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Spherical Agglomeration Formulation Development of Bilastine for Particle Engineering by Plackett-Burman Design

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Abstract
Purpose: The ambition of the present work was to development of Bilastine (BIL) spherical agglomeration (SA), BIL, peripheral histamine H1-antagonist used to treat seasonal allergic rhinitis and chronic spontaneous urticaria, which conceptualizes with certain technology based on principles of Quasi Emulsion Solvent Diffusion (QESD) method, to improve the Solubility, Absorbance of drug and micromeritic properties.
Method: Spherical agglomeration were developed by using different solvent system, Methanol as good solvent, water as poor solvent (bad solvent) and DCM as bridging liquid. Plackett-Burman design (PBD) could stimulate an economical experimental scheme that focuses on developing and determining the much relative significance. A Plackett-Burman (PB) screening design approach was utilized in which 7 factors at 3 levels were trial out at 12 runs to study the main effect of process and formulation variables on Saturated Solubility (SS), Particle Size (PS) and Angle of Repose(AR) of Spherical Agglomerates.
Result: Results show that of (GS-BL)-PS, Concentration of Polymer and stirring speed were the most fundamental factors that affect the saturated solubility (SS), particle size (PS) and angle of repose (AR). The surface morphology and crystalline nature of Agglomerates were also characterized by SEM, DSC and XRPD.
Conclusion: This study come to an closure and concluded with Plackett-Burman design (PBD) was a well systematized tool for screening of process and formulation variables which affecting the characteristic parameters of Bilastine Spherical agglomerates.

Keywords
Bilastine, Plackett-Burman design, Particle Engineering, Spherical Agglomerate, Screening design

INTRODUCTION

Solubility of drug in various solvents is a persistent characteristic for defined particle [1]. To attain a therapeutic activity, is must that particle exhibit certain solubility in physiological gastro-intestinal (GI) fluids and to be present in absorbed/dissolved state at the absorption site [2]. Beyond the 40% of new chemical entities having poor/less aqueous solubility likewise they show poorly soluble in water, which accelerates pharmacokinetic flexibility after the oral administration and thereby reveal poor bioavailability.[3] on that account, improvement in aqueous solubility or dissolution of these types of drug particles is great challenge to formulate or to develop a delivery system which provides essential oral bioavailability [4].

Spherical agglomeration can be defined as[5] “An agglomeration process which transmogrify crystalline drugs directly into a compacted spherical form for boosting the flowability[6], solubility[7] and compactability”[8] A different novel particulate technique by which crystallization and agglomeration can be accomplished simultaneously in one single step to convert the fine crystals directly into compacted spherical form. Kawashima[9] suggested that existing the size expansion of particles during the process of crystallization step by managing the crystal agglomerate with restrained properties. He introduced this process into pharmaceutical manufacturing and showed that spherically dense agglomerates which enhance the bioavailability, dissolution rate[10], wettability[11] of poorly soluble drug and hence the micromeritic properties are also enhanced.

Antihistamines are widely used for the treatment of allergic rhinitis (AR) and/or urticaria1-4. AR is a heterogeneous disorder characterized by one or more symptoms including sneezing, itching, nasal congestion and rhinorrhea as well as non-nasal symptoms such as tearing which can affect driving performance.[12] H1-antihistamines are functionally classified into two groups.[13] The sedating ones readily cross the blood-brain barrier (BBB) and occupy H1-receptors located on postsynaptic membranes of histaminergic neurons throughout the CNS13.[14] For this reason, sedating antihistamine have a potentially undesired impact on psychophysical performance. Bilastine is a highly selective, non-sedating second-generation antihistamine, indicated for the symptomatic treatment of allergic rhinoconjunctivitis (seasonal and perennial) and urticaria and generally well-tolerated.[15] It is safer and doesn't produce sedative effect and cardio toxic effect [16]

The present study was to select solvent system for Bilastine (BIL) spherical agglomeration. Thus, the first objective was to select right good solvent and poor solvent base on solubility study.[17, 18] In this study, we developed Spherical agglomerate with the aim of improving the solubility of Bilastine; a Biopharmaceutics Classification System (BCS) class II drug, highly lipophilic (log p 5.02, aqueous solubility 0.00203 mg/mL at 25ºC)[19-21]. Bilastine2 – [4-(2-{4[1-(2- ethoxyethyl) – 1 H- 1, 3 benzodiazol – 2yl] piperidine – 1 –yl} ethyl) phenyl] 2 – methyl propenoic acid is novel second generation antihistamine. Many investigators have been investigated different approaches to enhance the dissolution properties of Bilastine[22,23]. Here to fore, there is no work reported whereby dissolution profile and micromeritic properties of BIL have been improved by spherical crystal agglomeration technique. Hence, to study the influence of various processing and formulation variables on formation of Bilastine agglomerates and its properties like dissolution and micromeritic. Placket Burman design were utilized to screening factor and optimized theprepared agglomerates of Bilastine.

MATERIALS AND METHODS

Materials

Bilastine was obtained as a gift sample from Ajanta Pharmaceutical Ltd, Aurangabad. PEG 6000 was obtained from Ozone International, Mumbai (India); Methanol and DCM were obtained from Chemdyes Corporation, Rajkot and RFCL limited, Ankleshwar respectively. All other chemicals and reagents were used of Research grade.

Methods

Drug Characterization with Excipients

Drug characterization can be done by UV spectroscopy and Bilastine identification was carried out using Fourier Transform Infrared Spectroscopy-FTIR analysis[24]. For this, the FTIR spectra of pour drug were recorded in FTIR-8400S Shimadzu spectrophotometer. The Bilastine pure drug was mixed thoroughly with potassium bromide (KBr). Then the scans were obtained at a resolution of 4000-400 cm^{-1}[25].

