were analyzed by DSC and XRPD. Heat flow thermograms are depicted in Fig. 1A. CBZ exhibited one obvious endothermic peak at 191.79^0C, which represent close to melting point of pure CBZ (189-192°C) of the crystal forms of the drug. As other polymers forming POLAX and SMP possess a relatively high glass transition temperature (Tg), resulting in lower drug molecular mobility and crystallization tendency, which is more conducive to the preparation of stable CBX.[19-21] The characteristic endothermic peaks of CBZ disappeared in all of the CBZ-POLAX-SMPSD lyophilized formulations, which demonstrated the amorphous state of the drug in these dispersions. The melting point of a compound is a fundamental physical property determined for the purpose of characterization or purity identification of a compound. The DSC showed corresponding value of sharp endothermic at melting point. But DSC of lyophilized formulation showed the absence of any peak at totally the range of melting point of CBZ. It revealed that area of endotherm peak was decreased. Hence, it indicates that decreased in crystalline form of drug CBZ in formulation with polaxamer 407 and skimmed milk powder. CBZ have polymorphic form.

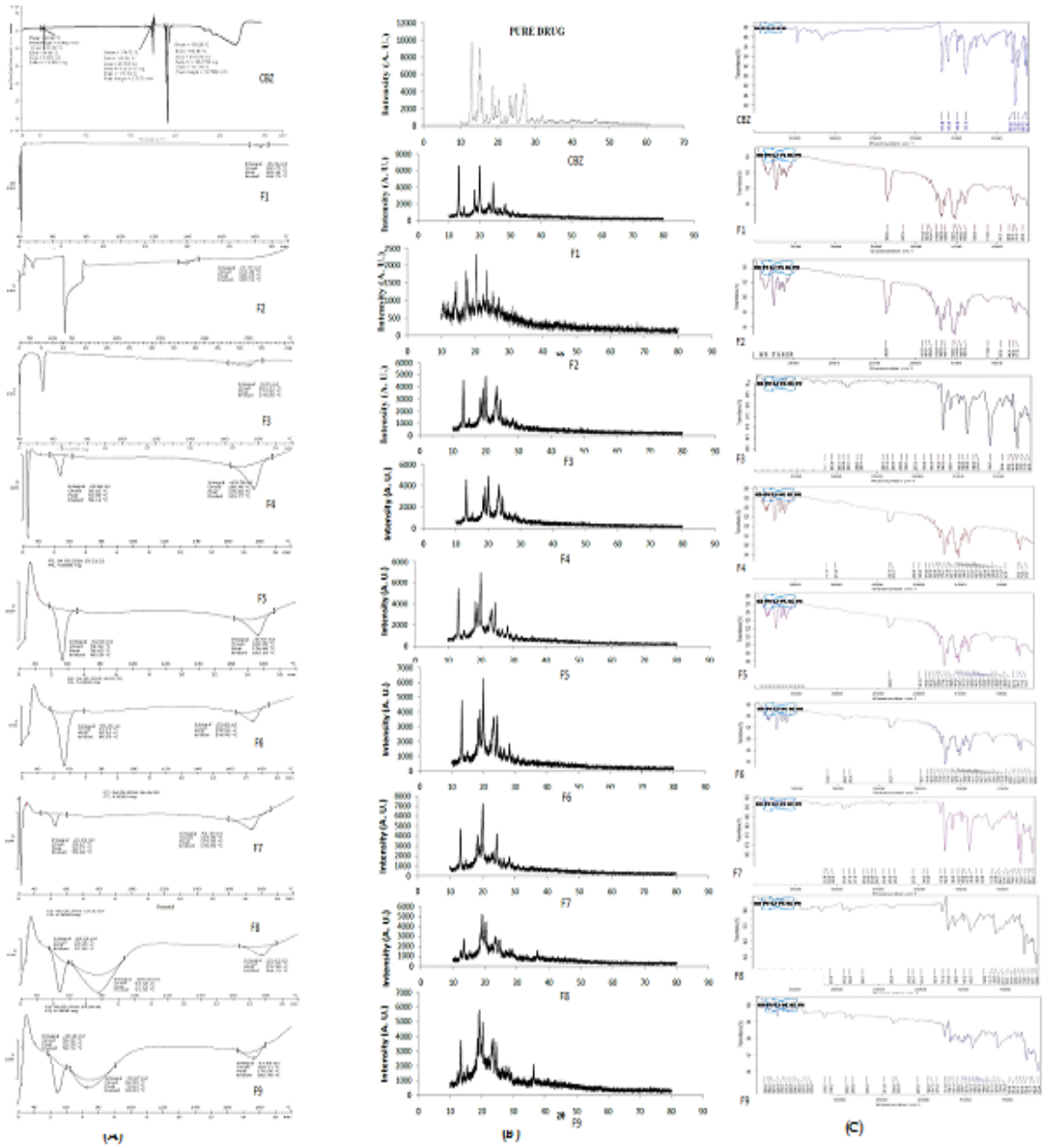

Figure 1: Characterization of CBZ-POLAX-SMPSD (A) DSC profiles of pure CBZ, and CBZ-POLAX-SMPSD formulation. (B) X-ray powder diffraction (XRPD) patterns of pure CBZ, and CBZ-POLAX-SMPSD formulation (C) FTIR of CBZ and CBZ-POLAX-SMPSD formulation.

Depending on their relative stability, one of the several polymorphic forms will be physically more stable than others. Such a stable polymorphs represents the lowest energy state, has highest melting point and least aqueous solubility. The remaining polymorphs are called as metastable form which represent the higher energy state, have lower energy state; the metastable form cannot be called unstable because if it kept dry, it will remain stable for year. The DSC of all lyophilized formulation shows decreased melting point with compare to pure CBZ. It also shows that decreased endothermic peak i.e. sharp crystalline form as compare to pure CBZ. It shows increases aqueous solubility of CBZ as compare to pure CBZ. According to study DSC, it might be contributed to decreasing crystallinity of CBZ and amount of CBZ might have converted to amorphous state. DSC of lyophilized Carbamazepine might have converted to stable form to metastable form which has more solubility than the stable form.

XRD pattern (XRD) is used for identification and determination of nature of particles. If the nature of particles is crystalline the diffraction pattern is sharp and show high intensity at particular 2θ range. CBZ is available in different polymorphic forms and they are identified by XRD. As a highly crystalline powder, crude CBZ showed distinctive peaks in the 2θ range of 13–28^0 and major characteristic peaks at 2θ values of 25.0 and 27.4^0 (Fig. 1B).However, none of these correlative characteristic drug peaks could be identified in the XRD

