

Formulation and Optimization of Immediate Release Pellets of Antiplatelet Drugs Using Design of Experimentation

Deshkar S S*, Pore A R

Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, Maharashtra, India, 411018.

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ABSTRACT

Platelets play an important role in hemostasis during tissue injury, which blocks the defect and terminates blood loss. Platelet aggregation inhibitors are widely used in treatment of cardiovascular disorders and Peripheral arterial disease. Clopidogrel bisulphate and Cilostazol, are FDA approved BCS class II drugs, used in treatment of Platelet aggregation, peripheral arterial disease and intermittent claudication. The aim of the present study was to develop an immediate release pellets for combination of Clopidogrel bisulphate and Cilostazol using extrusion spheronization technique. The effects of spheronization speed (X_1) and binder concentration (PVP K30) (X_2), on size of pellets, disintegration time and drug release were studied using 3^2 full factorial design. The surface response and counter plot were drawn to facilitate an understanding of the contribution of the variables and their interaction. From the results, speed of spheronization of 1100 rpm and 5% concentration of PVP K30, were selected. *In vitro* drug release studies revealed more than 80% of clopidogrel bisulphate release and more than 75% of cilostazol release within 30 min of dissolution which complied with the pharmacopoeal limits. Film coated pellets did not show significant change in the drug release. DSC and FTIR studies revealed no interaction of drugs and excipient during pellet formulation. The pellet formulations of clopidogrel and cilostazol were found to be stable when stored at $40^\circ\text{C}\pm 2^\circ\text{C}/75\%\text{RH}\pm 5\%\text{RH}$ for 2 months. Conclusively, clopidogrel bisulphate and cilostazol pellet fixed dose combination could be successfully developed by design of experimentation and complied with pharmacopoeal limits.

Keywords: Clopidogrel, Cilostazol, Pellet, Multiparticulate, immediate release, Design of Experimentation.

INTRODUCTION

During tissue injury, platelets play a key role in hemostasis. By interacting with activated plasma clotting factors, platelets help to form a mechanical plug at the site of blood vessel injury and terminate blood loss¹. It is now well understood that platelet hyperactivity has an important role in developing cardiovascular diseases and peripheral arterial diseases².

Peripheral arterial disease (PAD) is an important manifestation of systemic atherosclerosis and is caused by atherosclerotic occlusion that if not treated well may progress to critical limb ischemia (CLI)³. PAD affects men and women equally and prevalence is reported to be approximately 12%⁴. PAD is associated with mortality rate of 25% annually in case of patients with CLI, without proper treatment. It remains as a major cause for critical illness and loss of limb, resulting in social burden and affecting the overall quality of patient's life⁵.

Treatment of CLI remains a challenge for clinicians and it is reported that the patients with PAD are undertreated as compared to patients with coronary artery disease⁶. Current treatment and prevention strategies include risk factor modification along with the treatment of antiplatelet drugs. Antiplatelet drugs are reported to reduce the risk of cardiovascular events, ischemic strokes and resulting mortality rate⁷. The most commonly prescribed

antiplatelet drugs are Aspirin, Dipyridamole, Ticlopidine, Clopidogrel and Cilostazol⁸.

Clopidogrel is a thienopyridine that along with its active metabolite prevent binding of adenosine diphosphate (ADP) to its platelet receptor, impairing the ADP-mediated activation of the glycoprotein GPIIb/IIIa complex⁹. Cilostazol and several of its metabolites are cyclic AMP (cAMP) phosphodiesterase III inhibitors (PDE III inhibitors), inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation¹⁰. Cilostazol has shown to improve the symptoms in patients with intermittent claudication (IC) with significant improvement in walking distance of patient. Recently, cilostazol has been demonstrated as an effective and safer alternative to aspirin for long term prevention of CLI¹¹. Recent reports have shown the use of dual antiplatelet agents such as, clopidogrel and aspirin or cilostazol and aspirin, in combination, as more effective than either one used alone for reducing future recurrences and major cardiac events¹². A study by Sheu et al has demonstrated synergistic effect of combined therapy of clopidogrel and cilostazol in rat CLI³. Study by Wilhite et al suggested that as cilostazol is relatively weak platelet inhibitor compared to aspirin and clopidogrel, its combined use with these

agents does not alter the platelet function significantly, however, it can be safely used in IC and CLI without modification of dose for both drugs¹³. In view of this, in the present study, an attempt has been made to develop an immediate release pellet formulation of clopidogrel and cilostazol in a fixed dose combination.

Multiparticulate systems like pellets have gained a lot of attention and are preferred for oral drug delivery when compared to single unit dosage forms. The advantages of multiparticulates include, uniform distribution in the gastrointestinal tract, better *In vitro* and *in vivo* release of the drug substances with reproducible release characteristics, increase in the bioavailability, reduced risk of dose dumping and flexibility to modify the drug release¹⁴. In the present study, clopidogrel and cilostazol pellets were separately prepared by extrusion-spheronization technique using microcrystalline cellulose as the diluents. The formulation of pellets was optimized for formulation and process variables using 3² full factorial design. The effect of variables on particle size, disintegration and drug release was studied. The pellets were film coated with HPMC E50 LV using fluidized bed coater. The pellets were evaluated for size, hardness, friability, disintegration, *In vitro* dissolution for single drug and for combination used in fixed dose.

MATERIAL AND METHODS

Material

The sample of clopidogrel bisulphate was a generous gift from SAVA Pvt. Ltd., Pune, India and Cilostazol was gifted by Amsal chem Pvt. Ltd., Gujrat, India. Microcrystalline cellulose (Avicel pH 101), polyvinyl pyrrolidone K30 (PVP) were purchased from Himedia, Mumbai, India. Ac-di-sol was purchased from Loba chem., Mumbai, India. All the solvents used were of analytical grade.

