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Significance of Formulation and Process Variables on Development of Drug Loaded Micro-Granules

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Abstract:-Present study evaluated three independent factors amalgam of two process variable such as atomization, and product temperature; and one formulation variable such as binder concentration of the drug dispersion. Central Composite Design (CCD) with three factors, two levels with5 centre points was used to generate experimental plan for optimization of these three independent factors with target constraints of three dependent factors such as % Efficiency (R1), % agglomeration (R2),and % drug release(R3). Target constraints used for the optimization was; 80% ≤ *R1* ≥ 100%; 0% ≤ *R2* ≥ 10%; 85% ≤ *R3* ≥ 100%.Study indicated the responses were significant for the functional of the change in the level of the independent variables. Analysis of the design generates overlay plot to obtain design space.Binder concentration found significant for all the responses with its main impact and other factors like atomization and product temperature found significant with their main and two way interaction effect on drug loaded micro-granules. Prepared drug loaded micro-granules can be used for the further enteric coating.

I. INTRODUCTION

Solid drug delivery system is the most prevailing system for the drug delivery. After many years of research it is most acceptable form for drug delivery and that why it is referred as gold standard out of all dosage forms. There are many advantages associated with this drug delivery system. However there are some limitation associated with this system are less bioavailability; first pass metabolism; difficulty of swallowing (dysphagia); difference in the GIT pH etc. [1]

Looking at all the limitation of the solid oral dosage form a novel approach named multiunit particulate delivery system (MUPS) for the modified as well as immediate release solid oral dosage forms have been developed. This system consist of combination of thousands of spherical shaped micro-granules belonging to similar particle size, surface characteristic, in the form of tablet, capsules and sachets. Drug substances may be present in the matrix form or reservoir form of the micro-granules. MUPS have better behaviour of absorption and lesser inter and intra subject variability and ease of swallowing by modifying final dosage form containing micro-granules. Oral disintegrating tablets containing these micro-granules are the best solution for problem in swallowing. [2-4]

Functional relationships between various factors can be derived from response surface design of experiments and central composite design (CCD) is the most acceptable design to demonstrate the functionality between factors on the basis of the desired target constraints of the responses. [5] In the present study, CCD is used to optimized the limits of three factors for the manufacturing of the drug loaded micro-granules using drug dispersion.

Model drug is not soluble in the aqueous system and therefore drug dispersion was prepared to manufacture the drug loaded micro-granules. Independent factors selected in this study are based on the preliminary study performed for drug loading. Other formulation and process variables are selected based on the trials performed. This article only elaborates optimization of the drug loading based on the target constraints of the desired attributes.

II. MATERIAL AND METHOD

Materials

Drug substances was received from IPCA laboratories,Celphere CP 102 (microcrystalline cellulose spheres), Talc were received from Signet Chemicals, Hydroxypropyle methyl cellulose (HPMC E 5) was received from Colorcon, Citric acid from Merck, Sodium Lauryl sulfate (SLS) from Cognis. All other excipient and solvents used were of standard pharmaceutical and analytical grade.

A. Experimental Design for the Drug Loading

Central composite design (CCD) with three factor (Independent variables) two level, five replicates at the centre point with alpha rotatable value of 1.41421 was used to prepare the design for drug loaded micro-granules.Small type of the CCD with single block was used. Atomization (A), Product temperature (B) and Binder concentration (C), were

the independent factors while % efficiency (R1), agglomeration (R2) and % drug release (R3) were the dependent factors. Table 1 summarizes the factors with level, and responses with target constraints for optimization. Response surface was constructed using design expert software and a suitable polynomial model was selected based on statistical evaluation of the data. CCD suggested experimental plan of 15 trials.

Independent factors	Level		Dependent factors	Target Constraints	
	-1	+1		Lower	Upper
A – Atomization (bar)	0.50	2.50	R1 - Efficiency (% w/w)	80.00	100.00
B - Product Temperature (^{0}C)	25.00	45.00	R2 - Agglomeration (% w/w)	0.00	10.00
C - Binder concentration (%w/w)	1.00	6.00	R3 - % drug release in 30 minutes at pH 6.8 (% of label claim)	85.00	100.00

Table 1: Independent Factors with Level and Dependent Factors with Target Constraints.