patterns of the CBZ-POLAX-SMPSD samples, highlighting that the drug was completely amorphous in these three dispersions (Figure 2B), which is consistent with the DSC results. The obtained spectra showed that pure CBZ was identical to that of form III reference slandered that reported by the international center for diffraction data. The most providing identification is the absence of peaks from 20 to 100 at these 2θ ranges. The powder diffraction pattern (PDP) of pure CBZ spectrum showed that the drug was crystalline in nature as demonstrated by numerous distinct peaks observed at 2θ of 13.02, 15.22, 18.65, 20.33, 23.38, 24.90 and 27.15 these were matched to known PDP of CBZ form III. XRD pattern of solid dispersions showed no sharp peak. Intensity at these 2θ indicating amorphous nature. From result, we can conclude that the CBZ present in F_2 batches is in substantial amorphous form. Amorphous form is high energy and low melting point from due to free energy available for dissolution process and hence dissolution rate is faster than crystalline form. Relative degree crystallinity is defined as ratio of highest peak intensity of formulation to the highest peak intensity of formulation. It is always less than one. The smaller the particle size lowers the value of relative degree of crystallinity. Spray dried formulation ratio F_2 shows low value RDC. Reduction of crystallinity might have achieved due to reasons viz, amorphous hydrophilic polymer, skimmed milk powder and lyophilization technique. FT-IR spectrum of lyophilized formulation showed all the characteristic peak of CBZ, polaxamer 407 and skimmed milk powder. It showed the peaks at 3441.35, 1636.09, 2300.03 cm^{-1}. In the FT-IR graph, there was absence of any extra peak other than characteristic peaks of CBZ and polaxamer 407 and skimmed milk powder (Fig. 1C). Hence, it might confirm that there was no chemical interaction between CBZ, POLAX and SMP.

Drug content and flowability

The drug content, Carr's index and flowability of CBZ and CBZ-POLAX-SMPSD the related capsule formulations are listed in Table 5. The drug contents of formulations were in between 87.14 ±1.0 – 98.17 ±1.0%, which is consistent with the ratio of CBZ/excipients. The drug contents of the corresponding F_8 capsule formulations were slightly reduced 0.8714 due to the added small quantities of supporting agents during the preparation of dry granulates. It was observed that polymer concentrations were not affecting the percentage drug contents. The physicochemical properties of CBZ-POLAX-SMPSD the related capsule formulations were investigated and compared to crude CBZ. The flow properties of CBZ powder are considered critical to the quality of the final formulation.[22] In general, CI<20 represents a free flowing nature.[23]

Table 5: Drug contents, residual water contents and flow properties of CBZ and the related formulations.