Methods

Drug –Excipients Compatibility study¹⁵

Preformulation studies were conducted with various commonly used excipients at 40°C±2°C/75%±5% RH and 30°C±2°C/65%±5% RH. The drug along with different excipients in 1:1 ratio was mixed, sealed in clear glass vials with LDPE stoppers, which were then charged into stability chambers (Remi, CHM 16S, India) at the above-mentioned conditions. The vials were inspected periodically to observe physical changes and discoloration. For comparison of physical changes and discoloration, initial samples were used as reference sample. The physical observation was done after one month. At the end of one month the sample were analyzed by FTIR with DRS attachment (Shimadzu, 8400S, Japan), over the range of 4000-400 cm⁻¹ with a resolution of 5cm⁻¹, to observe any chemical changes in the blend¹⁶.

Preparation of pellets by extrusion and spheronization

The pellets were prepared using the extrusion and spheronization technique¹⁷. The drug and excipients were weighed and mixed intimately in a mortar for 15 min. PVP K30 solution as binder was added to the mixture and wet mass was prepared. The wet mass was extruded using twin screw extruder and was spheronized for 3 min at varying

speed in a spheronizer (Shakti, SSP 120 GMP, India), fitted with cross-hatched plate (2 mm depth). The pellets were dried in a lab scale pan Coater.

Formulation and optimization of clopidogrel bisulphate pellets by using 3² factorial design¹⁸

In order to study the effect of processing and formulation variable on clopidogrel pellets 3² factorial design was utilized. Design consisted of 9 experimental trials, with 3 levels of 2 independent variables. One variable was, spheronization speed (X₁) at levels, 900, 100 and 1300 rpm and second variable was binder concentration (X₂) at levels, 2, 3.5 and 5%. The composition of pellet formulation is shown in table 1.

Formulation of Cilostazol Pellets

Cilostazol loaded pellets was formulated using composition given in table 2 at spheronization speed of 1100 rpm and at varying binder (PVP K30) concentration (2, 3.5 and 5%).

Coating of Clopidogrel bisulphate and Cilostazol pellets by Fluidised bed processor^{19,20}

The coating of Clopidogrel bisulphate and Cilostazol pellets was done in the fluidized bed processor (ACG, Miniquest F, USA) using HPMC E15 polymer and triethylcitrate as plasticizer (table 3). The coating solution was prepared by dissolving HPMC E15 in the solution of IPA and water mixture (7:3) and stirred to obtain a clear solution. The plasticizer was added further in the solution with continuous stirring for 30 min. The uncoated drug loaded pellets were charged in fluidization basket and air flow of 1 bar was allowed to circulate in order to fluidize the pellets. The prepared solution was sprayed at the flow rate of 1 ml/min at the inlet temperature of 40°C for 30 min.

Evaluation of pellets

Micromeritic properties, Percent yield and hardness²¹

The bulk properties of pellets, viz. bulk density, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose were evaluated using standard procedures. Formulated batch was weighed accurately and the percent yield of each batch was calculated using following formula.

$$\text{Percent Yield} = \frac{\text{Actual weight of batch X 100}}{\text{Theoretical weight of ingredients}}$$

Hardness of pellets was measured using digital hardness tester (Veego, 0110, India).

Size analysis by sieving²²

The size analysis of pellets was done by the sieve shaking method. A series of sieves were arranged in the order of their decreasing pore diameter (sieve mesh size, 1400, 1180, 1000, 850, 710, 425, 355, 250, 180µm). Ten grams of pellets were sieved for 10 min on a sieve shaker. The weight fraction of pellet retained on each sieve was weighed and size distribution curves were plotted from the data.

Disintegration test²³

A 100 mg pellets were selected from each batch for disintegration test. This test was performed in tablet disintegration apparatus (Esico, 1901, India) without disc in water at 37±0.5°C temperature at speed of 30 dips. A 500 µm mesh cloth was placed at the bottom of the disintegration tubes. Disintegration test was carried out in

Table 1: DOE trials for clopidogrel bisulphate pellets.

Batch code*	Spheronization time (X ₁)	Ingredients				Pellet size (µm) (Y ₁)	Responses	
		Drug (%w/w)	MCC (%w/w)	Ac-di-sol (%w/w)	PVP K30 (%w/w) (X ₂)		Disintegration time** (Y ₂) (sec)	Drug release after 30 min** (%) (Y ₃)
F1	900	18.7	69.3	10	2%	852.5	49±0.86	98.1±0.35
F2	900	18.7	67.8	10	3.5%	852.5	52±0.95	99.9±0.23
F3	900	18.7	66.3	10	5%	1007.5	58±0.65	82.4±0.25
F4	1100	18.7	69.3	10	2%	852.5	51±0.56	98.0±0.18
F4	1100	18.7	67.8	10	3.5%	670	55±0.86	93.9±0.15
F6	1100	18.7	66.3	10	5%	670	58±0.86	83.9±0.25
F7	1300	18.7	69.3	10	2%	475	52±0.45	98.0±0.25
F8	1300	18.7	67.8	10	3.5%	475	56±0.65	99.3±0.15
F9	1300	18.7	66.3	10	5%	320	59±0.52	82.5±0.38

*Solvent system in pellet formulations was IPA:water mixture (1:1) ** Mean ± SD (n=3)

Table 2: Composition of cilostazol pellets.

Ingredients	Formulation Code		
	C1	C2	C3
Cilostazol	25%	25%	25%
MCC	63%	61.5%	6%
Ac-di-sol	10%	10%	10%
PVP K30	2%	3.5%	5%
IPA:Water (1:1)	q.s	q.s	q.s

Table 3: composition of coating solution.

Ingredients	Pellet formulation	
	A1	A2
	Clopidogrel bisulphate pellets (F2)	Cilostazol Pellets (C2)
HPMC E15 LV	2.5%	2.5%
Triethyl citrate	0.25%	0.25%
IPA:Water mixture (7:3)	q.s.	q.s.

triplicate and average readings were noted.

Friability²³

A sample of pellets (5 g) was placed in the drum of friability tester (HMK, HMK 1601, China). Drum was rotated at 25 rpm for 100 revolutions. Fines were removed using 250 µm sieve, intact pellets were reweighed and percentage friability was calculated as follows.