B. Preparation of Drug Loading Dispersion

Table 2 summarizes the different compositions used for the drug loading process. These compositions were prepared based on the five different levels of the HPMC E5 i.e., two axial points (0.00% and 7.04%), two factorial points (1.00 % and 6.00 %) and one centre point (3.50 %). Drug loading dispersion number D1 and D5 were based on the axial points, D2 and D4 with factorial points, and D3 with centre point. Required quantity of the HPMC E 5 was dissolved in the required quantity of the water with continuous stirring followed by addition of citric acid, SLS and talc with continuous stirring till uniform dispersion formed. Drug substance was added in the uniform dispersion formed and stirred continuously. Homogenous dispersion was sieved through ASTM # 80 to remove any extraneous material.

C. Preparation of Drug Loaded Pellet

Celphere CP 102 (less than 150 micron) was used as starting material for drug loading. Composition for drug loaded micro-granules per unit dose consist of Celphere CP 102 (50.00 mg / unit dose), DS (75.00 mg / unit dose), citric acid (8.75 mg / unit dose), SLS (0.38 mg / unit dose), Talc (21.88 mg / unit dose) and HPMC E5 (in concentration range of 0.00 %, 1.00 %, 3.50 %, 6.00 % and 7.04% w/w of drug dispersion). Each batch was starting usingCelphere CP 102 (100 g). Fluid bed processor with wurster was adopted for the development of the drug loaded micro-granules. 100 g of starting material was charged to the wurster container and process was switched "ON" followed by spraying of the respective drug dispersion once product temperature reached at 40°C ± 2°C. Adjustment of the inlet and exhaust temperature was performed to maintain the required product temperature, and adjustment of inlet air flow was also done to get enough fluidization, as with the progress of the drug loading particle size of the micro-granules increases and air flow needed to be increased, 0.8 mm of the spray nozzle was used at spray rate ranges from 3 g/min to 12 g/min (process started with the slowest spray rate with gradual increase with process progress upto 12 g/min). 22 -28 % of RH was maintained to overcome the static charge. Product temperature and atomization was set as per design trial shown in the table 3. After completion of the process drying of micro-granules was performed at 25^{0}C with airflow 25 m^3/htill LOD reachesbelow 2.0 % w/w.

ISSN No: - 2456 – 2165

Name of the ingredients	D1		D2		D3		D4		D5	
	% w/w	mg / unit	% w/w	mg / unit	% w/w	mg / unit	% w/w	mg / unit	% w/w	mg / unit
DS*	6.00	75.00	6.16	75.00	6.00	75.00	5.84	75.00	5.78	75.00
HPMC E 5	0.00	0.00	1.00	12.20	3.50	43.75	6.00	77.00	7.04	91.30
Citric acid	0.70	8.75	0.72	8.75	0.70	8.75	0.68	8.75	0.67	8.75
Sodium Lauryl Sulfate	0.03	0.38	0.03	0.38	0.03	0.38	0.03	0.38	0.03	0.38
Talc	1.75	21.88	1.80	21.88	1.75	21.88	1.70	21.88	1.69	21.88
Water	88.02	1100.25	90.30	1100.25	88.02	1100.25	85.74	1100.25	84.79	1100.25
Total dispersion	96.50	1206.25	100.00	1218.45	100.00	1250.00	100.00	1283.25	100.00	1297.55

*DS: Drug Substance

Table 2: Composition of Drug Dispersion sat Five Different Concentrations of CCD.

Run	**Independent factors**			**Dependent factors**		
	A – Atomization (bar)	**B – Product temperature (^{0}C)**	**C – Binder concentration (% w/w)**	**R1 – Efficiency (% w/w)**	**R2 – Agglomeration (% w/w)**	**R3 – Drug release in 30 minutes at pH 6.8 (% of label claim)**
1	1.50	35.00	3.50	90	4	90
2	0.09	35.00	3.50	90	14	92
3	2.50	45.00	1.00	50	0	94
4	1.50	35.00	7.04	95	45	71
5	1.50	20.86	3.50	90	18	90
6	1.50	35.00	0.00	42	0	96
7	1.50	35.00	3.50	89	2	94
8	1.50	35.00	3.50	88	5	92
9	2.91	35.00	3.50	84	1	89
10	0.50	45.00	6.00	94	30	83
11	1.50	35.00	3.50	87	5	90
12	1.50	49.14	3.50	64	4	91

ISSN No: - 2456 – 2165

Run	Independent factors			Dependent factors		
	A – Atomization (bar)	B – Product temperature (^{0}C)	C – Binder concentration (% w/w)	R1 – Efficiency (% w/w)	R2 – Agglomeration (% w/w)	R3 – Drug release in 30 minutes at pH 6.8 (% of label claim)
13	2.50	25.00	6.00	92	26	85
14	1.50	35.00	3.50	92	3	92
15	0.50	25.00	1.00	50	1	93

Table 3: CCD Experimental Plan with Responses.

