Drug Development and Industrial Pharmacy, 33:1008–1023, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040601179749

informa
healthcare

Investigations on Factors Affecting Chitosan for Dissolution Enhancement of Oxcarbazepine by Spray Dried Microcrystal Formulation With an Experimental Design Approach

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In the present work effect of chitosan on microcrystal formulation for dissolution enhancement of oxcarbazepine using controlled crystallization technique coupled with spray drying was explored. The work was extended for exploration of simplified approach for stable particle size reduction. The study was performed with an experimental design approach i. e. a fractional factorial design of resolution 5 (with all 2 factor interaction) for the screening of predefined independent variables drug concentration, chitosan concentration, feed rate, inlet temperature and percent aspiration for spray drying. Whereas percent drug dissolved, wettability time, flowability in terms of angle of repose and particle size were designated as response variables. Resultant models were analyzed using multiple linear regression analysis, which generated equation to plot response surface curves along with desirability function. Results showed that chitosan concentration had significant effect on dissolution enhancement of oxcarbazepine at a level of 2% w/v. Increase in drug concentration showed decreased dissolution rate however on particle size it did not show statistically significant effect. Topographical characterization was carried out by SEM which showed that feed rate, percent aspiration and inlet temperature had significant effect on particle morphology. For deriving optimized formulation results were analyzed using desirability function for the maximum percent drug dissolved and least drug polymer matrix particle size. DSC studies showed that drug was molecularly associated with chitosan matrix or particles.

Keywords oxcarbazepine (OCBZ) microcrystals; chitosan; fractional factorial design; desirability function; enhancement; scanning electron microscopy (SEM)

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INTRODUCTION

Paradigmatic shift in the process of new drug discovery towards synthesis of higher molecular weight entities and higher lipophilicity has emerged the development of several new techniques as well as more exploration of conventional techniques for the dissolution enhancement of drugs. Oxcarbazepine is classified as BCS class II drug. Drugs having low aqueous solubility but high intestinal permeability are classified as BCS class II drugs (Lobenberg & Amidon, 2000; Yu et al., 2002). It is 10-keto analogue of carbamazepine, an antiepileptic drug with similar effectiveness to standard first choice treatments in partial and generalized tonic-clonic seizures. Oxcarbazepine has linear and non-time dependent pharmacokinetics that makes dose titration simpler than carbamazepine - UK Medicines Information Pharmacists Group (Aug 2000). It is a white to faintly orange crystalline powder practically insoluble in water.

Conversion of a crystalline form of drug to an amorphous form is nothing but the alteration of molecular architecture of drug which helps in rapid solubilization with high energy advantage (Kaushal et al., 2004). To take the solubility and rapid dissolution advantage of amorphous drugs from their crystalline counterparts there are several techniques available for reducing the particle size of drugs which utilize high energy processes such as micronization by jet milling, cutting, freeze drying, spray drying, melt extrusion etc. But the systems resulting from use of such techniques are often referred to incomplete or partially amorphous products (Hancock & Zografi, 1997). It is because the high internal energy and specific volume of the amorphous state compared to original crystalline state eventually have enhanced dissolution and bioavailability but there is always danger of getting converted back of the amorphous systems to a more stable crystalline state during processing or storage (Ambike et al., 2005). They are also susceptible for agglomerations resulting decrease in

drug dissolution. In milling there is fracture of crystalline drug due to which electrostatic effects can occur. The products obtained with these techniques have their disadvantages posing serious questions for stability of amorphous systems. It is reported that changes in surface energy influences processing properties such as the powder flow of the system which is undesirable for compression or hard gelatin capsule fill. Micronized powders which have a higher energy on surface (measured by inverse gas chromatography) showed poorer flow properties (Feeley et al., 1998). High specific surface of micronized particles always make them. A further disadvantage of jet milling processes is a broad size distribution (Müller et al., 1996). It has been reported that amorphous systems are more unstable and highly susceptible for drug degradation than crystalline systems (Byrn et al., 2001).

In the present work an attempt was made to reduce the crystalline particle size of oxcarbazpeine using controlled crystallization technique which was coupled with hydrophilic polymeric coprecipitation and spray drying. Till the date several hydrophilic polymers like hydroxypropylmethyl cellulose (Kapsi & Ayres, 2001; Mitchell et al., 2003; Yamada et al., 2000), hydroxypropylmethylcellulose phthalate (Ishikawa et al., 2000; Qi & Ping, 2004; Sertsou et al., 2002), lactose (Abberger et al., 2002; Chidavaenzi et al., 2001; Christensen et al., 2001; Cilurzo et al., 2002; Corrigan et al., 2002), polyvinyl alcohol (Wiedmann et al., 1997), polyvinyl pyrollidone (Ambike, Mahadik, & Paradkar, 2005; Corrigan, Healy, & Corrigan, 2002; Doherty & York, 1989) have been utilized for the controlled crystallization of drugs but use of chitosan have been found to be with more promising results (Bodek, 2002).

Chitosan [(1–>4)-2-amino-2-deoxy-b-D-glucan] is a linear cationic polysaccharide obtained by *N*-deacetylation of chitin, a naturally-occurring structural polysaccharide abundant in crab and shrimp shells. Several authors have proposed its effectiveness in enhancing the dissolution properties and bioavailability of poorly-soluble drugs (Acarturk et al., 1993a,b; Giunchedi et al., 2000; Giunchedi et al., 2002; Portero et al., 1998; Sawayanagi et al., 1982; 1983; Shiraishi et al., 1990). Chitosan has also been reported as an excipient for direct compression (Ritthidej et al., 1994; Upadrashta et al., 1992). It has been reported that grinding of chitosan with poorly soluble drugs, such as griseofulvin or prednisolone, enhances their dissolution properties and for low solubility acidic drugs (indomethacin), it is proposed that gel forming reaction of the positively charged amino sugar groups of chitosan and the negatively charged drug to increase the solubility of the drug (Sawayanagi, Nambu, & Nagai, 1982). (Hou et al., 1985) found that granules, formed from chitosan and indomethacin, released the drug faster at pH 7.5 after exposure to acid stomach pH, than if the granules had not been exposed to the low pH. The reason for it was the swelling and gel formation ability of chitosan at this low pH.

Chitosan can easily form solid dispersions using a casting technique and a spray-drying technique and the release of the pharmaceutical prepared by the spray-drying technique was related to the crystallinity of the drug - chitosan systems (Asada et al., 2004). Literature has also revealed that spray drying of drug in solution with a hydrophilic polymer for coprecipitation has several advantages and efficiency over well reported conventional way of obtaining the amorphous form of drug i.e., solid dispersion (Corrigan, 1995; Lian, 2001; Paradkar et al., 2004; Rasenack et al., 2003). With all these points taking into account and considering the several favorable biopharmaceutical properties of chitosan, to explore in depth the idea of extending chitosan applications to pharmaceutical preparations, this polymer was selected as a potential carrier for improving oxcarbazepine dissolution behavior.

If there are several variables (multivariable) in a system design of experiments (DOE) a powerful statistical tool should be used for improvement or enhancement of output of such systems. In past several years, it is observed that pharmaceutical industry has used experimental designs more for the optimization of pharmaceutical agents; but, only a few are reported in the literature for the development of dosage forms (Nazzal et al., 2002.; Rotthäuser et al., 1998). The present study was performed with fractional factorial design of resolution 5 (with all 2 factor interaction) for the screening of drug concentration, chitosan concentration, feed rate, inlet temperature and percent aspiration for spray drying. Whereas percent drug dissolved, wettability time, flowability in terms of angle of repose and particle size were designated as response variables.

MATERIALS AND METHODS

Pure oxcarbazepine was received as gift sample from Torrent Research Centre (Ahmedabad, India), Chito Clear® chitosan was received as gift sample from Primax Biopolymers, Irland. Solvents and chemicals used were of analytical grade and were used without further purification, purchased from Loba Chemie (Mumbai, India). Double distilled water was used throughout the study.