Friability (%)

$$= \frac{(\text{Initial weight of pellets} - \text{Final weight of pellets}) \times 100}{\text{Initial weight of pellets}}$$

Estimation of drug content for clopidogrel and cilostazol²³

Pellets, equivalent to 75mg of clopidogrel and 100 mg of cilostazol, were weighed, triturated and then dissolved in 100 ml of methanol with continuous ultrasonication for 15 min. The dispersion was then filtered and the filtrate was then suitably diluted with methanol, the analysis was performed using UV spectrophotometer (Shimadzu, 1700, Japan) at λ_{max} of 271 for clopidogrel bisulphate and λ_{max} of 258 for cilostazol.

In vitro release studies for clopidogrel and cilostazol coated and uncoated pellets and their fixed dose combination

In vitro drug release study from pellets was performed using a USPXXIV Type 1 dissolution testing apparatus (Veego, VDA 8D, India). Pellets equivalent to 75mg of clopidogrel bisulphate and 100 mg of cilostazol were filled into different capsules and placed in the dissolution flask containing 900ml of dissolution medium (0.1% SLS with 0.1 N HCL for clopidogrel) and (0.3% SLS medium for cilostazol) at the temperature of 37±5° C. After specific time intervals, the aliquots of dissolution media were removed, filtered, suitably diluted and absorbance was measured on UV spectrophotometer at 271nm on for clopidogrel bisulphate and 257 nm for cilostazol. Drug release was calculated using the calibration curves of both drugs in the dissolution media.

In order to study the dissolution profile of Clopidogrel bisulphate and Cilostazol, when used in fixed dose combination (75 mg to 100 mg), pellets equivalent to 75mg of clopidogrel bisulphate and 100mg of cilostazol were filled in the same capsule. The *in-vitro* dissolution study was conducted using the USPXXIV type 1 apparatus (Veego, VDA-8D, India). The dissolution study was carried out in 900ml of solution containing 0.3% SLS as dissolution medium at 37±0.5°C and 75 rpm for 60 minutes. Aliquot of 10ml was withdrawn after interval of every 5 minutes and immediately replaced with same volume of dissolution medium to maintain sink condition. The aliquot was filtered using Whatman filter paper and absorbance was measured at 271nm of Clopidogrel bisulphate and 257nm of Cilostazol by UV spectrophotometry. Drug release was calculated using the calibration curve. The concentration of two drugs in the dissolution medium was determined by previously developed simultaneous equation method as given below^{24,25}.

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Differential Scanning Calorimetry (DSC)²⁶

For studying the possibility of interaction between drugs and excipients during pelletization process, the thermal analysis of drugs (clopidogrel bisulphate and cilostazol), their physical mixture with excipients and formulated pellets was performed using differential scanning

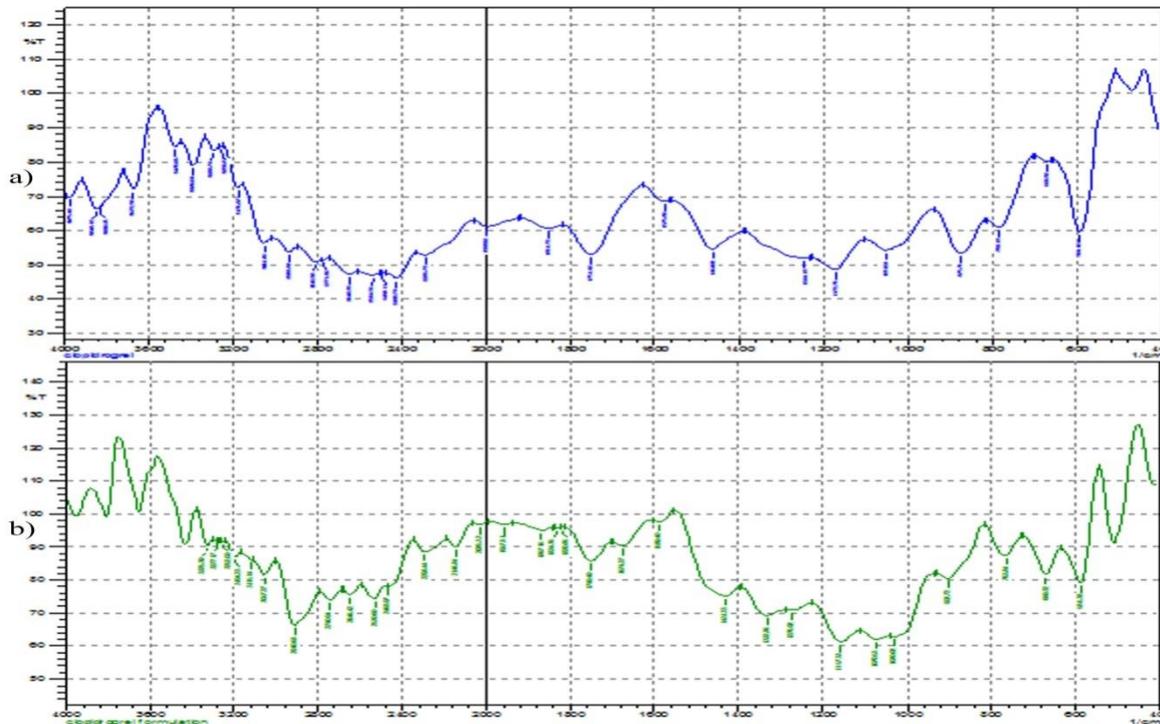


Figure 1: FTIR spectra of (a) clopidogrel bisulphate (b) physical mixture of clopidogrel bisulphate and excipients.