Selection of solvent system and Polymer

Various solvents i.e. Tween 80, DMF, Cyclohexane, Triethanolamine, acetone, methanol, dichloromethane, isopropyl alcohol, dimethyl sulfoxide, PEG 400, PEG 200, chloroform, water were screen with different polarity for selection of good solvent, bridging liquid and poor solvent[27]. An excess quantity of Bilastine was added to each selected solvent (5ml) and all saturate solution was kept for 24 hr at constant room temperature on shaker bath (Remi RSB12) at 120 RPM with constant stirring. individual solvent was filtered, diluted with methanol and concentration of drug in each solvent was determined by UV-Visible spectrophotometer(Simadzu UV-1800) at 283 nm against methanol as blank.[28] Preliminary trials were performed for selection of Polymer; i.e. HPMC E5LV, polyethylene glycol 4000, polyethylene glycol 6000, PVP 40,000, β-cyclodextrin, poloxamer 407 and 108; where Polymers were dissolved in water at different concentration 0.5% and 2% w/v; simultanesiously Selection of polymer, another processing condition like stirring speed (800 rpm) and ratio of solvent system were kept constant. After the selection, spherical agglomerates were prepared by using different concentration i.e. 0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/v of selected polymer for selection of level of concentration of polymer.

Spherical Agglomeration (SA) of Bilastine

Formulation of Bilastine spherical agglomerates by Quasi Emulsion Solvent Diffusion technique.[29]. According to solubility studies; good solvent and Poor solvent were categorized for the screening of agglomerates. In Agglomeration process, Bilastine(1 gm) dissolved in good solvent(methanol) afterwards a bridging liquid(DCM) was added. Subsequently the above mixture was added to poor solvent (Water) which containing selected hydrophilic polymer(PEG 6000) with continuous stirring. The mixture was then stirred until clear supernatant was generated and agglomerates get settled down. The generated agglomerates filtered by vacuum filter and dried at room temperature.

Experimental design (Plackett–Burman design)

Identification of the formulation and processing variables Plackett-Burman (PB) experimental design was used. In the Present Study, Seven different independent variables which affect the properties of SA were specified and included. The average Saturated Solubility, Particle Size and Angle of Repose were scrutinized as critical properties of SA therefore these properties are expected to influence its efficacy. Plackett-Burman designs are orthogonal-main-effects plans for (N− 1)

factors at two levels each using N experimental units when N is an integer multiple of 4. When N is a power of 2[30]. To study the effect of Independent variables on Saturated Solubility, particle size and Angle of repose, seven factors at two levels were tested at 12 runs. High and low levels of factors were selected according to preliminary trial, error study and also review of data. Design expert® software V-10.0.1.0, State-Ease Inc., Minneapolis, MN, USA was used to generate and randomize the matrix of design which is statistically analysed[31]. By ANOVA and multiple regression analysis the significance factor of coefficients for the model were analyzed.

Characterization of Spherical Agglomeration

Flow characteristics

Flow characteristics like angle of repose(AR), Compressibility Index (Carr's Index)[33] and Hausner's ratio[34] for spherical agglomerates were investigated by fixed funnel method[32], tapping stable number of agglomerates by Tapped density equipment (Electrolab, Mumbai, India) respectively.

In Vitro Drug release study

In vitro drug release study of Bilastine spherical agglomerates was carried out [23]by USP dissolution Type-II(paddle type) apparatus (TDT-06 model,Electrolab, Mumbai) in 0.1N HCl 900 mL at 50 rpm. 5 mL of sample withdrawn at 10min time interval and replaced with 5 ml fresh medium, sample withdrawn was filtered through whatman filterpaper. The absorbance of samples was determined at maximum wavelength λ_{max} using UV- Visible spectrophotometer against 0.1N HCl as blank.

Particle size measurement

The size of pure drug particles and individual batch spherical agglomerates was measure by Stage micrometer.

Scanning Electron Microscopy (SEM)

The surface morphology and shape of pure drug Bilastine and optimized spherical agglomerates was determined by scanning electron microscopy (SEM). The Drug sample and optimized sample was fixed on brass/metal stub with help of double side adhesive tape then they were made electrically conductive by coating in vaccum with thin layer of gold. Then agglomerates were detected by scanning electron microscope(SEM)(JSM-5610) at various scale bars with stimulating voltage of 15kV in order to explore the effect of additives on surface morphology and competency of agglomerates.[35]

Differential Scanning Calorimetry analysis

Differential Scanning Calorimetry (DSC) was carried out in order to investigate the thermotropic attributes and the thermal behaviours of the Bilastine Pure drug, excipients(additives) used in the formulation of spherical agglomerates system. DSC spectra of Bilastine drug and optimized batch agglomerates were collected by Differential Scanning Calorimeter (DSC) equipment (ShimadzuDSC 60 TWS60). That was calibrated by indium standard previously. Sample (~5–10 mg) was esoterically sealed in an aluminium crucible and subjected to throw out of nitrogen gas at flow rate of 50ml/min. The spectra collected was analyze for endothermic and exothermic transitions in drug agglomerates. The change in physical and chemical characteristics of agglomerates can be studied.[36]

X- Ray powder diffraction

The form of crystallinity and intensity of Bilastine drug crystals in agglomerates was determined using X-ray powder diffraction (Bruker D8 ADVANCE Bruker India Scientific Pvt. Ltd., Mumbai). The XRPD structures were noted using Diffractometer with Cu (Copper) target and scintillation detector counter for pure drug and optimized agglomerates.[37]

RESULTS

Selection of Independent variables for placket-burman design, experimental conditions, Bilastine spherical agglomerates characterization study, solubility studies and *in vitro* drug release study was discussed in this section.

Selection of Independent variables

Bilastine was carried out by utilizing different polymer i.e. HPMC E5LV, PEG 4000, PEG 6000, PVP 40,000, β-cyclodextrin,poloxamer 407 and poloxamer 188 at different concentration (Table 1). Spherical agglomeration was attempt with HPMC E5LV, PEG 4000 and β-cyclodextrin the flowability of agglomerates were not much improved, furthermore an agglomeration carried out with Poloxamer 407 (batch T_3 to T_6), PEG 6000 (batch T_9 to T_{12}) and Poloxamer 188 (batch T_{15} to T_{18}) the flowability of agglomerates were greatly improved and as the concentration of Carrier get increased the Solubility, Drug release and partical size were also improved. Whenever the agglomerates prepared by utilizing PEG 6000 (batch T_{10}, T_{11}& T_{12}) showed much improved in flowability, compressibility, and solubility compare to Poloxamer 407 and Poloxamer 188. PEG 6000 between 0.5 to 1.5%concentrations (batch T_9, T_{10}& T_{11}) imparts better flowability, compressibility and drug release to the agglomerates. Hence, PEG 6000 was selected in concentration between 0.5 to 2 %w/v for agglomeration of Bilastine. The details of selected variables are presented in Table 2.