METHODS

Experimental Design

For the experiments performed as per design coded and actual values of independent variables are depicted in table 1. Two levels -1 and 1 were used for study and for centre points a third level was set at '0' for all the independent variables (drug concentration, chitosan concentration, feed rate, inlet temperature and percent aspiration). Table 2 shows the experiments performed as per the fractional factorial design. The optimum concentration of sodium lauryl sulphate as a stabilizing agent was screened previously and it was found to be 0.25% w/v.

Microcrystal Preparation

Microcrystals of oxcarbazepine were prepared using solvent change method. In this method the previously molecularly

TABLE 1
Independent Variables for Fractional Factorial Design and Their Coded and Actual Levels

Independent Variables	Low Level (–1)	Middle Level (0)	High Level (1)
Chitosan Conc. (A)	1	1.5	2
Drug conc. (B)	0.5	0.75	1
Feed rate (C)	2.5	5	7.5
% Aspiration (D)	50	75	100
Inlet temp (E)	105	115	130

dispersed (dissolved) drug is rapidly precipitated by addition of miscible solvent in presence of stabilizing agent. (Rasenack, Hartenhauer, & Muller, 2003; Rasenack & Muller, 2002) The formed microcrystals were grown naturally in medium and spray dried to completely dry the microcrystal product and not for formation of the particles or microcrystals. Weighed amount of oxcarbazepine was dissolved in 250 mL of acetone. The drug was dissolved in solvent with constant stirring for 15 min. on magnetic stirrer. The solution was added with 0.25% aqueous solution of sodium lauryl sulfate as a stabilizing agent. In a separate beaker weighed amount of chitosan was dissolved in 5% glacial acetic acid. The chitosan solution was kept for 4 to 6 hr to allow complete hydration and obtain a clear solution. To this solution drug solution was added with vigourous stirring over magnetic stirrer for 5 to 10 min. The resultant mixture was allowed to stand for further 10 min. to allow association of chitosan molecules with naturally growing microcrystals dispersion. This microcrystals' dispersion was then spray dried (JISL Lab Mini Spray Dryer, Mumbai, India). Spray drying parameters like feed rate, inlet temperature and percent aspiration decide the quality of final product like particle size, flow properties as well drug loading capacity of the system. In the present work these parameters were termed as independent variables.

CHARACTERIZATION AND EVALUATION OF MICROCRYSTALS

Particle Size Measurements

The volume Particle size of microcrystals was determined with the laser diffraction particle size analyzer (MAN 0244/ HYDRO 2000 SM, Malvern Instruments Ltd., UK). Petroleum ether was used as dispersion medium for carrying out measurements.

In Vitro Dissolution Studies

The dissolution study was conducted in USP XXVII simulated gastric fluid (without enzymes) having pH 1.2 ± 0.02. Accurately weighed amount of microcrystals, containing equivalent 100 mg of drug were wrapped in cloth pouch of approximate 2–5 μm mesh. This pouch was placed in basket of USP dissolution apparatus (Type I, TDT-06P, Electrolab, Mumbai, India) with 900 mL deareted dissolution medium. Microcrystals were placed in cloth pouches to avert floating of them on dissolution media surface. Deareation of dissolution media were done with the help of ultrasonication (Ultrasonics—2.2, India) for 15 min. The dissolution apparatus was run at 50 RPM keeping the temperature (37 ± 1ºC) constant throughout the experiment. Samples (5 mL) were withdrawn at 0, 5, 10, 15, 20, 25, and 30 min and were filtered through 0.45 μm whatmann filter paper, diluted suitably and analysed spectrophotometrically at 303 nm (Shimadzu, UV-1601 UV, Visible spectrophotometer, Japan). An equal volume of fresh dissolution medium maintained at the same temperature was added after withdrawing sample to maintain the sink conditions. The absorbance values were transformed to concentration by reference to a standard calibration curve obtained experimentally (r^2 = 0.9998). The dissolution test was performed in triplicate for each batch.

Angle of Repose Measurements (Flowability Indicator)

For measurement of angle of repose of microcrystals, they were passed through a funnel on the horizontal surface. The height (h) of the heap formed was measured with a cathetometer and the radius (r) of the cone base was also determined. The angle of repose (Φ) was calculated from equation 1.

$$\Phi = \tan^{-1}\left(\frac{h}{r}\right) \quad (1)$$

Wettability Studies

For determination of powder wettability two approaches were used. All the experimental batches were evaluated for wettability time whereas the contact angle, well reported measure of powder wettability; of optimized batch of microcrystals and pure drug was determined using tensiometer (GBX Tensiometer, France equipped with Mettler Toledo balance and 3S software). For wettability time determination sample powder weighed about 1 g was placed in sintered glass funnel of 27 mm. internal diameter. Bridge was formed at the neck of funnel with the help of cotton plug. The funnel was held in upright position in a beaker filled with water such that water level in beaker just touches the cotton plug. Methylene blue powder was layered over surface of pure drug in funnel. Time required to rise the water through drug till wetting of methylene blue powder occurs was recorded (Gohel & Patel, 2003). In case of contact angle determination the cell constant for system was

TABLE 2
Matrix of the Experiments and Results for the Measured Responses and the Desirability for the Fractional Factorial Design

Formulation No.	Pattern	Chitosan (A)	Drug (B)	Feed Rate (C)	Aspiration (D)	Inlet Temp. (E)	Percent Drug Dissolved	Particle Size (μm)	Wettability Time (min.)	Angle of Repose (°)	Predicted Percent Drug Dissolved	Desirability (D)
1	---+	–1	–1	–1	–1	1	60.55	20.66	2.31	30.00	59.47	0.00
2	---+-	–1	–1	–1	1	–1	58.93	24.21	2.01	36.54	57.85	0.00
3	--+--	–1	–1	1	–1	–1	56.26	30.09	1.87	35.36	55.18	0.36
4	--+++	–1	–1	1	1	1	62.70	28.65	1.92	30.28	61.62	0.48
5	-+---	–1	1	–1	–1	–1	53.32	22.34	2.21	35.45	52.24	0.00
6	-+-++	–1	1	–1	1	1	58.14	22.56	2.01	34.00	57.07	0.42
7	-++-+	–1	1	1	–1	1	75.54	26.54	0.87	31.25	74.47	0.76
8	-+++-	–1	1	1	1	–1	80.75	27.86	1.50	30.25	79.68	0.69
9	+----	1	–1	–1	–1	–1	83.73	21.25	1.42	27.89	82.66	0.79
10	**+--++**[1]	**1**	**–1**	**–1**	**1**	**1**	**93.27**	**16.78**	**0.59**	**25.69**	**92.19**	**1.00**
11	+-+-+	1	–1	1	–1	1	78.26	21.34	1.24	29.40	77.19	0.78
12	+-++-	1	–1	1	1	–1	73.36	30.54	1.18	29.80	72.29	0.66
13	++--+	1	1	–1	–1	1	88.54	26.47	0.90	32.58	87.47	0.82
14	++-+-	1	1	–1	1	–1	72.56	22.07	1.36	29.80	71.49	0.72
15	+++-	1	1	1	–1	–1	79.33	29.13	1.09	29.25	78.26	0.73
16	+++++	1	1	1	1	1	80.14	40.25	0.98	24.15	79.07	0.00
17	CP 1[2]	0	0	0	0	0	62.38	30.24	1.29	33.09	71.13	0.55
18	CP 2	0	0	0	0	0	68.56	20.89	1.48	31.69	71.13	0.67
19	CP 3	0	0	0	0	0	65.27	24.56	1.36	32.25	71.13	0.63

[1]Optimized batch of microcrystals.
[2]CP – Centre Point.

determined using hexane. Contact angle was determined using equation 2 which was proposed by Washerburn.

$$\frac{m^2}{t} = \frac{C\rho_L^2\ \gamma_L\ \text{Cos}\ \theta}{\eta_L} \quad (2)$$

where, m – mass with capillary rise
t – time in sec.
ρ_L – liquid density (g/m^3)
γ_L – liquid surface tension (N/m)
C – cell constant (m^5)
η_L – liquid viscosity (Pa.s)
θ – angle of contact

Scanning Electron Microscopy (SEM)

To get a topographical image analysis of microcrystals SEM study was performed. The beads were mounted on brass stubs using double-sided adhesive tape. SEM photographs were taken with scanning electron microscope (JSM-5610LV, Jeol Ltd., Japan) at the required magnification at room temperature. The working distance of 39 mm was maintained and acceleration voltage used was 15 kV, with the secondary electron image (SEI) as a detector.