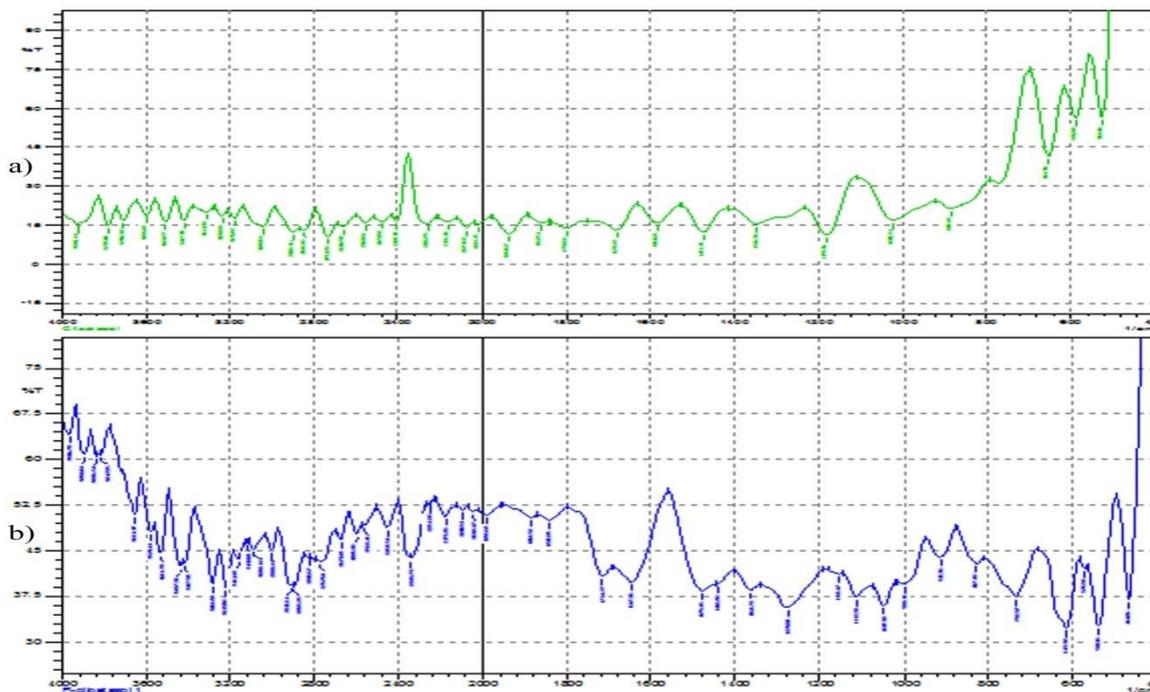


Figure 2: FTIR spectra of (a) cilostazol (b) physical mixture of cilostazol and excipients.

Calorimeter (Hitachi, DSC 7020, Japan). The sample was heated from 40°C to 220°C at the rate of 10°C/min. The inert atmosphere was maintained by purging gas throughout the experiment at the rate of 10ml/min. The sample (1-4mg) was carefully transferred and heated in a crimped aluminium pan for accurate results.

Stability study²⁷

To assess the stability of optimized formulation, it was filled in capsule and kept at 40±2° C temperature and 75

± 5% RH in a stability chamber (Remi, CHM 16S, India) for two months. At the end of studies, samples were analyzed for the physical appearance, drug content and for drug release.

RESULT AND DISCUSSION

Drug and Excipient compatibility study

Drug excipient compatibility was assessed through FTIR analysis of mixtures of excipients and drug, stored at

Table 4: Physical characterization of Clopidogrel bisulphate pellets.

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Yield	86%	92%	95%	89%	85%	90%	92%	90%	90%
B.D*	0.61±0.2	0.65±0.5	0.60±0.6	0.55±0.2	0.60±0.5	0.5±0.5	0.65±0.2	0.64±0.1	0.64±0.1
T.D*	0.66±0.4	0.70±0.4	0.65±0.6	0.59±0.2	0.65±0.3	0.6±0.4	0.70±0.5	0.69±0.6	0.69±0.86
Carrs index*	7.51±0.2	7.14±0.5	7.69±0.32	6.77±0.56	7.69±0.57	16.66±0.23	7.14±0.65	7.24±0.5	7.24±0.50
Hausners ratio*	1.081±0.4	0.928±0.2	1.083±0.23	1.072±0.56	1.08±0.56	1.20±0.89	1.07±0.25	1.07±0.36	1.07±0.56
Angle of repose	25.25 °	28.06 °	29.30 °	31.40 °	28.60 °	28.40 °	26.30 °	24.20 °	25.30 °
Hardness*	0.398±0.6	0.705±0.2	0.975±0.25	0.378±0.36	0.801±0.89	0.989±0.56	0.423±0.56	0.801±0.65	0.999±0.85

*Mean ± SD (n=3)

Table 5: Data of ANOVA for Clopidogrel bisulphate pellets.

Source	Pellet size (µm) (Y ₁)		Disintegration Time (sec) (Y ₂)		Drug release after 30 min (Y ₃)	
	F value	p value Probe ≥ F	F value	p value Probe ≥ F	F value	p value Probe ≥ F
Model	18.32	0.0028	87.9	0.0001	14.05	0.0271
Speed of spheronization (X ₁)	36.07	0.0010	18.89	0.0048	0.0099	0.9268
Binder concentration (X ₂)	0.58	0.4761	156.10	0.0001	53.54	0.0053
X ₁ X ₂	-	-	-	-	0.9609	-
X ₁ ²	-	-	-	-	0.4870	-
X ₂ ²	-	-	-	-	0.0278	-

40°C±2°C/75%±5%RH and 30°C±2°C/65%±5%RH for one month. After one month of storage at these temperatures, there were no significant changes were observed in the samples. FTIR spectra analysis indicated absence of physical interaction or incompatibility in drugs, clopidogrel bisulphate and cilostazol and excipients. All the major characteristic peaks of clopidogrel bisulphate at 3389.0 for -N-H stretch, 2534.5 for -SH, 783.1 for -C-Cl stretch and 1751.4 cm⁻¹ for -C=O stretch were indicated in the spectra of drug-excipient mixture (fig 1). The major characteristic peaks of cilostazol at 3284.8 for -N-H stretch, 3088.1 for -CH stretch, 1714.7 at -C=O stretch and 1276.9 cm⁻¹ representing -C-O stretch were indicated in the spectra of mixture (fig 2). This indicated compatibility of both the drugs with all the excipients.