Formulation	Drug Content (%)	Residual Water Content (%)	Angle of repose* (^{0})	Carr Index	Flowability*	Hausner's ratio*
CBZ	-	0.162 ± 0.18	38.15± 0.5	25.14±0.1	Fair	1.32±0.01
F1	88.00 ± 1.3	0.762 ± 0.12	24.12± 0.12	12.23±0.65	Good	1.21± 0.01
F2	92.25 ± 0.8	0.593 ± 0.05	25.42± 0.05	12.25 ±0.82	Good	1.12 ± 0.05
F3	96.15 ±0.3	0.186 ± 0.01	28.60 ±0.40	11.70 ±0.58	Good	1.15 ±0.01
F4	98.17 ±1.0	0.574 ± 0.15	26.90± 0.30	12.05 ±0.22	Good	1.35 ±0.01
F5	93.23 ±0.9	0.482 ± 0.04	28.00± 0.55	15.19±0.02	Good	1.00 ± 0.06
F6	90.12 ±1.0	0.563 ± 0.12	29.50± 0.20	15.13 ±0.8	Good	1.18± 0.01
F7	89.20 ±0.5	0.522 ± 0.13	28.60 ±0.40	15.97±0.06	Good	1.25 ±0.02
F8	87.14 ±1.0	0.526 ± 0.01	28.45± 0.32	14.18±0.02	Good	1.17 ±0.02
F9	94.20 ±1.5	0.536 ± 0.01	29.59± 0.70	11.19±0.03	Good	1.28 ±0.01

**SD (n=3), * Flowability: Evaluated by the Carr index, <10 Excellent, 11–15 Good, 16–20 Fair, 21–25 Passable, 26–31 Poor, 32–37Very poor, >38 extremely poor.*

For all the CBZ-POLAX-SMPSD, the flowability was fair or passable, with CI values in the range of 11.70 to 25.14. The corresponding CI values of the CBZ-POLAX-SMPSD related capsule formulations were much smaller (from 11.17 to 15.97), indicating that those capsule contents showed better fluidity and stronger compressibility than did CBZ. The angle of repose for all the lyophilized batches were within 25°-29.5° indicating good flow properties. The angle of repose is function of internal friction and cohesion of particle. If value of angle of repose is high, particles are cohesive and if it is low, particles are non-cohesive or free flowing. The relationship between angle of repose and flow property of powder is shown in Table 5. The bulk density of powder depends on particle size distribution, particle shape and tendency of particle to adhere together. The taped density of particle indicates packing capacity. The flow property behavior all crystals and co-crystals were studied by Carr's Index (%) and Hausner's ratio. The Carr's Index (%) and Hausner's ratio of all the samples were found to be less than 25% and according to Carr's Index the material having value less than 25% will be free flowing (Table 5).

Solubility and *in-vitro* dissolution by response surface method

From the study of response variables following equation (2) is generated for the formulations containing polaxamer 407 and skimmed milk powder.

Solubility=1.04+0.11 *A+0.15*B) ……. (Eq. 2)

Where, A and B represents the effect of variables i.e. concentration of POLAX and SMP concentration respectively. The counter and surface plot for solubility profile is shown in Fig. 2. All the polynomial equations were found to be statistically significant, as determined using ANOVA. The Model F-value of 78.86 in above equation implies the model is significant. P value was found to be 0.0001 ($P<0.05$) which indicates model terms A and B are significant.