Table 1 Selection of Polymer

Trials	Polymer	Concentration of Carrier (%W/V)	Saturated Solubility (mg/ml)	Partical Size (µm)	Angle of repose (°)
T1	HPMC E5LV	0.5	0.28±0.03	28.28±0.32	39.73±0.28
T2		1.0	1.36±0.005	25.36±0.14	35.37±0.47
T3	POLOXAMER 407	0.5	1.53±0.13	33.53±0.07	39.86±0.34
T4		1.0	0.61±0.32	27.61±0.29	36.50±0.27
T5		1.5	0.15±0.11	45±0.16	26.37±0.19
T6		2	0.08±0.01	18.5±0.21	30.41±0.01
T7	β-CYCLO DEXTRIN	0.5	1.48±0.08	22.48±0.36	33.89±0.35
T8		1.0	0.16±0.01	48.16±0.19	29.62±0.16
T9	PEG 6000	0.5	0.50±0.03	103.47±0.31	26.39±0.28
T10		1.0	3.52±0.006	298.68±0.05	24.01±0.33
T11		1.5	4.86±0.21	354.35±0.14	25.84±0.24
T12		2.0	2.12±0.37	121.61±0.23	26.73±0.07
T13	PEG 4000	0.5	0.73±0.24	28.28±0.05	35.21±0.46
T14		1.0	0.37±0.31	45.36±0.09	28.18±0.01
T15	POLOXAMER 108	0.5	0.06±0.09	33.53±0.14	26.18±0.56
T16		1.0	1.05±0.19	67.61±0.02	25.0±0.61
T17		1.5	0.03±0.37	65.2±0.21	24.17±0.44
T18		2	0.004±0.29	38.5±0.07	31.22±0.28
T19	PVP 40,000	0.5	0.01±0.13	22.48±0.05	39.61±0.71
T20		1.0	0.006±0.28	37.16±0.03	35.61±0.48
T21		1.5	0.002±0.16	43.54±0.17	38.03±0.52

Table 2 Plackett-Burman(PB) screening design setup

Factor Code	Factor Name	Level	
		Low (-1)	High (+1)
Independent Factors			
➢ ***Formulation variables***			
X1	Type of Good solvent	Methanol	Cyclohexane
X2	Type of bridging liquid	DCM	CHFM
X3	Concentration of Polymer	0.5	2.5
X4	Ratio of GS-BL	0.1	1
X5	Ratio of (GS-BL)-PS	0.1	2
➢ ***Process variables***			
X6	Stirring Speed	500	1000
X7	Stirring Time	20	60
Dependent Factor			
Y1	Saturated Solubility (mg/ml)		
Y2	Particle size (µm)		
Y3	Angle of repose (°)		
Variables kept constant			
Drug quantity	50 mg		

For all formulated batches: The quantity of drug was 50 mg, water was used as a poor solvent (PS) and the total volume of Solvent system was 50mL.

Screening of variables using Plackett-Burman design

PB experimental design was used for analyzing experiments because in a PB design, main effects are in general; steadily staggered with two-factor interactions. The PB design exhibit 12 runs, for example, may be used for an exploration containing upto 11 factors. For Present research 7 factors, 12 run Plackett-Burman screening design was originated (Table 2) using Design expert® software (V-11.0.1.0, State-Ease Inc; Minneapolis, MN, USA).

Independent variables scanned were Type of Good solvent (X_1), Type of bridging liquid (X_2), Ratio of GS-BL (X_3), Ratio of (GS-BL)-PS (X_4), Concentration of Polymer (X_5), Stirring Speed (X_6), and Stirring Time (X_7). The Saturated Solubility (SS), partial size (PS), Angle of repose were selected as dependent variables. The parameter level of screening was based on preliminary trial studies and literature survey report. The Plackett-Burman(PB) design setup and design construction matrix with outcome are summarized in Table 2 and Table 3 respectively. The polynomial equation for persistence of the effect of factors on outcome is given following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7$$

Where, Y is the dependent variable, X_1 stand for levels of independent formulation variables while b_0 is the intercept, b_1 (b_1 to b_7) represents as the coefficient of regression for the 1st order polynomial. The immensity and recommendation of factors in the earlier equation elucidate the nature of the factor's effect on the outcome Y.

Table 3 Plackett-Burman (PB) screening design production matrix with responses

Batch Code	Independent variables							Dependent variables		
	Type of Good solvent	Type of bridging liquid	Ratio of GS-BL	Ratio of (GS-BL)-PS	Concentration of Polymer	Stirring Speed	Stirring Time	SS (mg/ml)	PS (µm)	Angle of repose (°)
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y_1	Y_2	Y_3
SA1	Methanol	DCM	0.1	2	0.5	1000	60	0.47±0.017	478.7±0.03	23.57±0.71
SA 2	CHX	DCM	1	2	0.5	1000	60	0.82±0.004	269.49±0.07	25.91±0.65
SA 3	CHX	CHFM	0.1	0.1	0.5	1000	20	0.27±0.026	78.49±0.57	34.86±0.77
SA 4	CHX	CHFM	0.1	2	2.5	1000	20	3.65±0.007	59.74±0.62	32.21±0.52
SA 5	Methanol	CHFM	1	2	0.5	500	20	0.38±0.005	38.62±0.09	46.39±0.17
SA 6	Methanol	DCM	1	0.1	2.5	1000	20	2.37±0.015	87.59±0.32	35.11±0.28
SA 7	Methanol	CHFM	1	0.1	2.5	1000	60	1.27±0.029	187.39±0.27	24.76±0.84
SA 8	CHX	DCM	1	2	2.5	500	20	1.89±0.021	69.21±0.15	37.43±0.24
SA 9	CHX	CHFM	1	0.1	0.5	500	60	0.21±0.003	267.15±0.04	39.69±0.39
SA 10	Methanol	DCM	0.1	0.1	0.5	500	20	0.13±0.014	49.71±0.43	44.89±0.69
SA 11	CHX	DCM	0.1	0.1	2.5	500	60	0.54±0.034	187.43±0.01	36.21±0.23
SA 12	Methanol	CHFM	0.1	2	2.5	500	60	2.49±0.012	154.27±0.13	35.87±0.56

Effect analysis of variables on Saturated Solubility (Y_1)

Saturated solubility (SS) of all developed batches was in ranges from 0.130 to 3.654 mg/mL. The analytical significances of independent variables were originate in all the circumstances while conduct the research. The regression productivity for SS (Y_1) is appeared in Table 4. The value of R^2 was 0.9933 whichever identifying a good fit. ANOVA divulged a statistically heterogeneity in the middle of all formulation. Regression coefficient is asserted to be significant whether the *P*-value is lower than 0.05 (95% CI). In accordance with the outcomes shows in Table 4, which prove the Ratio of GS-PS (*P*=0.040), Concentration of Polymer (*P*= 0.001), and Stirring speed (*P*=0.046) affect significantly to the Solubility which is most committed to respective value of % contribution shows in Table 4 and Pareto chart shows in Figure 1 (A). The term effect plot in Figure 1 (B) shows the main effect of factors and the significant effect of factors on the Saturated Solubility.