Preparation and evaluation of Clopidogrel pellets

Physical characterization of pellets

Physical characterization of all the prepared clopidogrel bisulphate pellets is shown in table 4.

Percent yield

All pellet formulations indicated good percent yield ranging from 86 to 95%. Yield was higher at lower speed of spheronization and higher binder concentration.

Micrometric properties

Flow properties of pellets were found to be excellent. There was no significant difference observed in loose bulk and tapped bulk density. This indicated uniformity in size

distribution of pellets. This was further supported by lower values of angle of repose indicating good flow. Lower values of Carr's index indicated higher compressibility of pellets.

Hardness

Hardness was influenced by binder concentration. Increase in binder concentration increased the hardness of the pellets.

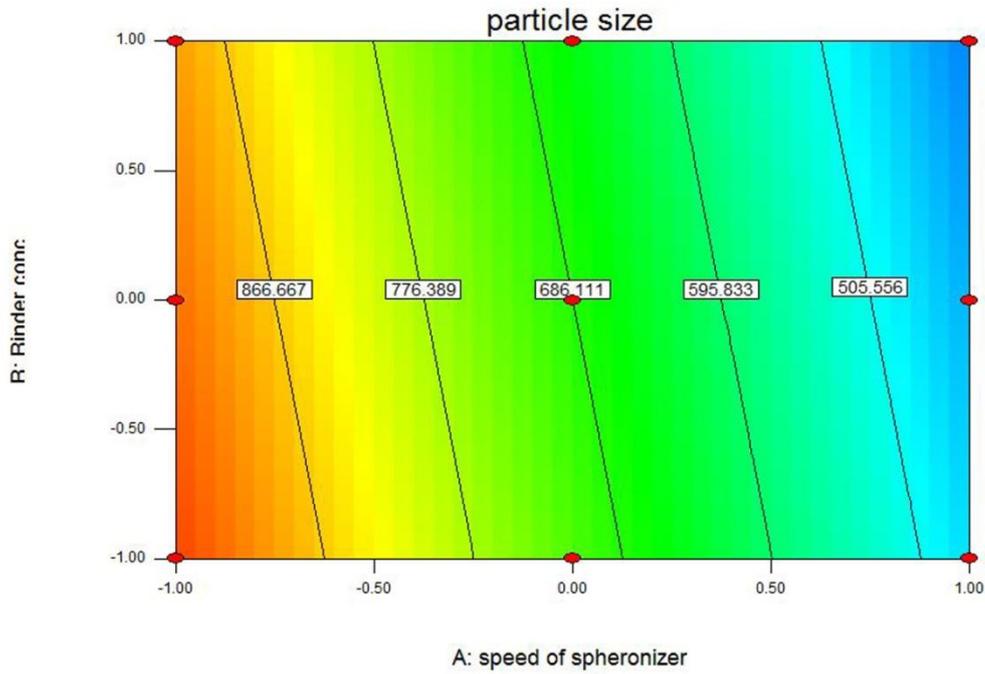
Optimization of clopidogrel bisulphate pellets using design of Experimentation (DOE)

Effect of spheronization speed (X₁) and PVP K30 concentration (X₂) on pellet size, disintegration and *In vitro* dissolution was studied using 3² full factorial design. Table 1 shows the data for independent variables and responses for nine experimental trials. Mathematical relationship between the independent variables and responses was generated using Design Expert software (Stat Ease, version 9.0, USA). The polynomial equations were obtained for the observed responses and are given as equation 1, 2 and 3. Equation 1, 2 and 3 represented mathematical equations showing effect of variables, X₁ and X₂, on Pellet size, disintegration and drug release after 30 min, respectively.

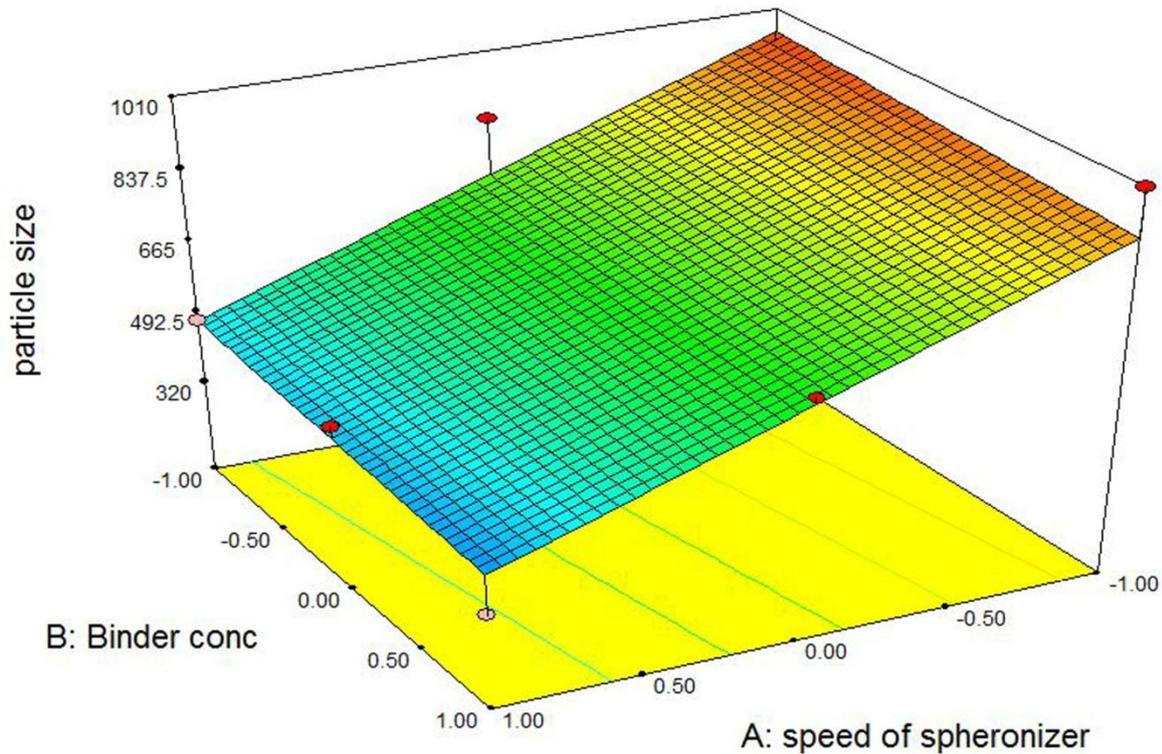
$$Y_1 (\text{Avg pellet size}) = +68.6 - 240.4 X_1 - 30.4 X_2 \dots \dots \dots 1$$

$$Y_2 (\text{disintegration time}) = +54.4 + 1.3 X_1 + 3.8 X_2 \dots \dots \dots 2$$