XRay Powder Diffraction Studies (XRPD)

XRPD studies were used for getting a defining idea about the crystallinity of the drug in the dosage form or formulation. In present work also XRPD studies were performed (Philips PW 1830) to get the crystalline morphology of the drug in microcrystals formed. The samples were scanned from a starting angle of 5° to an end angle of 60° with a step size of 0.0160°.

Differential Scanning Calorimetry (DSC)

To have an idea about the amount of presence of free crystalline drug along with change in energy of the system DSC thermograms for different formulations were obtained using an automatic thermal analyzer system (DSC-60, Shimadzu, Kyoto, Japan). The samples were heated with a heating rate of 10°C per min. with constant nitrogen flow of 25–30 mL.min^{-1}.

Factorial Design and the Desirability Function

The study was performed with a fractional factorial design of resolution 5 i.e., with all 2 factor interaction. The independent variables selected for predefined screening were drug concentration, chitosan concentration, feed rate, inlet temperature and percent aspiration for spray drying. Whereas percent drug dissolved, wettability time, flowability in terms of angle of repose, and particle size were designated as response variables. For determination of the experimental error, the experiment at the centre point was replicated five times at different intervals. The results for these centre points showed comparable results which is an indication of reproducibility of the experiment.

The results were statistically evaluated with the help of analysis of variance (ANOVA) using statistical software package (JMP 5.1, The Statistical Discovery Software). The quadratic model was selected for this analysis.

For understanding the improvement of the process desirability function was used. In case of the desirability function all the responses were combined in one measurement, this gives the possibility to predict the optimum levels for each of the independent variable. To combine all the responses in one desirability function it is necessary to calculate the individual desirability function. In the present study there were no exact requirements for the particle size of the optimum formulation, but to achieve minimum of it was the set target (lesser the particle size—greater the surface area—greater is the interaction of solute with dissolution medium). Whereas maximum percent drug dissolved, minimum wettability time and maximum powder flowability i.e., minimum angle of repose were the set criteria for enhancement of the process. The individual desirability for each response was calculated using the following methods.

The percent drug dissolved value was targeted to maximize in the procedure, as the higher values of this parameter are desirable. So the desirability function of this parameter was calculated by using equation 3.

$$d_1 = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}} \quad (3)$$

where d_1 is the individual desirability of percent drug dissolved and Y_i is the experimental result for all desirability functions. The values of Y_{max} and Y_{min} for percent drug dissolved were 93.265 and 53.315, respectively.

The calculation of the desirability function for wettability time was carried out using equation 4.

$$d_2 \text{ or } d_3 = \frac{Y_{max} - Y_i}{Y_{max} - Y_{min}} \quad (4)$$

where d_2 is the individual desirability of wettability time and d_3 is the individual desirability of particle size. The values of Y_{max} and Y_{min} for wettability time were 2.31 and 0.59 respectively whereas for particle size they were 16.78 and 40.25, respectively.

For the determination of desirability for angle of repose non-linear partial desirability function was selected. The value was minimized as lower angle of repose was desirable. In all the experiments performed all the experimental values were acceptable, however, the values far from the target, were little

penalized, by choosing 0 < s < 1 (0.1 in this case) in equations 5, 6 and 7.

$$d_4 = 1 \qquad \text{if} \quad Y_i \le Y_{\min} \tag{5}$$

$$d_4 = \left(\frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \right)^s \qquad \text{if} \quad Y_{\min} \le Y_i \le Y_{\max} \tag{6}$$

$$d_4 = 0 \qquad \text{if} \quad Y_{\max} \le Y_i \tag{7}$$

where d_3 is the individual desirability of angle of repose and Y_i is the experimental result. The values of $Y_{\max}$ and $Y_{\min}$ for angle of repose were 36.54 and 24.15, respectively.

The overall desirability value was calculated from the individual values by using Eq. 8.

$$D = (d_1 \times d_2 \times d_3 \times d_4)^{\cdots} = \left[\prod_{i=1}^{4} d_i \right]^{\cdots} \tag{8}$$

RESULTS AND DISCUSSION

The crystallization process involved creation of a hydrophobic surface with a tendency to achieve a low energy stable condition. However in this process due to the surface energy of the seeds energy of the system increased. In such condition chitosan, being in physical immediacy had an attachment with new born particles which prevents further crystal growth of the particles which were also sterically stabilized (Schott, 1985). Thereby small particles failed to aggregate for lowering the surface energy. Thus surface energy and consequently the enthalpy of the system were reduced.

Figure 1 shows the leverage plots of predicted and actual values for the experimental design with percent drug dissolved, particle size, wettability time, and angle of repose as responses or dependent variables. The points on a leverage plot for simple regression are actual data coordinates, and the horizontal line for the constrained model is the sample mean of the response. But when the leverage plot is for one of multiple effects, the points are no longer actual data values. The horizontal line then represents a partially constrained model instead of a model fully constrained to one mean value. The