Table 4 Regression analysis for Saturated Solubility (Y_1), Particle size (Y2), Angle of repose (Y3)

Independent variables	Saturated Solubility (SS) (Y_1)			Particle size (Y_2)			Angle of Repose (Y_3)		
	Coefficient	*P*-Value	% Contribution	Coefficient	*P*-Value	% Contribution	Coefficient	*P*-Value	% Contribution
Intercept	1.21	0.001		231.11	0.00580		72.68	0.0007	
X1-Type of Good solvent	0.009	0.960	0.09	-28.84	0.02269	2.43	1.14	0.172	0.79
X2-Type of bridging liq	0.129	0.384	0.77	61.14	0.001802	3.79	0.95	0.103	0.88
X3-Ratio of GS-BL	-0.190	0.328	0.89	-9.19	0.016282	0.054	1.11	0.117	1.17
X4-Ratio of GS-PS	0.417	**0.031**	**8.02**	79.31	**0.008109**	**19.93**	-5.37	**0.0009**	**32.84**
X5-Con. Of Polymer	1.564	**0.007**	**88.67**	143.36	**0.001632**	**47.74**	-0.69	0.331	0.93
X6-Stirring speed	-0.465	**0.027**	**7.63**	-124.16	**0.003961**	**26.12**	-4.52	**0.0026**	**30.65**
X7-Time	0.006	0.909	0.03	-29.18	0.002325	1.019	-8.013	**0.0010**	**51.39**

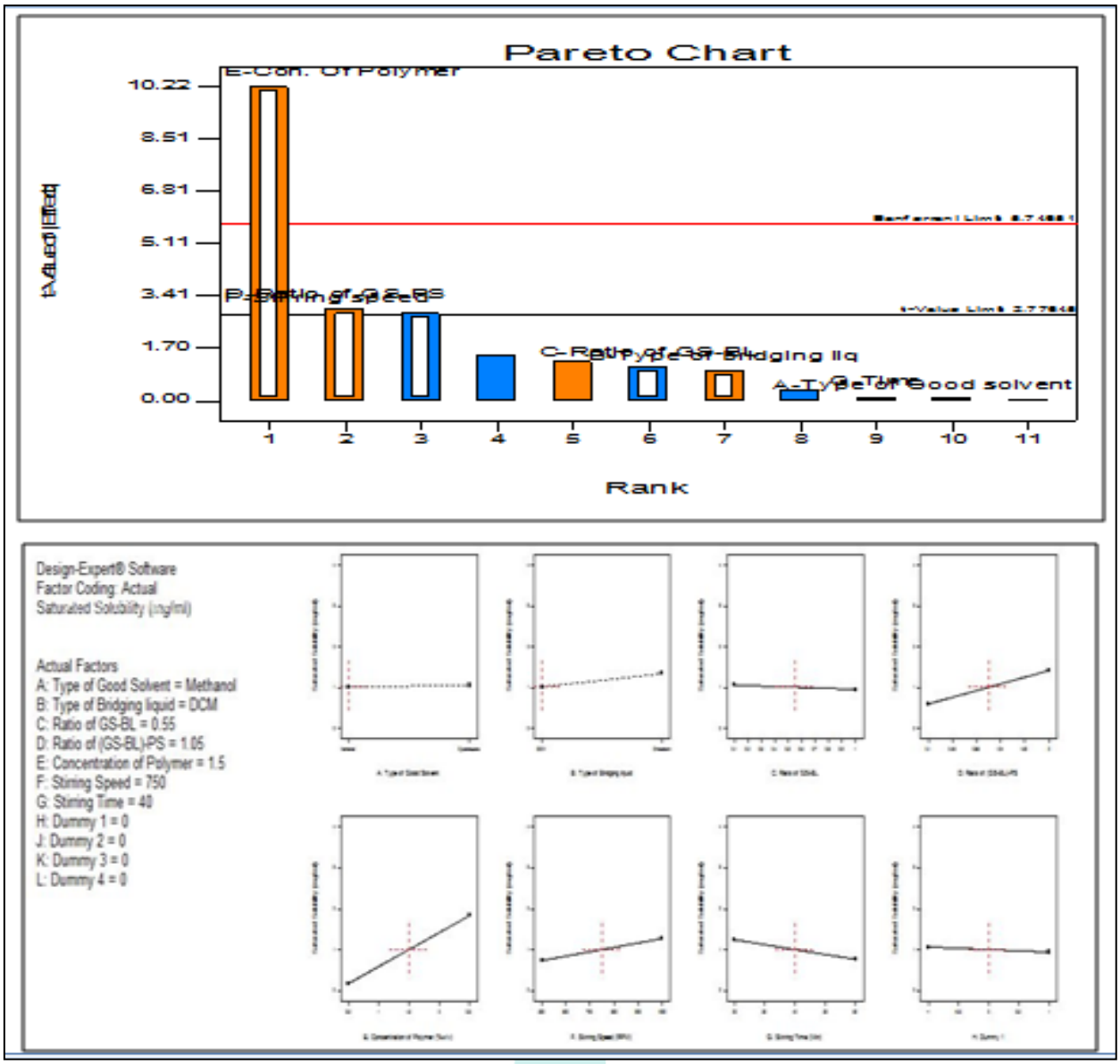

Figure 1 (A) Pareto chart; **(B)** Term effect plot for Saturated Solubility (Y_1)

Effect analysis of variables on particle size (Y_2)

All batches for Particle Size (PS) were in the ranges from 11.1 to 323.3 µm. The logical analytical significances for independent variables were obtained in all formulations while executing the experiment in arbitrary sequence. Response of Regression for Particle size (Y_2) is represented in Table 4. The R^2 value was found 0.9997 that indicate a good fit. ANOVA shows a statistical variation in the middle of all batches. If the *p*-value is lower than 0.05 of a regression coefficient is significant. As result shows in Table 4, it is observed that ratio of GS-PS ($P = 8.19\times 10^{-5}$), concentration of polymer ($P = 1.46 \times 10^{-5}$), and stirring speed ($P = 3.16 \times 10^{-5}$) which significantly affect the PS which is once most committed by respective value of %contribution shows in Table 4 and Pareto chart shows in Figure 2(A). The term effect plot for main effect of factors and significant effect the factors on the particle size shows in Figure 2(B).