Y_3 (Drug release after 30 min) = $+96.7 - 0.1 X_1 - 7.6 X_2 + 0.06 X_1 X_2 + 1.4 X_1^2 - 7.1 X_2^2$3
 Equations obtained for particle size (Y_1) and disintegration
 Effect on pellet size



a



b

Figure 3: a) Contour plot b) Response surface area showing influence of binder concentration and speed on particle size of formulation.

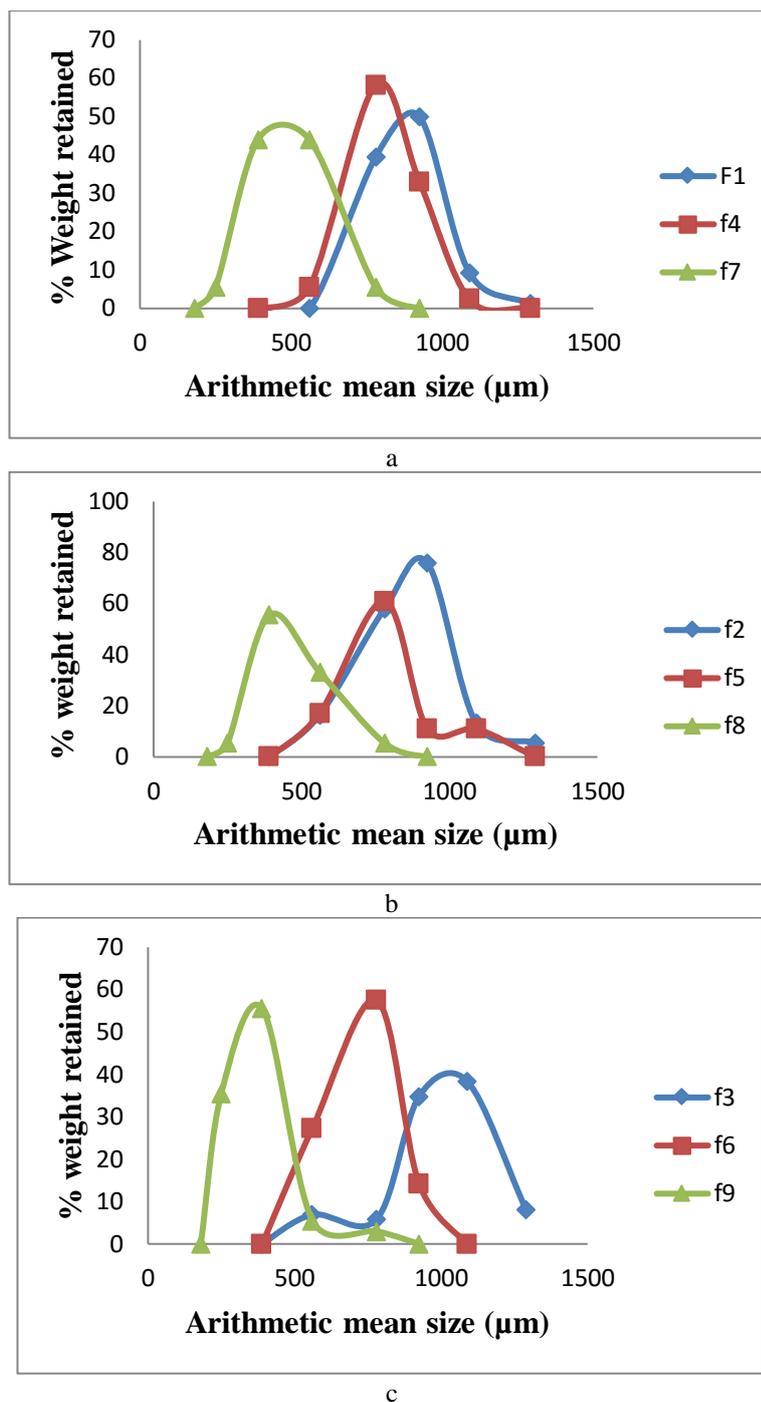


Figure 4: Particle size distribution analysis of Clopidogrel bisulphate pellets (a) for batches F1, F4, F7 (b) for batches F2, F5, F8 (c) for batches F3, F6, F9.

time (Y_2) were linear equations in which interactive and quadratic terms were present indicating that the independent variables did not reveal any synergistic or antagonistic effect.

The significance of the model was estimated by applying ANOVA followed by student t-test. The significance of the model was confirmed from higher F values and lower p values. The model and terms were considered significant when $p > F$ value was less than 0.05. Table 5 indicates the data for ANOVA. For response Y_1 (pellet size), the effect

of spheronizer speed was found to be significant as evident from the p value (0.0028) whereas the effect of binder was not significant on average pellet size.

In case of disintegration (Y_2), both the variables, spheronization speed and binder concentration, exerted significant effect (p value, 0.0048 and 0.0001 respectively).

For drug release after 30 min (Y_3), the coefficient of X_1 (spheronization speed), X_1X_2 (interactive term), and X_1^2 (quadratic term) were not significant due to higher p value.