D. Process % Efficiency

It was calculated with the following formula;

% Efficiency (w/w) = $[(W_d – W_S/ W_{SC}] \times 100$

Where, $W_d$is total weight of the drug loaded micro-granules; W_S is weight of the starting material taken; W_{SC} is weight of the total solid content of the drug dispersion used for drug loading.

E. Sizing

Drug loaded micro-granules sized using ASTM # 60, retained portion of micro-granules was considered as agglomerates and it was calculated with following formula:

% Agglomeration (w/w) = $(W_R / W_d) \times 100$

Where, W_R is weight of the ASTM # 60 retained drug loaded micro-granules; $W_d$is total weight of the drug loaded micro-granules.

F. In-Vitro Drug Release

In-vitro drug release was performed using capsules filled with drug loaded microgranules (quantity equivalent to 75 mg of the DS) in 1000 ml of phosphate buffer pH 6.8 in USP II (paddle) at 100 rpm at 30 minutes at analysis by UV at 265 nm wavelength.

G. Pugh Matrix Analysis to Identify the Sequence of Significant Independent Factor

It is a tool for quantitative analysis to identify the sequence of significantly impacting independent factors. It was constructed to identify the completesequence of significance order of the independent factor of drug loaded micro-granules. Main effects were scored 3 while interaction was scored 2. Overall significance order is identified through this analysis.

III. RESULT AND DISCUSSION

A. Experimental Design for Drug Loading

Experimental plan of CCD suggested 15 unique combinations of the independent factors level. Difference in the results of the responses indicated the significance of the different level combination of the independent factors. Different models such as linear, 2FI, Quadratic and Cubicwere tried to analyse the responses individually and validity of the selected quadratic model was confirmed by ANOVA terms like model ‘F-value’ (p value), the sequential model sum of square, Lack of fit, *R*-square, adjusted *R*-square, predicted R-square and adequate precision. P value should be less than 0.05, while lack of fit should be not less than 0.05 for the model to fit along with reasonable difference (not more than 0.2) between ‘predicted R-square’ and ‘adjusted R-square’. ‘adequate precision’ should be more than 4 to adequate fit the model. Table 4summarises the statistical terms of the selected model for each response and confirms the suitability of the selected model.

ISSN No: - 2456 – 2165

Model Statistical Value	R1 – Efficiency	R2– Agglomeration	R3 – Drug release
F-Value	122.70	5761.05	8.09
Prob> F (P-Value)	< 0.0001	< 0.0001	0.0230
Lack of fit (p-Value)	0.9289	0.5525	0.1696
R – square	0.9969	0.9999	0.9635
Adjusted R – Square	0.9912	0.9998	0.8978
Predicted R – Square	0.9945	0.9991	-0.6563
Adequate precision	37.687	237.063	15.692
PRESS	25.90	0.77	864.14
Selected model	Quadratic	Quadratic	Quadratic

Table 4: Summary of Statistical Value for Each Response

Quadratic equation for the selected model is as follows;

$R1/R2/R3 = X_0 + X_1A + X_2B + X_3C + X_4AB + X_5AC + X_6BC + X_7A^2 + X_8B^2 + X_9C^2$

Where, X_0(intercept) and $X_1 – X_9$ (coefficient of respective factors and interaction terms). Table 5 shows individual quadratic equation of all dependent factorsand table 6 summarises standardize main effectof all independent factors on dependent factors.

$$\text{Efficiency} = +89.22 -2.12A -9.19B +18.74C -2.76AB -9.69AC -1.62BC -1.14A^2 -6.14B^2 -10.39C^2$$

$$(1/\text{Agglomeration} + 0.04) = +0.28 +0.31A +0.068B -7.85C -2.07AB -5.25AC -5.00BC +0.13A^2 -0.054B^2+5.43C^2$$

$$\%\ \text{drug release} = +91.26 -1.06A +0.35B -8.84C -4.09AB +0.60AC -1.81BC +0.046A^2+0.046B^2 -3.45C^2$$