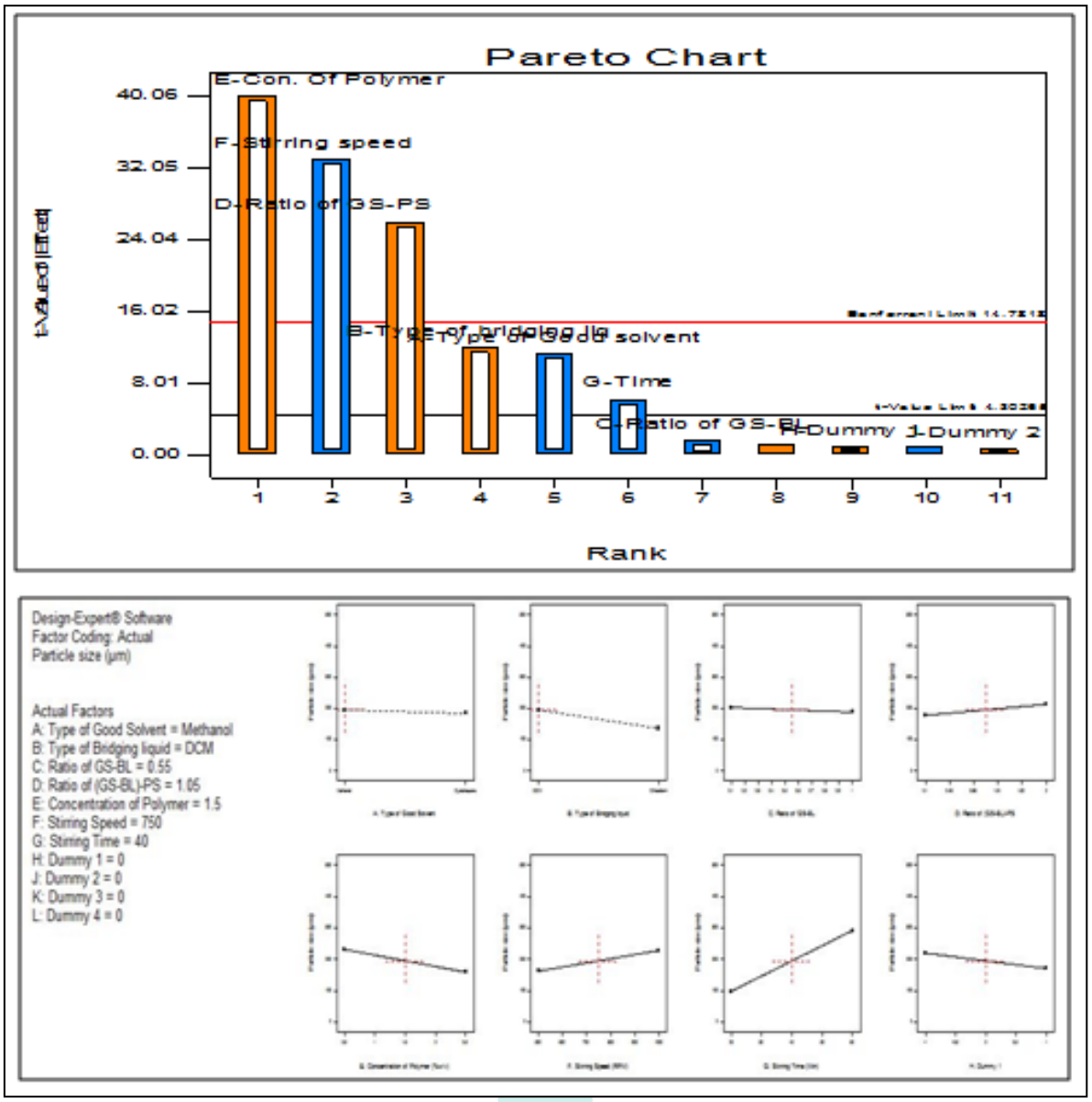

Figure 2 (A) Pareto chart; **(B)** Term effect plot of Particle size (Y2)

Effect analysis of variables on angle of repose (Y_3)

The results of angle of repose shows in Table 4 for all formulated batches of Plackett-Burman (PB) screening design. The regression analysis output for angle of repose (Y_3) is known in Table 4. The R^2 value was 0.9892 specifying a good fit. The analysis of variance (ANOVA) reported a difference in statistical value between the all batches. If the *p*-value is lower than 0.05(95% *CI*) a coefficient of regression is supposedly significant.

From the result shows in Table 4, it is generate the ratio of GS-PS ($P = 6.1 \times 10^{-3}$), stirring speed ($P = 0.0016$), and stirring time ($P = 0.0001$) significantly affect the particle size as well as its flow property which is once must committed by respective value of %contribution (Table 4) and Pareto chart (Figure 3 (A)). Term effect plot (Figure 3(B)) represents the main effect of factors and the significant effect for factor on angle of repose.