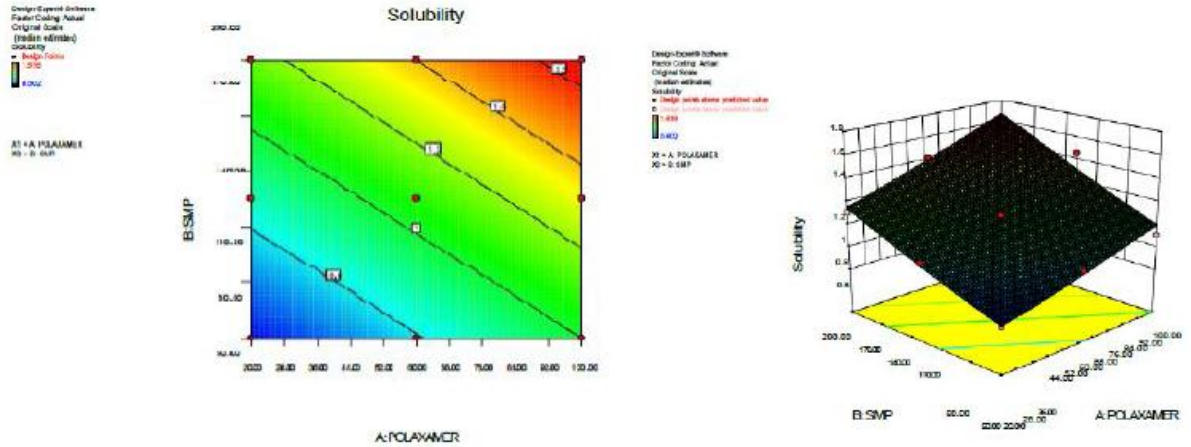

Figure 2: Counter and surface plot for solubility profile.

In-vitro dissolution testing under different pH conditions was conducted to assess the performance of the CBZ samples and CBZ-POLAX-SMPSD the related capsules. The resulting profiles of dissolution of CBZ and CBZ-POLAX-SMPSD the related capsules are shown in Fig. 3. Immediate burst release of drug from the formulation was observed within about 10 min. It might be due to the property of polaxamer 407 that adsorbs drug at the surface of lyophilized formulations. Hence, surface drug immediate exposure to the dissolution medium and showed highest drug release. This dissolution was related to the great water solubility of the excipient, which provided significantly greater media dissolution rates of the drug. Poor dissolutions (<50% for the CBZ) were revealed in an acidic medium of pH 1.2. In general, drug release from CBZ-POLAX-SMPSD showed a similar trend as that of the related capsules, with slightly increased accumulative dissolution rates. The formation of a high-energy amorphous material can increase the predicted solubility of the drug. [24, 25] Once the samples were exposed to the dissolution medium, the dissolution rates mainly depended on the solubility of the excipients.[26] All formulations showed increased dissolution profile of drug CBZ from lyophilized system as compared to pure CBZ. Percentage of drug release of pure drug was 28.38% and formulations showed 77.84 to 93.98 % drug release.

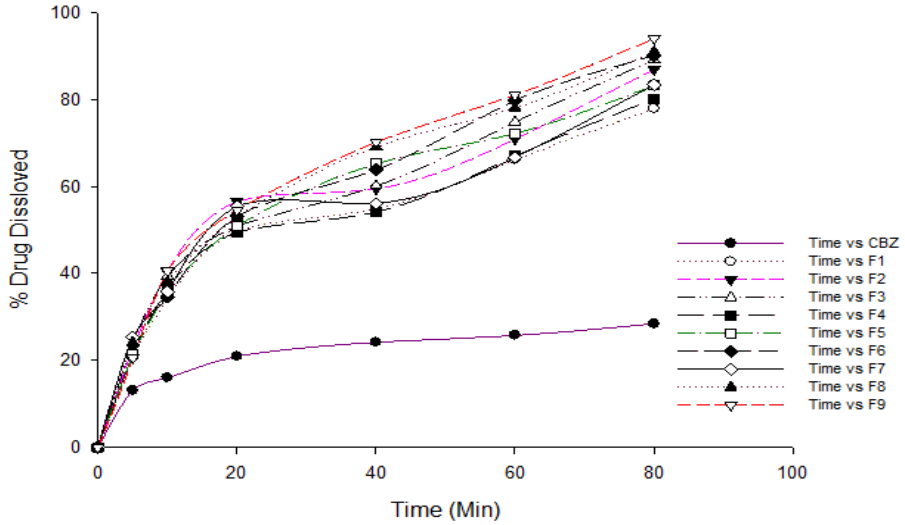

Figure 3: *In-vitro* dissolution profiles of CBZ-POLAX-SMPSD and the related capsules.

This increased drug release of formulation was might be due to enhancement of solubility in dissolution medium. From the study of response variables following equation (3) is generated for the formulations containing polaxamer 407 and skimmed milk powder % drug release=85.31 +5.30* A+2.43*B-0.12*A*B -127 *A^2+2.53 *B^2 …… (Eq. 3)

Where, A and B represents the effect of variables i.e. concentration of polaxamer and SMP concentration respectively. Figure 4 shows the profound effect of concentration of the polaxamer and SMP on the % drug release of the formulation.