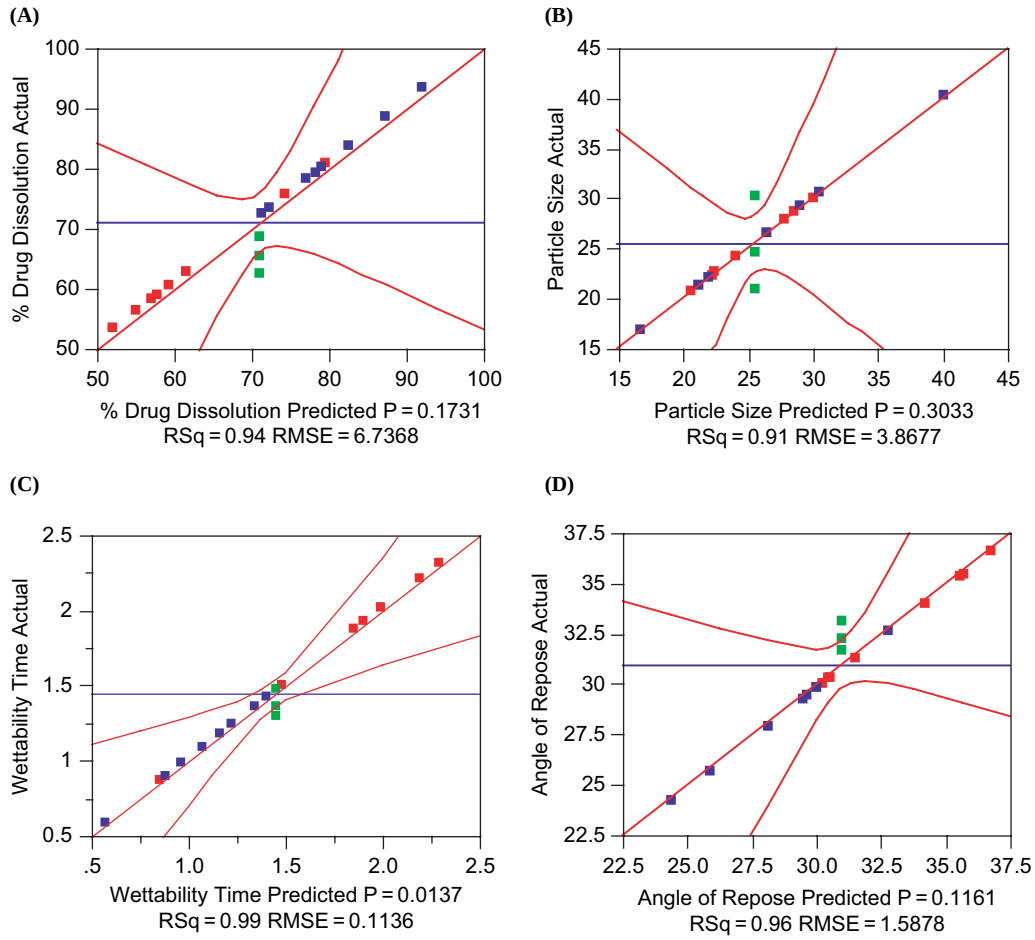

FIGURE 1. Actual vs. predicted values for (A) percent drug dissolved, (B) particle size, (C) wettability time, (D) angle of repose.

response values of all the formulations (except for 2 of 3 center points) lie between the confidence intervals of the leverage plot. Also the confidence region for the line of fit does contain the horizontal line of the mean; this predicts that effects (dependent variables) are significant.

The prediction profiles of the effect of all independent variables over dependent variables and overall desirability of the microcrystals are shown in Figure 2.

Effect of Formulation Independent Variables on Particle Size and Percent Drug Dissolved

Figure 3 (A to I) shows the contour profiles for effect of different independent variables on percent drug dissolved, particle size and overall desirability. Particle size analysis for all formulations was found to have direct relationship with chitosan and drug concentration. It may be attributed to increase in saturation level of solution polymer and drug. Rise in saturation of solution to be spray dried generally produce particles of greater size due to increase in cohesive force of droplets formed during spraying (Adler & Lee, 1999). Since particle size has direct relationship with drug dissolution, decrease in particle size led to increased drug dissolution due to increase in surface area available for wetting. In case of formulation 14, high level of chitosan did not have significant effect on reduction of microcrystals size. This shows that an optimum concentration of chitosan helps in controlling the particle size of microcrystals. Chitosan has physical properties like moisture retaining, swelling and distributing ability. These properties usually help in wetting of drug microcrystals thereby promoting their dissolution. However increase in chitosan concentration also resulted in decrease in wetting time of microcrystals formed, probably due to decrease in surface area available of particles. Figure 10 shows the particle size analysis of pure drug and optimized batch of microcrystals i.e., formulation 10.

Results from table 2 showed that increase in feed rate increased the particle size of microcrystals. Particle size of formulation no. 12 and 16, carried out with high levels of feed rate was found to be 30.54 and 40.25 µm both values are on uppermost side of particle size results of entire experimental study. Increase in feed rate for spray drying promoted particle growth because of excessive liquid supply and larger droplet size (Maury et al., 2005). Literature also has reported that feed rate for spray drying has reflective effect on particle size, since it determined

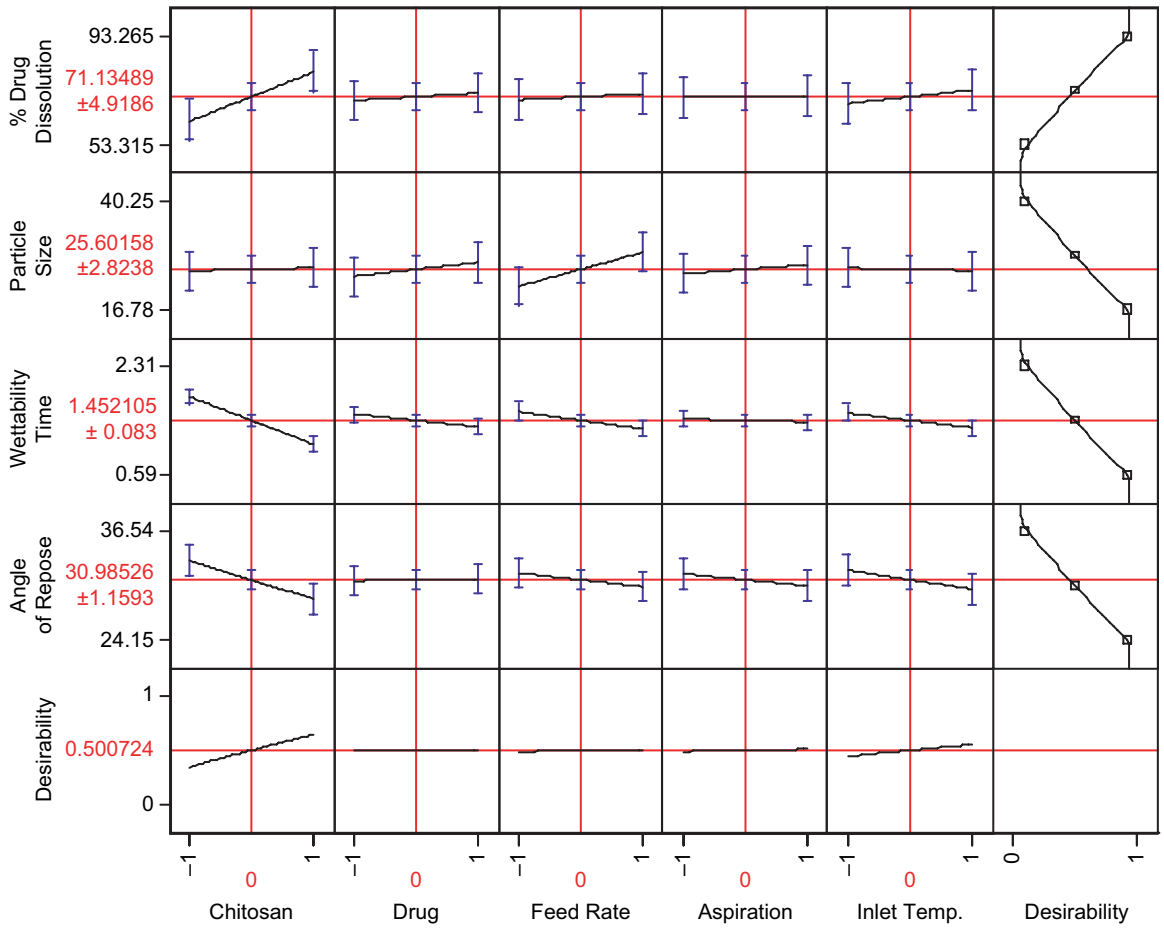

FIGURE 2. Prediction profiler for all the dependant variables and overall desirability of the microcrystals.