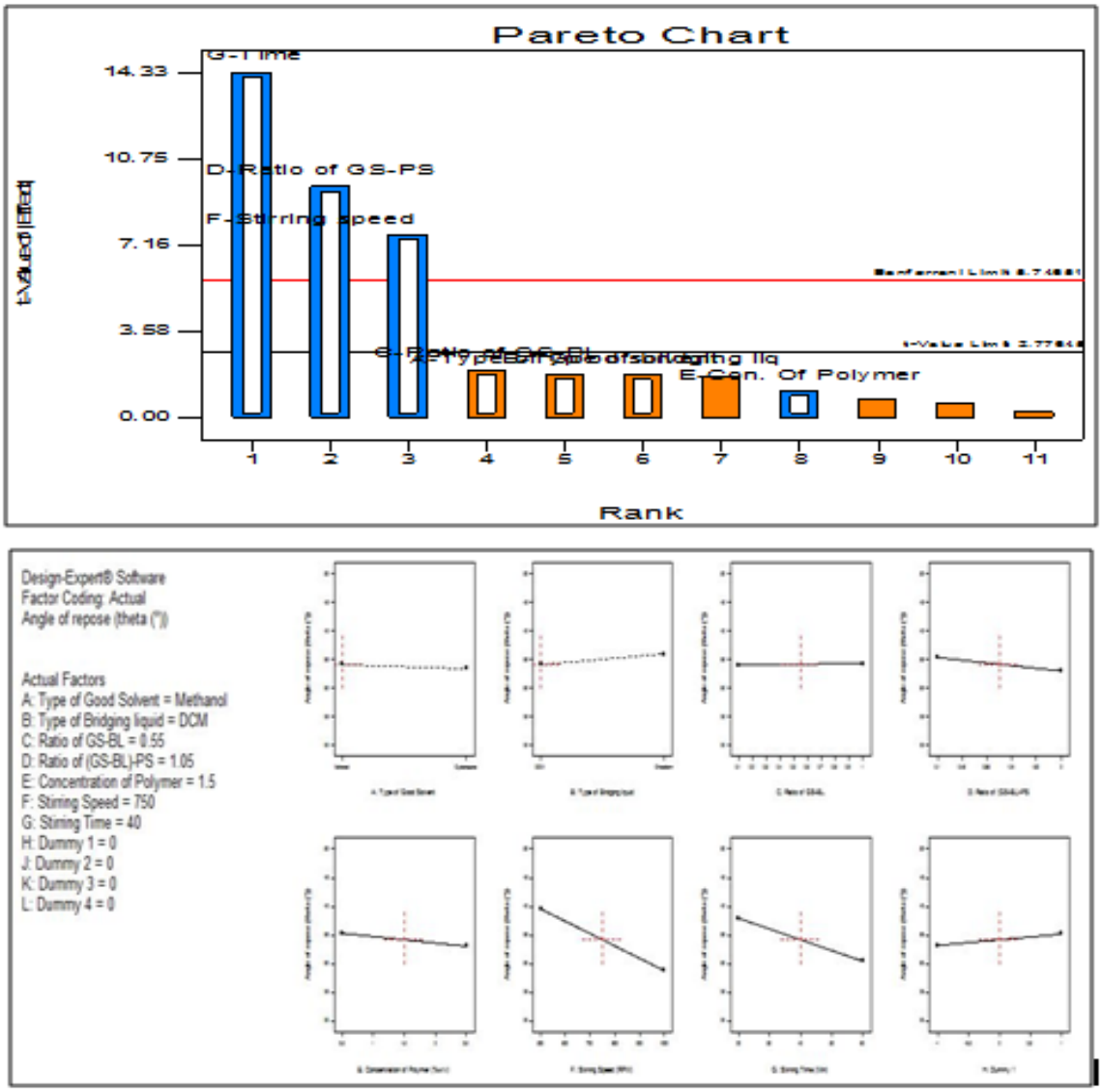

Figure 3 (A) Pareto chart; **(B)** Term effect plot for Angle of repose (Y_3)

DISCUSSION

Selection of Independent variables

The selection criteria were based on study of Saturated Solubility (mg/ml), Particle Size(um), and angle of repose(degree). In addition, selections of processing conditions various preliminary trials were performed. In selection of stirring speed along with stirring time, Spherical agglomerates of Bilastine were prepared at 500-1000 rpm and 15-60 min at room temperature and selection criteria were based on study of Saturated Solubility(mg/ml), Particle Size(um), and angle of repose(degree). The process and formulation factors were selected using Plackett-Burman (PB) design to find the few significant factors from a list of many potential ones.

Variables Screening using Plackett-Burman(PB) design

Effect analysis of variables on Saturated Solubility (Y1)

When the higher solubility required within the range of selected factors, X_4 and X_5 have positive coefficients effect that specifies the increase of the factor's value that increase the response which indicates that increasing ratio of Solvents and concentration of polymer that increases the solubility of formulated agglomerates. Unflatteringly, factor X_6 has negative coefficient effect which notify that decreasing the value of factors enhances the result which obtain the increasing of stirring speed enhance the solubility of formulated agglomerates. Apart from, the value of coefficient for factor X_5 is higher as collate to that of X_4 and X_6 factor, which indicates X_5 factor has higher significant effect on solubility of agglomerates comparison with the factor X_4 and X_6.

The types of good solvent, Cyclohexane fevers the lower solubility compared to that of Methanol. The type of Bridging liquid, chloroform favors the lower solubility as well as circularity of agglomerates compared to the DCM. This might be higher solubility of Bilastine in chloroform. Higher solubility promotes the intense driving force to the development of particles at the time of preparation by irregular interactions between molecule, Good solvents, bridging liquid and poor solvent. Additionally, the results show that higher ratio of only GS – BL without PS slightly favors (% contribution is 0.97) lower the solubility associated, might be it further reduces the concentration of drug in solvent system. The response of stirring time (X_7) was found to be insignificant ($P>0.05$) with a less % contribution (0.01).

Effect analysis of variables on particle size (Y2)

The particle size is given within the range of factor, X_4 and X_5 factors have positive coefficients that indicates that enhancing the value of factor enhance the response that notify increasing ratio of solvent and concentration of polymer which shows enlargement of the agglomerates particle size. Similarly, factor X_6 has negative coefficients which indicate decreasing value of factors; increasing of the response reported that decreasing the stirring speed and increasing of the particle size of agglomerates. Apart from that, the coefficient of X_5 factor is higher as well to the factor X_4 and X_6; comparatively the X_5 factor has larger remarkable effect on agglomerates particle size rather than of X_4 and X_6 factor.

The class of good solvent, cyclohexane fevers the lower solubility compared to that of DMSO. The type of Bridging liquid, Chloroform fevers the lower solubility as well as sphericity of agglomerates differentiated by DCM. This may be caused by higher saturated solubility of Bilastine in DCM. Greater particle size produce the intense driving force for the formulation of agglomerates throughout method on account of unbalancing molecular interactions between solvents, bridging liquid and Poor solvent. Moreover, the response indicate greater ratio of only GS – BL without PS slightly favours (%contribution: 0.054) lesser the size of particle attendant, might be it further decrease the drug concentration in solvent. The response of stirring time (X_7) was found to be significant ($P>0.05$) but it's give a less % contribution (1.019).