Table 5: Quadratic Equations of Dependent Variables Indication Quantitative Effect of Independent Variables

ISSN No: - 2456 – 2165

		R1 (efficiency)				R2 (agglomeration)				R3 (% drug release)			
		Estimated coefficient	Standard error	Standardized main effect***	P-Value	Estimated coefficient	Standard error	Standardized main effect***	P-Value	Estimated coefficient	Standard error	Standardized main effect***	P-Value
X_1	A	-2.12	0.86	-2.47	0.0570	0.31	0.057	5.44**	0.0028	-1.06	0.98	-1.08	0.3266
X_2	B	-9.19	0.86	-10.69**	0.0001	0.068	0.057	1.19	0.2909	0.35	0.98	0.36	0.7319
X_3	C	18.74	0.86	21.79**	<0.0001	-7.85	0.057	-137.72**	<0.0001	-8.84	0.98	-9.02**	0.0003
X_4	AB	-2.76	1.22	-2.26	0.0727	-2.07	0.081	-25.56**	<0.0001	-4.09	1.38	-2.96*	0.0314
X_5	AC	-9.69	1.22	-7.94**	0.0005	-5.25	0.081	-64.81**	<0.0001	0.60	1.38	0.43	0.6800
X_6	BC	-1.62	1.22	-1.33	0.2406	-5.00	0.081	-61.73**	<0.0001	-1.81	1.38	-1.31	0.2464
X_7	A^2	-1.14	0.62	-1.84	0.1257	0.13	0.041	3.17*	0.0272	0.046	0.70	0.07	0.9500
X_8	B^2	-6.14	0.62	-9.90**	0.0002	-0.054	0.041	-1.32	0.2517	0.046	0.70	0.07	0.9500
X_9	C^2	-10.39	0.62	-16.76**	<0.0001	5.43	0.041	132.44**	<0.0001	-3.45	0.70	-4.93*	0.0044

* Significant at 5% level

** Significant at 1% level

***Standardized main effect were calculated by dividing the estimated coefficient by the standard error of the estimated coefficient

Table 6: Standardized Main Effect of Factors on Response

Table 6 indicated that statistically significant coefficient ($P<0.05$) for R1 were X_2, X_3, X_5, X_8 and X_9; for R2 were X_1, X_3, X_4, X_5, X_6, X_7 and X_9; and X_3, X_4 and X_9 were significant for R3. Common significance of the estimated coefficients of binder concentration and its square (X3 and X9) was confirmed for all independent factors. A positive and negative sign before a coefficient in quadratic model indicates a synergistic effect or an antagonistic effect for the factor respectively.

B. Impact of Independent Factors

Atomization (X1) and its square (X7) werestatistically significant for agglomeration only with synergistic effect. Product temperature (X2)and its square (X8)were found statistically significant for efficiency (R1) with its antagonistic effect. Plausible reason for this significance may be spay drying of the droplet of the drug dispersion before reaching to particle surface at over-optimum product temperature, leads to high fine generation and lesser efficiency. Unlike this, it's interaction with atomization may leadsignificant effectonboth agglomeration (R2) and % drug release (R3) whereas interaction with binder concentration would lead to agglomeration only. Both the interaction will lead to antagonistic effects.Binder concentration (X3) and its square (X9) were found statistically significant for all dependent factors. Higher the concentration of the binder, slower would be the release; however there would be no impact with higher concentration of the binder interaction with change in atomization and product temperature. Whereas, efficiency and agglomeration were found significant for its interaction with product temperature and atomization with opposite effect.Table 7 shows order of significantly affected responses.

Responses Factors	Efficiency	Agglomeration	% drug Release
	Significantly affected priority number*		
Atomization	2	3	1
Product Temperature	3	2	1
Binder Concentration	2	3	1
**3: most effected; 2: less effected than 3 and more effected than 1;1: Least effected*			