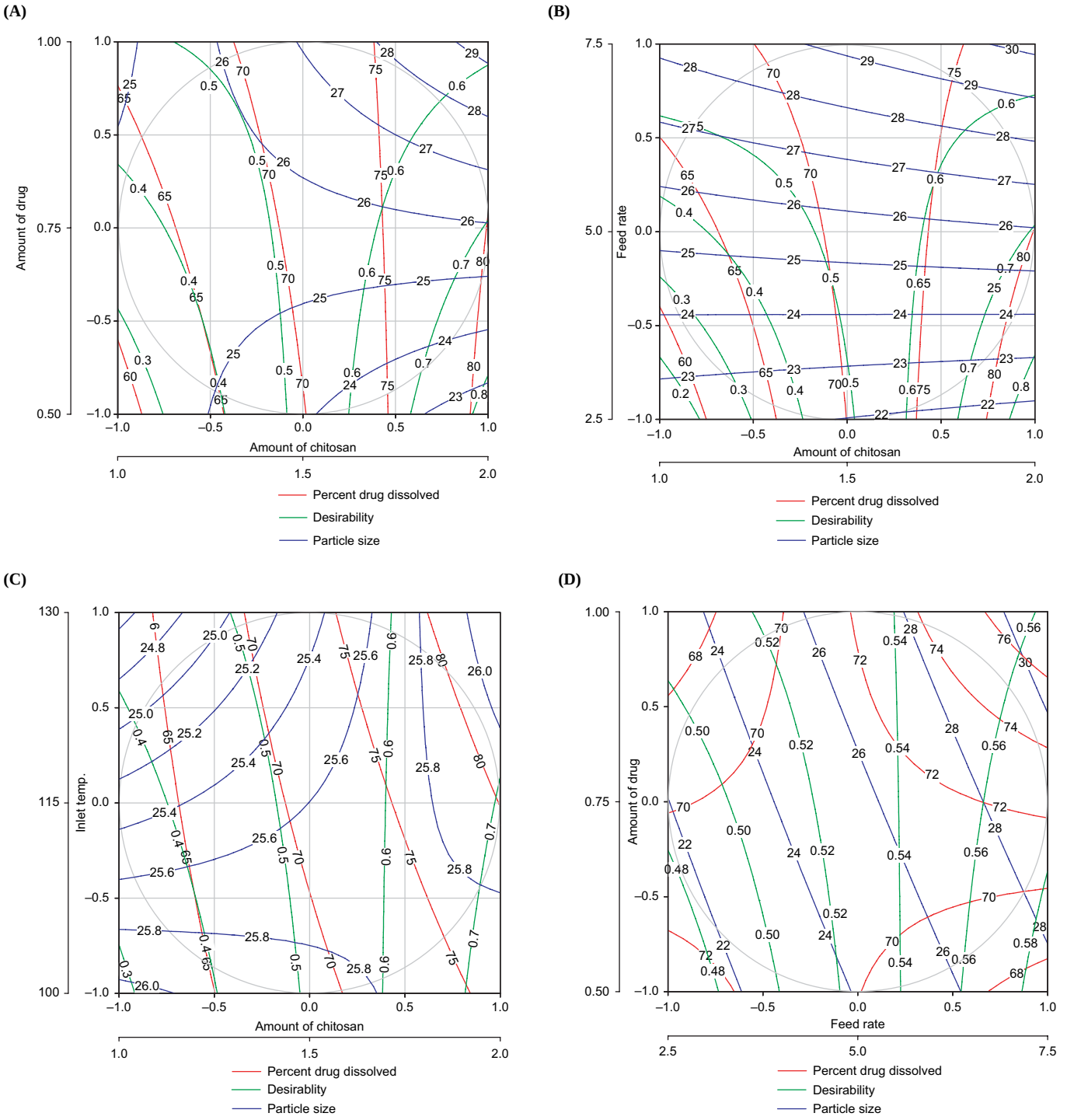

FIGURE 3. Contour plot showing effect of (A) amount of chitosan and amount of drug, (B) amount of chitosan and feed rate, (C) amount of chitosan and inlet temperature, (D) feed rate and amount of drug, (E) amount of drug and percent aspiration, (F) amount of drug and inlet temperature, (G) percent aspiration and feed rate, (H) inlet temperature and feed rate, (I) inlet temperature and percent aspiration on (i) percent drug dissolved (ii) desirability.

the moisture or liquid content and the droplet size of binder solution (Rambali et al., 2001; Vertommen & Kinget, 1997).

Increase in inlet temperature of inlet air during spray drying decreased the particle size of microcrystals formed since the moisture content of spraying solution was also decreased due to rapid evaporation of liquid. Particle growth has direct relationship with spray rate or feed rate and inverse relationship to the inlet air temperature (Menon et al., 1996). Scaled estimates,

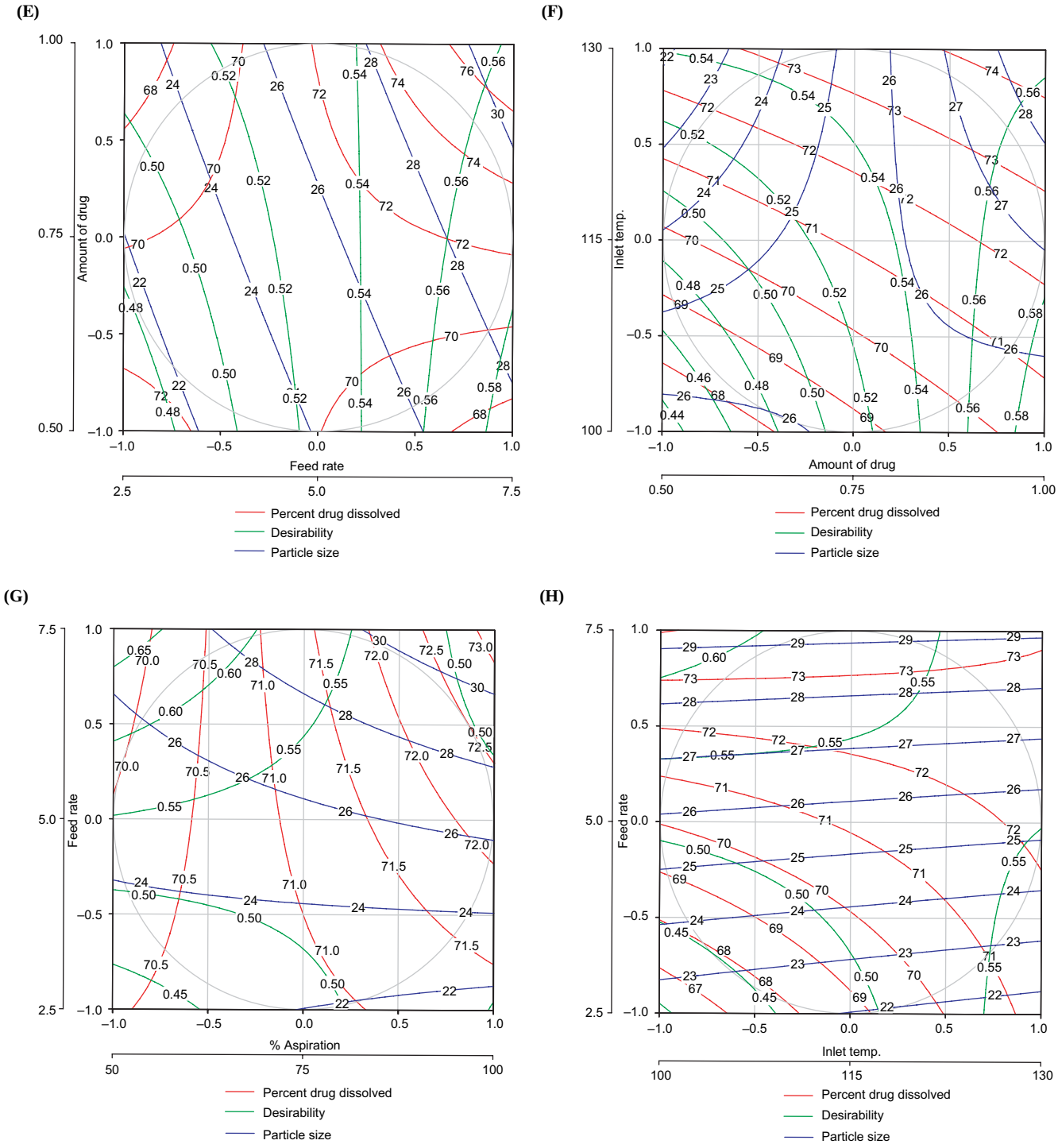

FIGURE 3. (*Continued*).

standard error, 't' ratio and probability for percent drug dissolved and particle size of the model analyzed are shown in Table 3a and b depicts the results for wettability and angle of repose as dependant variables.