Effect analysis of variables on angle of repose (Y_3)

Lower angle of repose is important within selected range; factors X_6 and X_7 have negative coefficients which preferable that decreasing factor value and increasing the response, similarly decreasing the stirring speed and time that increase the agglomerates value of angle of repose. In other side, factor X_4 has negative coefficients, shows that decreasing the factor value decrease the response mean while decreasing the ratio of GS-PS expanses the angle of repose of agglomerates. Moreover the X_7 factor has higher coefficient as compared to that of X_4 and X_6, which shows that with comparison of X_4 and X_6, factor X_7 has notable significant response on angle of repose of agglomerates. High energy surfaces of Particles are formed due to solvent evaporation that leads to the aggregation of particle or particle growth. Addition of polymer develop inflated energy surface response in the reduce of surface energy and also enthalpy which diminish uneven particle growth on account of steric stabilization. The increase in stirring speed effects in reduction of particle size since the increasing of micromixing. Higher micromixing efficacy increased the diffusion rate, Provides notable homogenous supersaturation on a very low time and though the faster, similar nucleation, developing compact drug particles through tapered size distribution.

The variety of good solvent, Cyclohexane significantly induces the angle of repose compared to that of Methanol. The type of bridging liquid, Chloroform favors the smaller particle size separated to that of DCM. The reason may be exchanging in the variety of solvent not only interchanges the supersaturation all over evaporation phases still also influence the surface tension, furthermore changes the rate of nucleation. Additionally, the results show that higher ratio of only GS – BL without PS slightly commendation (%contribution is 1.17) lower the particle size which associated increase the angle of repose, reason may be, it further decrease the drug concentration in the solvent system. Hence obstruct uneven particles growth. The concentration of polymer(X_5) was generated to have the lowest effect on angle of repose with a negative coefficient. The 'factors' accountable for the essential changes were stated as the influential variables, rather remaining were identified as a noise variables.

***In vitro* dissolution study**

In-vitro drug release study for Spherical agglomerates was accompanied out in 0.1N HCl using USP apparatus type II at 37 ± 0.5°C temperature. As shown in Figure 4, the SA4, SA9 and SA12 batches of spherical agglomerates shows more than 90 % drug release respectively which was higher drug release compare to other batches at 60 min. From the above results it was found that as concentration of poloxamer 108, ratio of (GS-BL)-PS and stirring speed increased, the cumulative % drug release was increased.

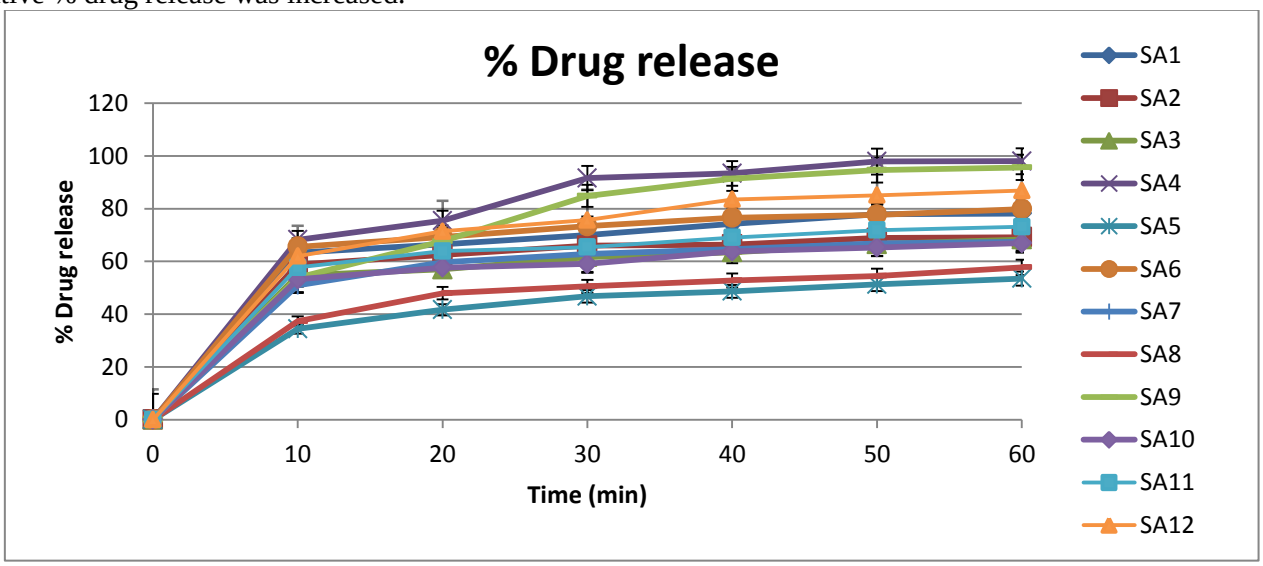

Figure 4 *In vitro* drug release study of spherical agglomeration of Bilastine

Scanning Electron Microscopy

The Scanning Electron Microscopy (SEM) figure 5 represent that Pure Bilastine powder shows to shaped irregular and fine crystalline. But in spite of that the Bilastine spherical agglomerates observed spherical in size with improved particle size which indicates that the drug particles are converted in spherical Agglomeration.