Table 7: Significantly Affected Priority of Responses

C. Impact on Dependent Factors

Statistical analysis indicated that efficiency found significantly affected by main impact of the product temperature and binder concentration; and interaction impact of atomization withbinder concentration. Contour plot presented in figure 1 indicated that all three independent factors play an important role in change in the efficiency of the process. Poor efficiency (NMT 70 %) observed with low binder concentration (1.00 %), at middle binder concentration (3.50 %) efficiency found improved and also indicated interaction with product temperature, as product temperature increases, efficiency decreased. No interaction with atomization was observed at this concentration of the binder. Whereas improved efficiency observed at higher concentration of binder (6.00 %) with interaction of product temperature and atomization observed after optimum product temperature and resulted in lesser efficiency. High atomization lead to the smaller droplet size and upon interaction with product temperature smaller droplet have comparatively faster rate of drying than larger droplets. Addition to this if viscosity of the dispersion is higher than it adds to this effect and all three factors like higher binder concentration, higher product temperature and high atomization leads to more faster drying and result in more spray drying and reduced efficiency. In the figure 1a, 1b and 1c the only difference is of binder concentration and viscosity of the dispersion. Effect of viscosity of dispersion is self-explanatory.

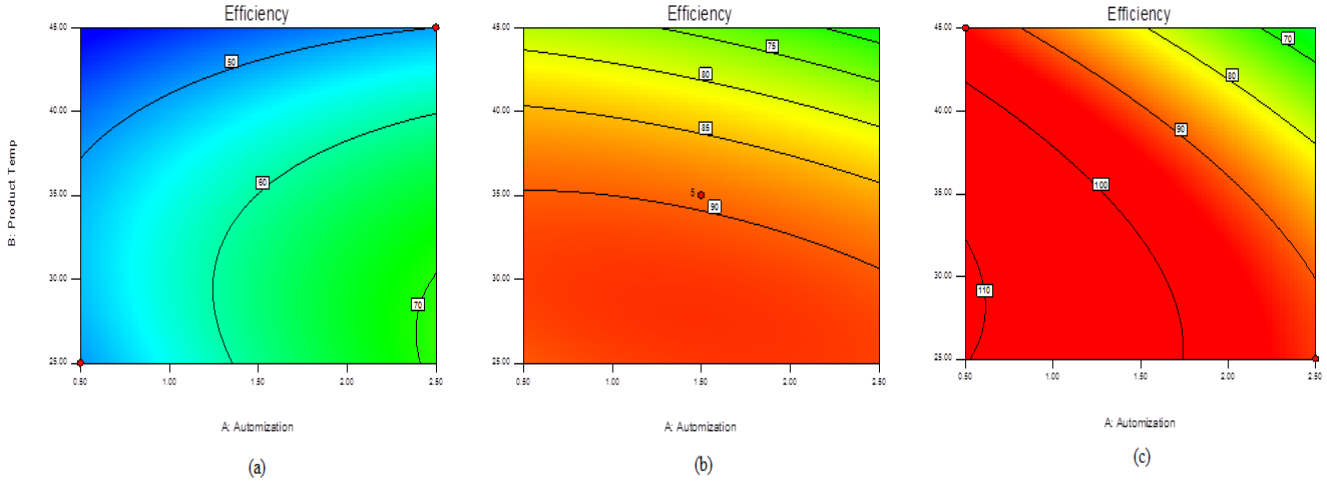

Figure 1: Contour Plot for Efficiency at Different Concentration of the Binder (a) 1.00 % w/w; (b) 3.50 % w/w; (c) 6.00 % w/w.

ISSN No: - 2456 – 2165

Table 7 indicated that dependent factor 2 which is agglomeration is the most impacted dependent factor amongst all three. Agglomeration becomes an independent factor for other dependent factors like % drug release, uniformity of content, particle size distribution of the micro-granules. Agglomeration impacts the surface area of drug loaded micro-granules and results in the changed drug release, density of the micro-particles also varies with agglomeration and tendency of segregation in the blend with extra-granular increases with that. Figure 2represents contour plots of the agglomeration at different binder concentrations. Less binder concentration shows least agglomeration, with the increase in the binder concentration tendency of the agglomeration also increases. Curve lines also show interaction impact of all three independent factors on agglomeration. However, binder concentration has main impact on the agglomeration.