Effect of Formulation Independent Variables on Wettability Time and Angle of Repose

Wettability time for microcrystals had a direct relationship with particle size of microcrystals (unpublished data).

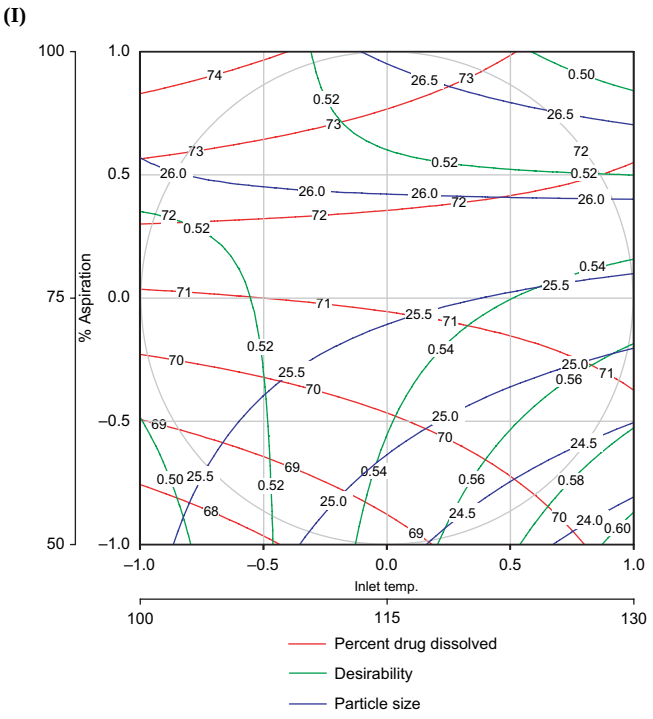

FIGURE 3. (*Continued*).

Due to this all the factors governing particle size had same effect on wettability time. As mentioned previously increase in feed rate resulted in increase particle size of microcrystals.

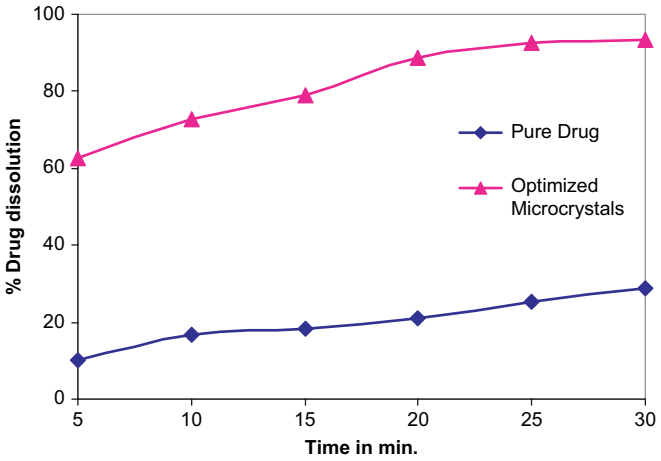

FIGURE 4. Powder dissolution of pure drug and optimized microcrystal formulation.

Feed rate has the same effect on particle size distribution also. However particle size distribution measurement was not the part of study, it showed effect on angle of repose of microcrystals which is a flowability measurement tool. High feed rate during the process lead to disorderly growth of particles since equal drying of liquid from droplet surfaces was not ensured, leading to local overwetting (Vertommen & Kinget, 1997). Figure 5 shows the contact angle sketch for pure drug and optimized batch of microcrystals. Figure 6

TABLE 3 (a)
Model Results for Scaled Estimates, Std Error, *t* Ratio and Probability of Percent Drug Dissolved and Particle Size as Dependant Variables

	Results for Percent Drug Dissolved			Results for Particle Size		
Term	Scaled Estimates	*t* Ratio	Prob>|t|	Scaled Estimates	*t* Ratio	Prob>|t|
Intercept	71.13	46.03	<.0001	25.60	28.85	<.0001
Chitosan	8.94	5.31	0.01	0.31	0.32	0.77
Drug	1.33	0.79	0.49	1.48	1.53	0.22
Feed rate	1.08	0.64	0.57	3.63	3.75	0.03
Aspiration	0.27	0.16	0.88	0.94	0.98	0.40
Inlet temp.	2.43	1.44	0.24	–0.27	–0.27	0.80
Chitosan*drug	–2.34	–1.39	0.26	2.02	2.09	0.13
Chitosan*feed rate	–4.46	–2.65	0.08	0.71	0.73	0.52
Drug*feed rate	4.32	2.56	0.08	0.16	0.17	0.88
Chitosan*aspiration	–1.59	–0.94	0.42	0.49	0.50	0.65
Drug*aspiration	–0.91	–0.54	0.63	0.09	0.09	0.93
Feed rate*aspiration	0.68	0.40	0.72	1.58	1.64	0.20
Chitosan*inlet temp.	1.47	0.87	0.45	0.50	0.51	0.64
Drug*inlet temp.	–0.38	–0.23	0.84	2.07	2.14	0.12
Feed rate*inlet temp.	–1.56	–0.93	0.42	0.16	0.17	0.88
Aspiration*inlet temp.	–1.35	–0.80	0.48	0.71	0.73	0.52

TABLE 3 (b)
Model Results for Scaled Estimates, Std Error, *t* Ratio and Probability of Wettability and Angle of Repose as Dependant Variables

	Results for Wettability			Results for Angle of Repose		
Terms	Scaled Estimates	*t* Ratio	Prob>|t|	Scaled Estimates	*t* Ratio	Prob>|t|
Intercept	1.45	55.70	<.0001	30.99	85.06	<.0001
Chitosan	–0.37	–13.07	0.00	–2.16	–5.44	0.01
Drug	–0.10	–3.56	0.04	0.11	0.28	0.80
Feed rate	–0.14	–4.75	0.02	–0.76	–1.92	0.15
Aspiration	–0.02	–0.79	0.49	–0.67	–1.68	0.19
Inlet temp.	–0.11	–4.00	0.03	–1.06	–2.68	0.08
Chitosan*drug	0.09	3.12	0.05	0.26	0.67	0.55
Chitosan*feed rate	0.16	5.72	0.01	0.34	0.86	0.45
Drug*feed rate	–0.12	–4.22	0.02	–1.35	–3.41	0.04
Chitosan*aspiration	–0.05	–1.58	0.21	–0.54	–1.37	0.26
Drug*aspiration	0.12	4.22	0.02	–0.62	–1.57	0.21
Feed rate*aspiration	0.09	3.04	0.06	–0.68	–1.71	0.18
Chitosan*inlet temp.	–0.05	–1.89	0.15	0.45	1.13	0.34
Drug*inlet temp.	–0.06	–2.16	0.12	0.72	1.80	0.17
Feed Rate*inlet temp.	0.04	1.23	0.31	–0.14	–0.34	0.76
Aspiration*inlet temp.	0.05	1.58	0.21	–0.47	–1.19	0.32

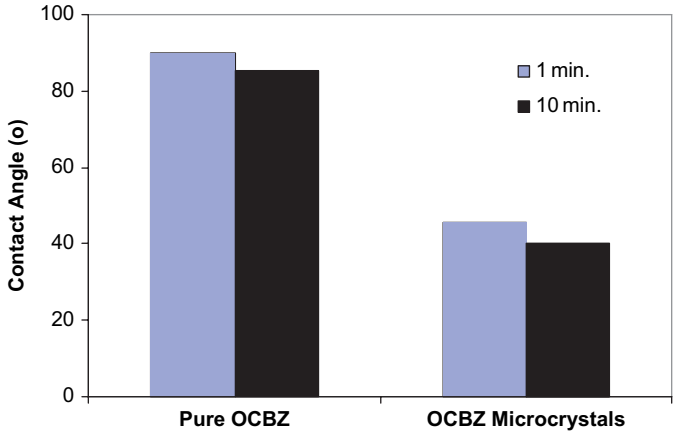

FIGURE 5. Contact angle of pure drug and optimized batch of microcrystals.

shows the optimized batch of microcrystals dispersed in water.