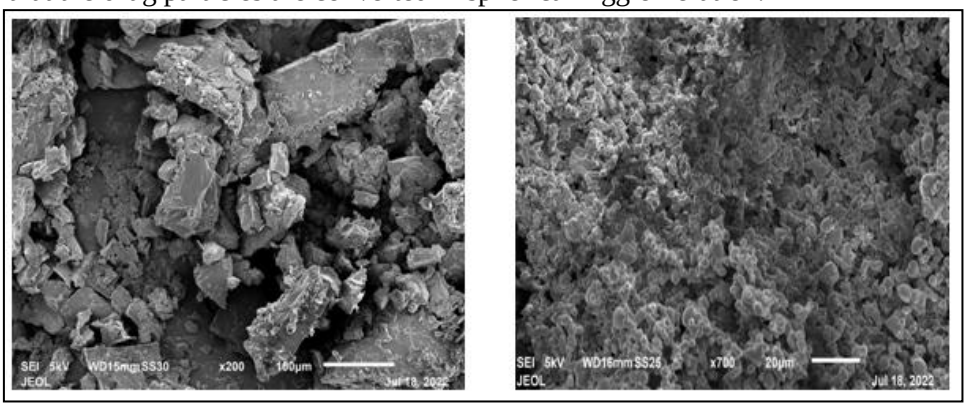

Figure 5 SEM image of A) pure Bilastine and B) Bilastine Spherical Agglomerates

Differential Scanning Calorimetry (DSC) analysis

Thermal behavior of pure Bilastine (A) and BilastineSpherical agglomerates (B) was measured by DSC which shown in Figure 6. Pure Bilastine shows characteristic sharp endothermic peak at 148.96°C that is differentiating its melting point. In Differential scanning calorimetry thermogram of spherical agglomerates of Bilastine with Polymer showed endothermic peak at 138.78ºC. A lower melting point of Bilastine spherical agglomerates indicating the presence of amorphousness in the optimized batch which might be due to weakening or disrupting of crystal lattice.

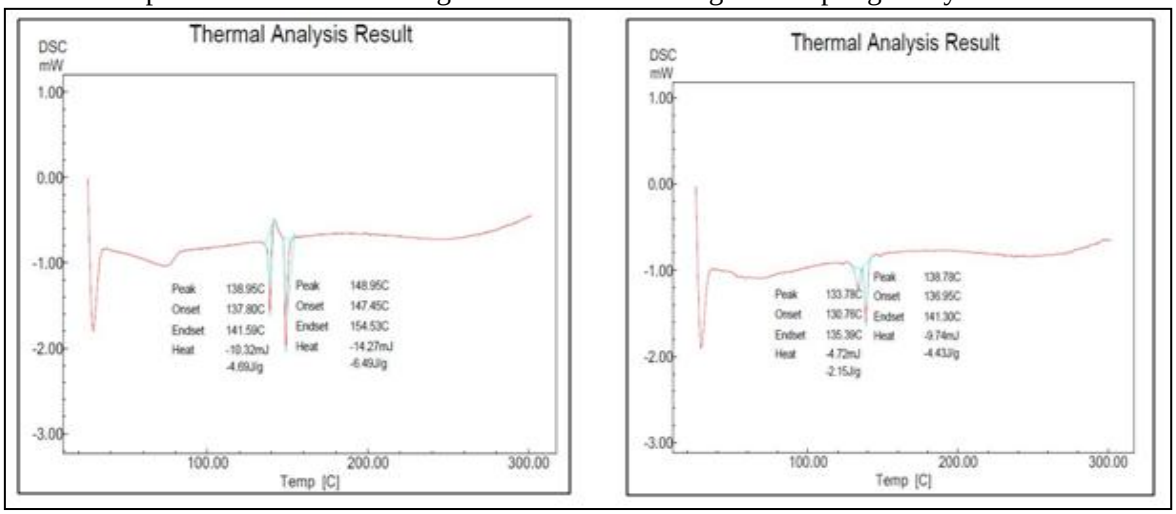

Figure 6 DSC of A) Pure Bilastine and B) Bilastine Spherical agglomerates

Powder X-ray Diffraction (XRD) analysis

The crystallinity intense peaks of Pure Bilastine was observed by X-ray diffraction (XRD) scan. whereas Bilastine spherical agglomerates exhibited a aureole pattern with biter intense and thicker peaks compared to Pure Bilastine (Figure 7). This pattern indicates that increase in crystallinity and increase amorphization of the Bilastine in its spherical agglomerates form.

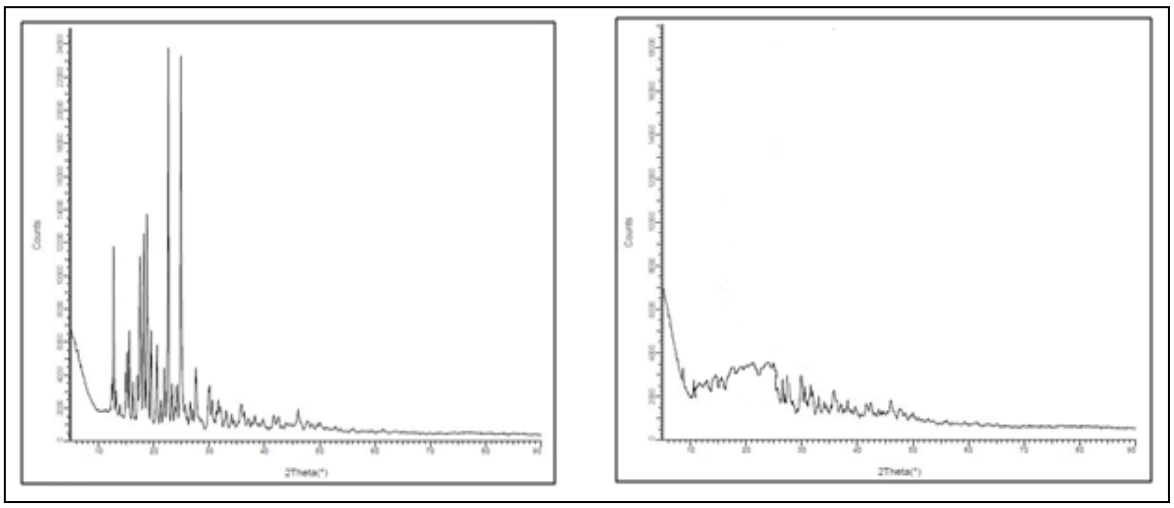

Figure 7 XRD plot of **A)** Pure Bilastine and **B)** Bilastine Spherical agglomerates

CONCLUSION

In the present study, various formulation and process variables successfully evaluated and screened using Placket-Burman (PBD) design. The Ratio of (GS-BL)-PS, Concentration of Polymer and stirring speed were the most criticism factors which affect the saturated solubility (SS), particle size (PS) and angle of repose (AR) of spherical agglomerates formulation. Additionally, less influential factors like type of Good solvent (Methanol), type of bridging liquid (DCM), ratio of (GS-BL)-PS (1.05), and stirring time (40 min) were set at a favorable level. SEM study reviled the substantial change in shape and surface morphology of Bilastine after formulated into spherical agglomerates. Moreover, the XRD study confirmed the amorphous nature of agglomerates.

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STATEMENTS AND DECLARATIONS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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