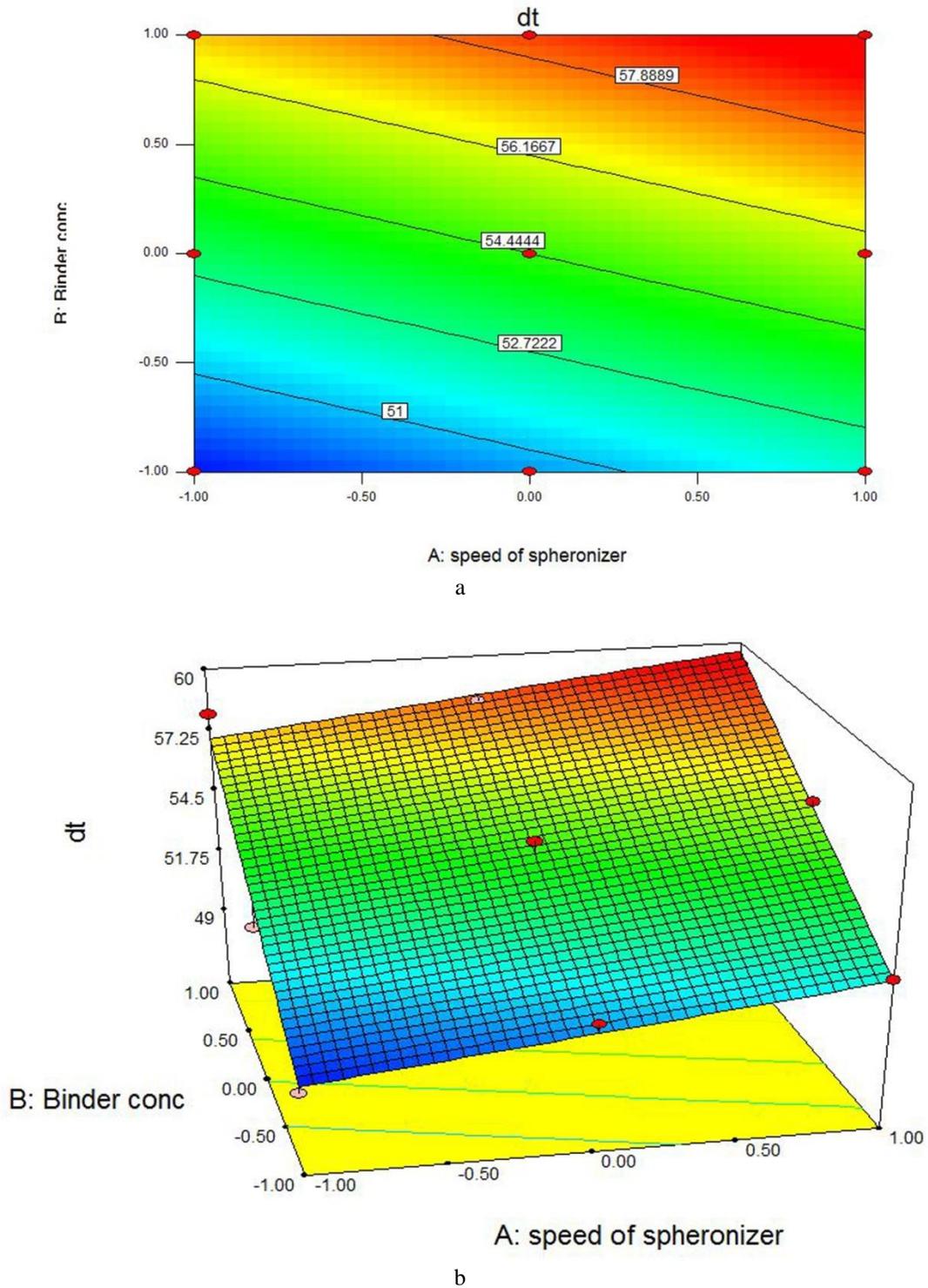


Figure 5: a) Contour plot b) Response surface area showing influence of binder concentration and speed on disintegration time of formulation.

Presence of significant term X_2^2 indicated nonlinearity of effect.

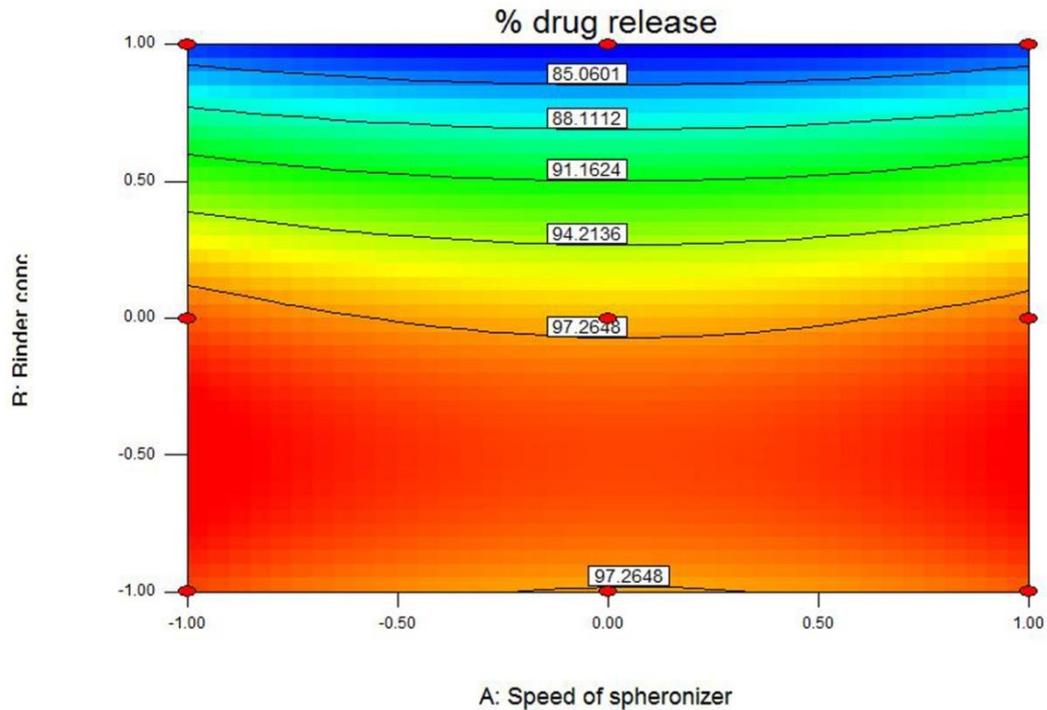
Response surface analysis

The effects of independent variables were further verified by response surface plots.