Results for Contact angle of pure drug powder and optimized batch of microcrystals showed that there was significant improvement in hydrophilicity of microcrystals produced. Figure 7 shows the uniform dispersion of optimized batch of microcrystals in water which is also a measure of wettability of dispersed phase.

FIGURE 6. Optimized batch of spray dried microcrystals dispersed in water.

Interaction Between the Factors

The ANOVA results are depicted in Table 3a and b gives idea about the significant effect of variables individually and in combination with each other. Here instead of using normal probability function '*t*' ratio, which lists the test statistics for the hypothesis that each parameter is zero. It is the ratio of the parameter estimate to its standard error. If the hypothesis is

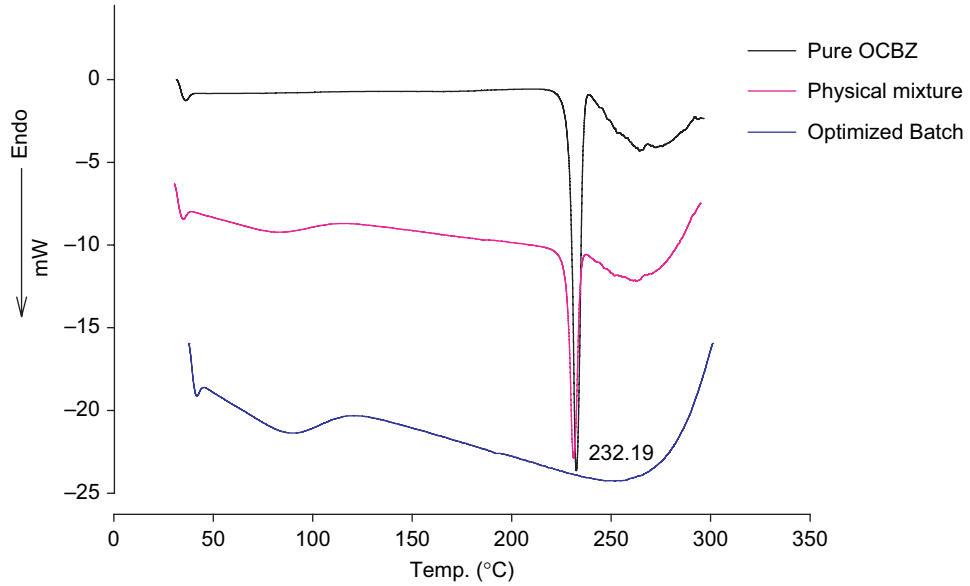

FIGURE 7. DSC thermograms for pure drug, physical mixture and optimized batch of microcrystals.

true, then this statistic has a Student's *t*-distribution. Looking for a '*t*' ratio greater than 2 in absolute value is a common rule of thumb for judging significance because it approximates the 0.05 significance level. Also the term 'Prob > |t|' lists the observed significance probability calculated from each '*t*' ratio. It is the probability of getting, by chance alone, a '*t*' ratio greater (in absolute value) than the computed value, given a true hypothesis. Often, a value below 0.05 (or sometimes 0.01) is interpreted as evidence that the parameter is significantly different from zero.

The results showed that chitosan has significant effect on percent drug dissolved, particle size, wettability time and angle of repose whereas drug concentration, feed rate, percent aspiration and inlet temperature during spray drying has significant effect on particle size, wettability time and angle of repose.

Thermal Analysis and XRPD Studies

Pure crystalline oxcarbazepine was characterized by a single, sharp melting endotherm at 232.19°C during DSC (Figure 7) and prominent diffraction peaks in the range of 8–30°2θ during XRPD (Figure 8). Thermogram of optimized batch of microcrystals revealed broadening of melting endotherm of the drug. Consequently, there was significant decrease in intensity of major OCBZ crystalline peaks (23.5, 14.5, 12, 10, 4°2θ) in diffractogram of optimized batch of microcrystals which suggests an interaction between oxcarbazepine and carrier chitosan. The partial loss of crystallinity of drug observed may also be attributed to physical presence of chitosan. The thermogram of optimized batch for microcrystals showed a very shallow endotherm at around 232°C which indicated the presence of residual crystallinity. SEM studies performed gave a strong support to the results of DSC and XRPD.

SEM Studies

The microphotographs of pure OCBZ and optimized batch of microcrystals are shown in figure 9. Pure drug was observed in the form of a mixture of some large crystals ranging from 20 to 200 μm) with microparticles. Microcrystals formed in various batches of experimental design revealed significant changes in particle shape and surface topography due to impact of spray drying process. However, in figure only images of optimized batch are shown. The images for optimized batch appeared as smooth spherical agglomerates with slight particle aggregation as well as residual crystals, to a small extent adhered to particles.

Enhancement of the Process Using the Desirability Function

Any process can only be authenticated when optimum level of its variables (affecting the process) for a product of good quality characteristics is recognized. Desirability function is one excellent procedure for identifying the optimum levels of variables. In this procedure, all the measured responses for independent variables which are supposed to affect the quality of the product are taken into consideration. Some of these responses have to be minimized and some have to be maximized, in order to pour desired characteristics in the product. Using the desirability function, all the dependant

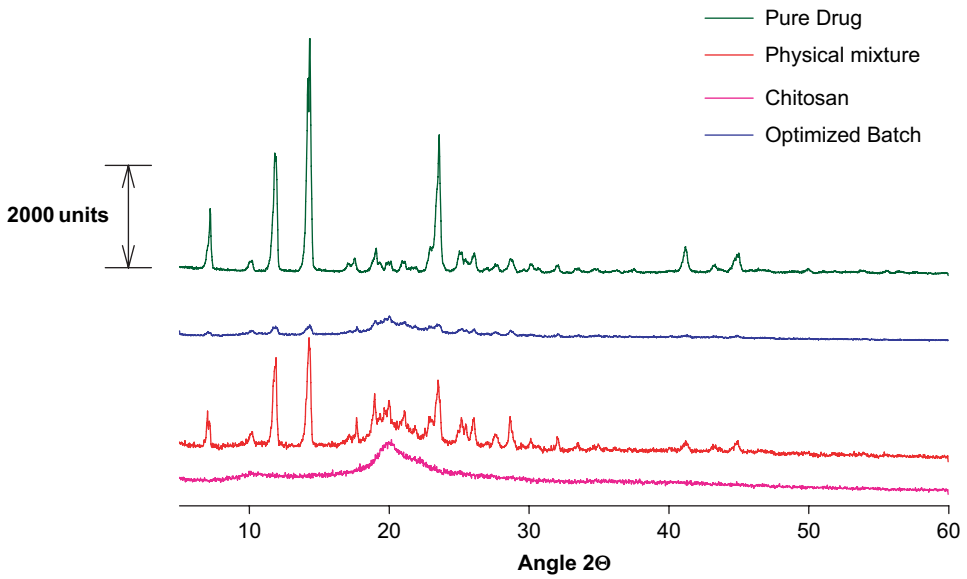

FIGURE 8. XRPD results for pure drug, chitosan, physical mixture and optimized batch of microcrystals.