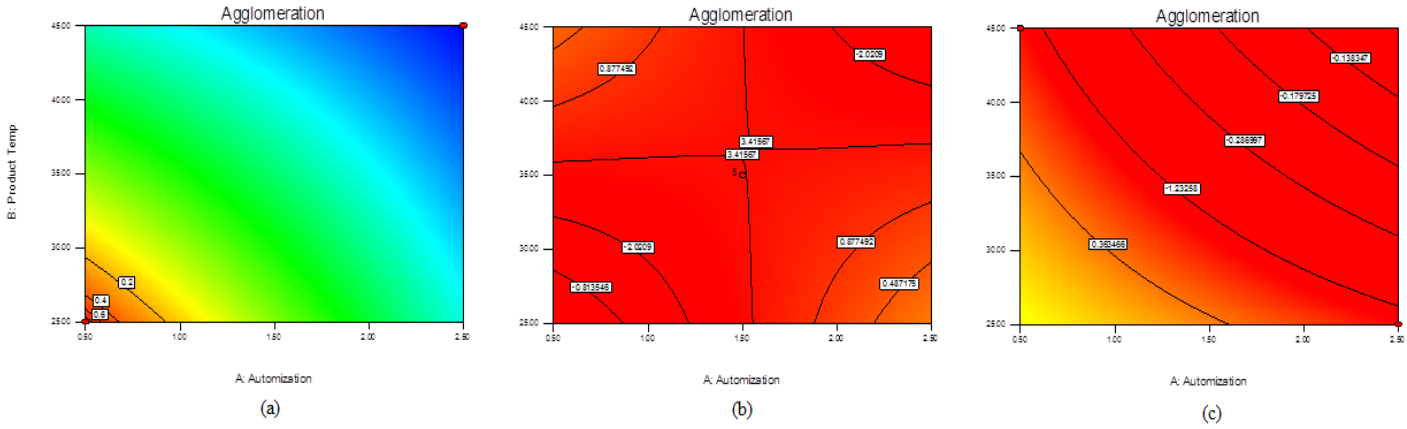

Figure 2: Contour Plot for Agglomeration at Different Concentration of the Binder (a) 1.00 % w/w; (b) 3.50 % w/w; (c) 6.00 % w/w.

Therapeutic safety and efficacy of the drug product is dependent on drug release quality attributes of the drug product. Drug release in phosphate buffer pH 6.8 was the last response analysed statistically by the design. This was the least affected response. Figure 3 shows contour plot of drug release profile of drug loaded micro-granules indicating significance of the binder concentration. Study indicated that increase in the binder concentration, slower is the drug release. At high binder concentration, molecules of the active substance are more firmly clamped by the binder and resulting drug release is slower. Binder concentration has main significant impact on the drug release with two way interaction of atomization with product temperature.

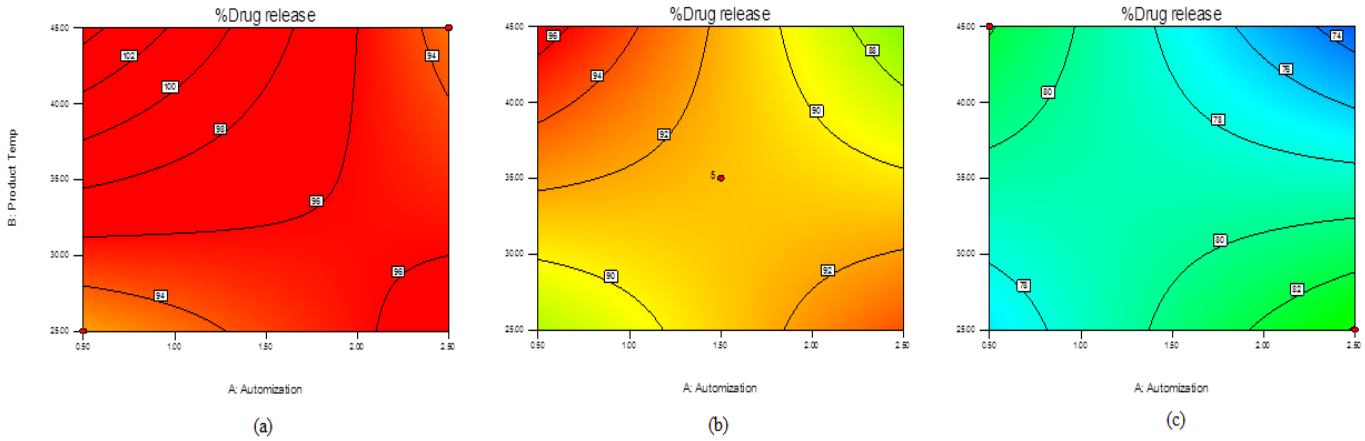

Figure 3: Contour Plot for % Drug Release at Different Concentration of the Binder (a) 1.00 % w/w; (b) 3.50 % w/w; (c) 6.00 % w/w.