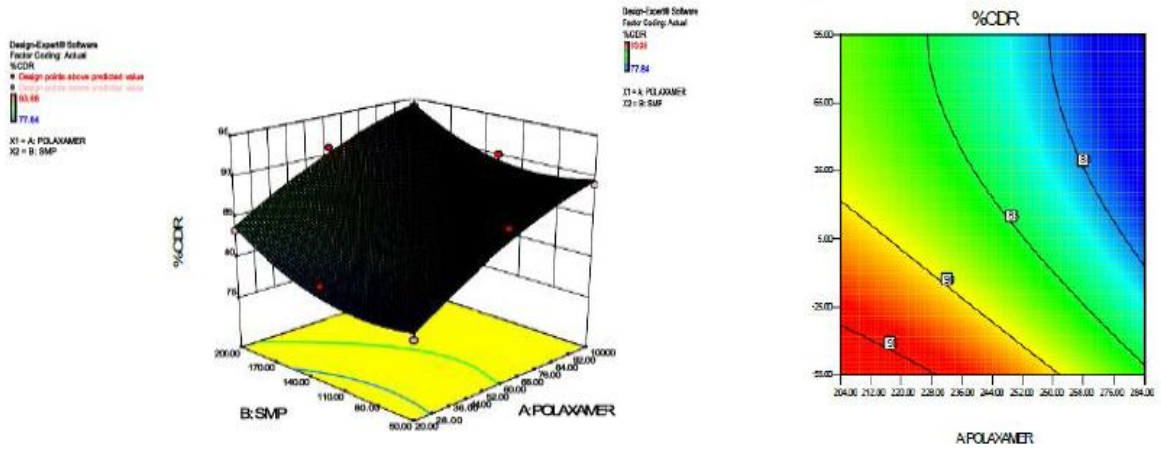

Figure 4: Contour and surface plot for effect of concentration of the polaxamer and SMP on the % drug release of the formulation.

All the polynomial equations were found to be statistically significant, as determined using ANOVA, as per the provision of design significant. P value was found to be 0.0343 (*P<0.05*) which indicates model terms A and B are significant. The counter plot clearly indicates that the % drug release is increased with increase in concentration of polaxamer and SMP. From the formulations, it was observed that the maximum % drug release was obtained with the formulation F_1. Formulation F_9 shows highest drug release 93.98 %.This is because of increased amount of polaxamer 407 and skimmed milk powder in drug. Formulation F_1 shows less drug release 77.84. This is because of decreased amount of polaxamer 407 and skimmed milk powder in drug.

Physical stability studies

As revealed in Figure 1A, 1B and 1C, compared to the related CBZ-POLAX-SMPSD, the drug remained in its amorphous state in all of the contents, which demonstrated that the preparation procedures should not affect the properties of these formulations with nearly unchanged main quality indexes. A further stability study was conducted with CBZ-POLAX-SMPSD-related capsules at 25°C/60% RH for 3 months to evaluate any changes in crystallinity and drug content. The samples were analyzed at time points of 1 and 3 months using DSC, XRPD and drug content, as described previously. The DSC results confirmed that the melting point of CBZ was absent, and therefore the XRPD patterns exhibited no modification within the peak intensity of the drug in any of the contents, after 3 months, which indicated an essentially amorphous state of CBZ. The F1 (dissimilarity value) and F2 (similarity value) for drug dissolved at 25±2°C/60±5% RH were 9.74 and 763.45 respectively indicated that the formulation was stable after three month and complied for the stability. The drug content gave the correlation coefficient values of 0.998 which indicated that the values were strongly correlated with each other. From stability study it was concluded that there were no major changes in the physicochemical parameters evaluated like drug content and *in-vitro* dissolution pattern at the various sampling points.

CONCLUSIONS

In the present investigation, lyophilized technique of solid dispersion was successfully used to enhance the solubility, saturation and dissolution of CBZ in distilled water. The related CBZ-POLAX-SMPSD was further processed for capsule formulations by a dry granulation method for convenient oral administration. The drug remained in an amorphous state in all of the preparations, and the drug dissolution rates from the CBZ-POLAX-SMPSD the related capsules were modified and enhanced compared to that of the pure (CBZ) drug. Compatible ternary complex of skimmed milk powder and polaxamer 407 with hydrophobic drug has proved to enhance physicochemical and micromeritics properties. Thus, bioavailability of CBZ, BCS class II drugs, can increase due to minimization of rate limiting step. Increased solubility and dissolution profile may responsible to reduce the dose of CBZ and hence increase in therapeutic concentration. Collectively, CBZ-POLAX-SMPSD prepared by lyophilized technique could be a promising delivery system to enhance the oral absorption of poorly water soluble drugs.

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