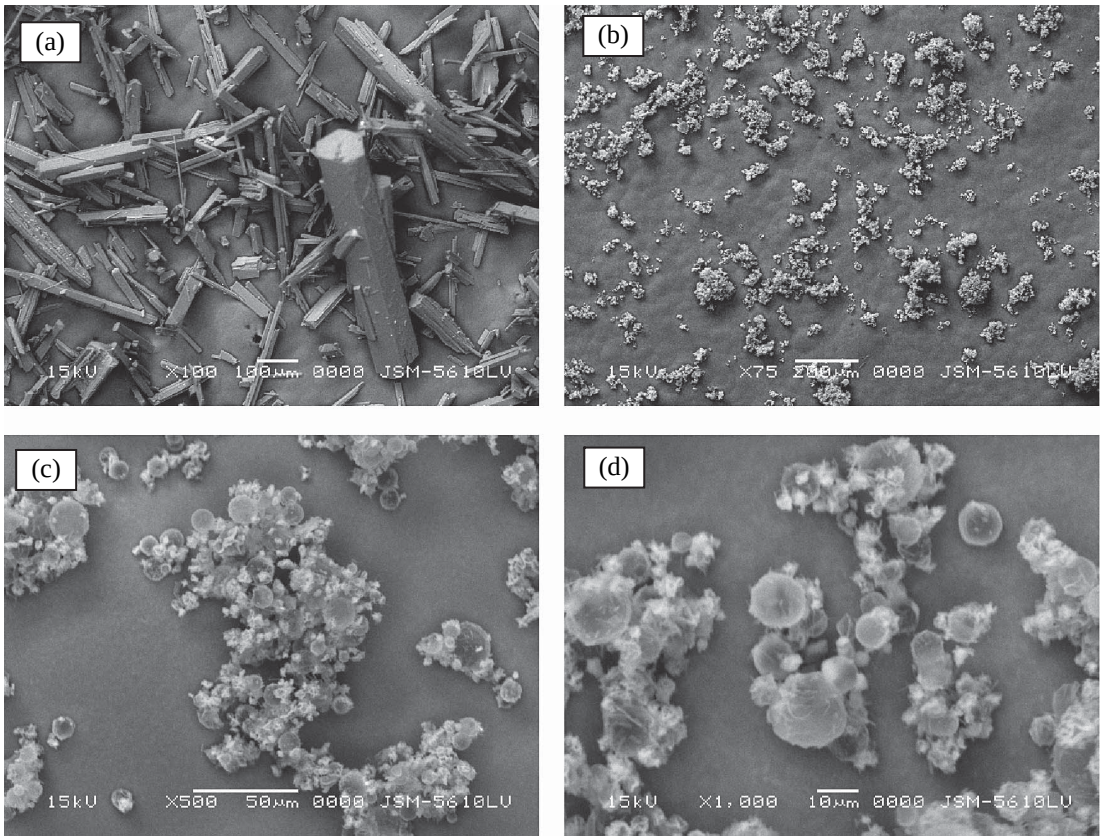

FIGURE 9. SEM images for pure drug (a) and of optimized batch of microcrystals at 75, 500 and 1000 × (b), (c) and (d), respectively.

variables were combined to get one overall response i.e., the overall desirability. The overall desirability response was calculated from the individual desirability of each of the responses using the equations 3 to 8. The results of each of these overall desirability responses construct a solitary equation which describes the overall enhancement of the

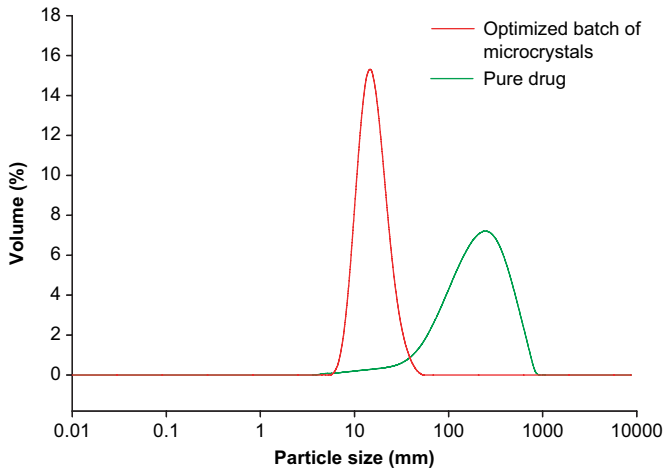

FIGURE 10. Particle size analysis of pure drug and optimized batch of microcrystals.

process. Equation 9 describes the influence of the factors on the overall desirability.

$$\begin{aligned} D = {} & 0.5304 + 0.1746A + 0.0045B + 0.0447C - 0.0173D \\ & + 0.0188E - 0.1246AB - 0.0179BC \\ & - 0.0832CD - 0.0412DE - 0.1889AC \\ & - 0.0760AD - 0.0562AE - 0.0421BD \\ & - 0.03619BE - 0.0729CE \end{aligned} \quad (9)$$

The optimized batch was identified with a desirability value of 0.9973. This batch was produced with high level of chitosan concentration, percent aspiration, inlet temperature during spray drying whereas low levels of drug and feed rate. Table 4 enlists the optimized values for all the independent process variables.

TABLE 4
Optimum Levels for the Independent Process Variables

Independent Variables	Optimum Values
Chitosan conc. (A)	2% w/w
Drug conc. (B)	0.5 g/250 mL
Feed rate (C)	2.5 mL/min
Percent aspiration (D)	100%
Inlet temp. (E)	130°C
Overall desirability (D)	0.9973

Cross Validation of the Model

The reliability of the equation that described the influence of factors on all responses was assessed by cross validation of the model. The response data for two independent check point batches was collected (Mashru et al., 2005). The experimental values and predicted values of each response are shown in table 5. The percent relative error between predicted values and experimental values of each response was calculated using equation 10.

$$\% \text{ Relative error} = \left(\frac{|\text{Predicted value} - \text{Experimental value}|}{\text{Predicted value}} \right) \times 100 \quad (10)$$

The percent bias obtained from checkpoint batches was in range of 3.6310–8.1759. A low value of percent bias depicts that in all cases there was a reasonable agreement in predicted values and experimental values.

CONCLUSION

Controlled crystallization of poorly water soluble drugs in presence of carrier like chitosan, which is rendered as a

TABLE 5
Comparison Between Predicted and Experimental Values for the Test Formulations

		Factors/levels							
Responses	Test	A	B	C	D	E	Predicted Values	Experimental Values	Bias %
Percent drug dissolved	1	–0.75	–0.6	–0.4	–0.6	–0.8	58.41	55.69	4.6612
	2	0.75	0.75	0.9	0.9	0.9	78.07	81.57	4.4834
Particle size	1	–0.75	–0.6	–0.4	–0.6	–0.8	26.15	24.68	5.6213
	2	0.75	0.75	0.9	0.9	0.9	36.65	37.98	3.6310
Wettability time	1	–0.75	–0.6	–0.4	–0.6	–0.8	2.02	2.19	8.1759
	2	0.75	0.75	0.9	0.9	0.9	1.04	1.10	5.7817
Angle of repose	1	–0.75	–0.6	–0.4	–0.6	–0.8	33.70	35.65	5.7845
	2	0.75	0.75	0.9	0.9	0.9	25.63	23.67	7.6303

protective hydrophilic polymer using spray-drying technique effectively enhances the drug dissolution. The process is cost effective, readily scalable and devoid of any harsh treatment to drug like in case of milling or grinding. Controlled crystallization of drug in presence of carrier leads to formation of molecularly dispersed form of drug which has significantly reduced particle size. In this way, a large surface area for a particle which is hydrophilized is generated. This surface area serves as a protective layer on the crystal surface. In case of OCBZ chitosan was found to be able to stabilize the surface of drug. Application of experimental design alongwith desirability function can be proved as an ideal tool to optimize various parameters like carrier and drug concentration, percent aspiration, feed rate and inlet air temperature which have significant effect on microcrystal's desired properties. In present work interactions between these factors were found to be statistically significant. It suggests that every variable has its own significant complimentary role in enhancement of the process rather than having exclusive effect. The multiple regression analysis of the results led to equations that described adequately the influence of the selected variables on the responses under study.

ACKNOWLEDGMENT

One of the authors Mr. Yogesh Rane wishes to thank AICTE, India for NDF. Authors are thankful to IIT, Mumbai for help in contact angle determination of powder samples.

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