ISSN No: - 2456 – 2165

D. Pugh Matrix to Identify the Sequence of Significant Independent Factor

Based on the statistical analysis performed, all the independent factors were scored for their impact on the dependent factors. Sign "+" was used for the positive impact, while sign "-" was used for no impact. Table 8summarizes the order of significance of independent factors. Overall significance sequence was as follows:

Binder concentration > Atomization > Product temperature

		Importance	Atomization	Product temperature	Binder concentration
R1	Efficiency	3	-	+	+
R2	Agglomeration	3	+	-	+
R3	% drug release	3	-	-	+
Interaction		2	+++	++	++
Sum of + (Significant)			4	3	5
Sum of - (Non-significant)			2	2	0
Weighed sum of + (affected)			9	7	13
Weighed sum of - (not affected)			6	6	0

Table 8: Pugh Matrix Analysis Identifying Sequence of Significance of Independent Factors.

E. Design Space Using Graphical Optimization

It was clearly demonstrated that every independent factor impacts responses either through its main effect and/or interaction with other factor. Based on the target constraints i.e, 80% ≤ *R1* ≥ 100%; 0% ≤ *R2* ≥ 10%; 85% ≤ *R3* ≥ 100% graphical optimization was performed and design space was generated. Figure 4 represents the overlay plot with design space in yellow area. Overlay plot also changes with the change in the value of any one of the independent factor. As observed, binder concentration was found the most significant factor and is also a formulation variable, therefore, it is better to fix the value of the binder to generate the design space. Hence this overlay plot was generated with a medium concentration (3.50 %) of the binder. The design space is the area of working to get desirability of 1 (all quality attributes would be in the desired specification limits).

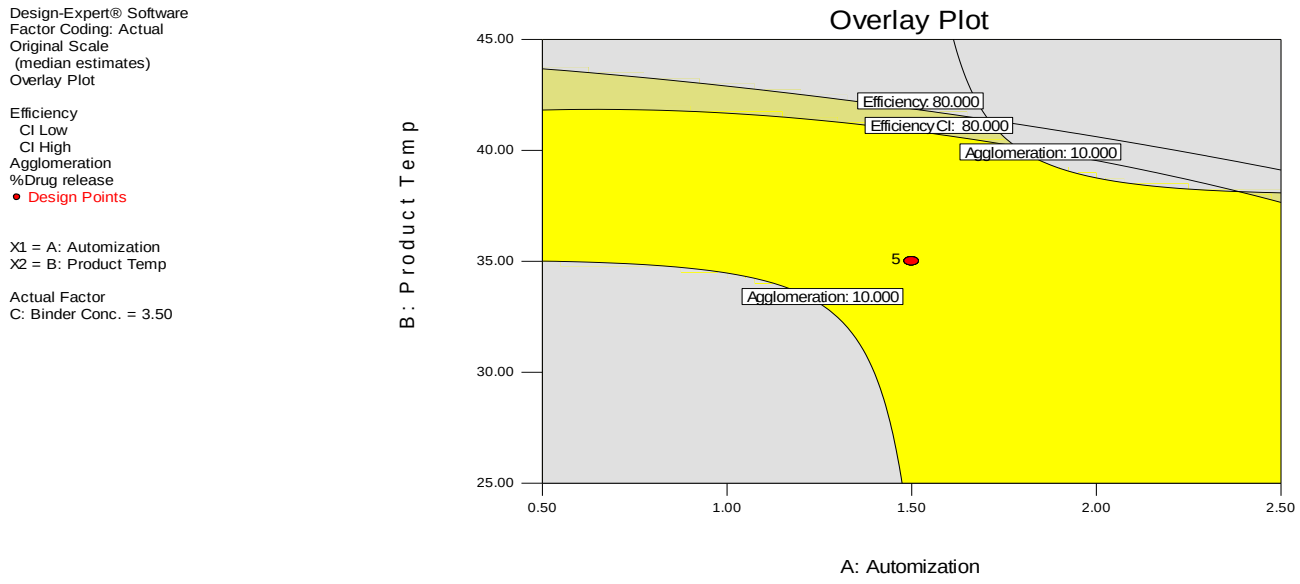

Fig 4: Overlay Plot Representing Design Space as Yellow Area

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IV. CONCLUSION

Present study successfully described impact of the formulation and process variables on the development of the drug loaded micro-granules using drug dispersion loading through wurster process. CCD successfully demonstrated the impact and identified design space.

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