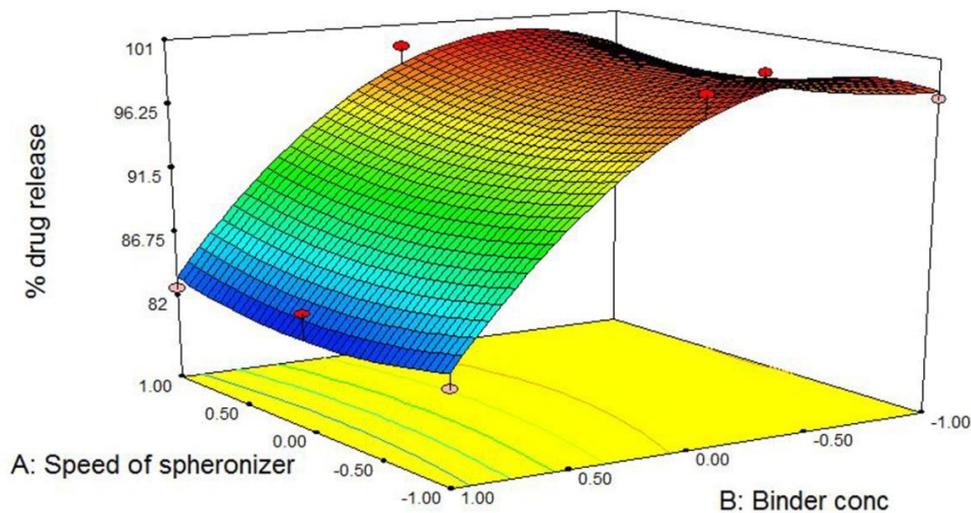
The response plot and counter plot in fig 3 indicate a relative effect of increase in speed and binder

concentration on average size of pellets. The pellet size distribution curves are indicated in fig 4.

Spheronizing speed exerted significant effect on mean size and size distribution of pellets. At lower spheronization speed i.e. 900 rpm, formation of larger size pellets with no sphericity was observed (1290 to 561 μm). At higher speed (1100 to 1300 rpm), there was formation of spherical pellets with smaller size range (1090 to 250 μm). The



a



b

Figure 6: a) Contour plot b) Response surface area showing influence of binder concentration and speed on disintegration time of formulation.

evolution of shape and size of the pellets during spheronization is a complex process. During spheronization, the cylindrical extrudates collide with each other, friction plate and the wall, break into short length and undergo plastic deformation resulting into round cylinders which further changes to dumb bells, ellipsoid and finally a sphere. During dumb bell phase, fines produced due to attrition of extrudates, attach in the waist region of pellet resulting in formation of larger spherical pellets. At lower speed of spheronization, there was lower attrition and excessive agglomeration during growth phase

producing pellets of larger size with no sphericity. At higher speed of 1300 rpm, excessive attrition lead formation of more number of fines resulting in uneven size distribution and presence of small particles in size distribution curves is evident. At 1100 rpm more uniform distribution of size was achieved.

When binder concentration was increased, particle size distribution was narrower than binder concentration at low concentration. Thus, higher binder concentration (5%) and 1100 rpm was suitable to obtain spherical shaped pellets with narrow size distribution.

Effect on disintegration time

The response plot and counter plot in fig 5 indicate a relative effect of increase in speed and binder concentration on disintegration time of pellets.

Disintegration was affected significantly by binder concentration. As binder concentration was increased, the hardness of resulting pellets was increased leading to higher disintegration time. There was no significant effect

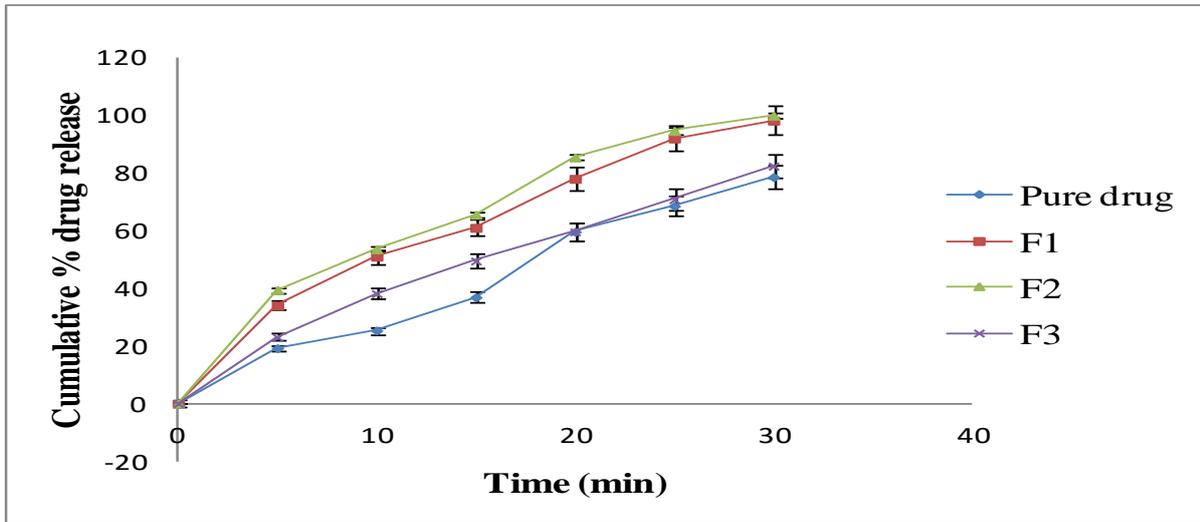


Figure 7: Drug release profile of clopidogrel bisulphate DOE formulations (F1-F3).

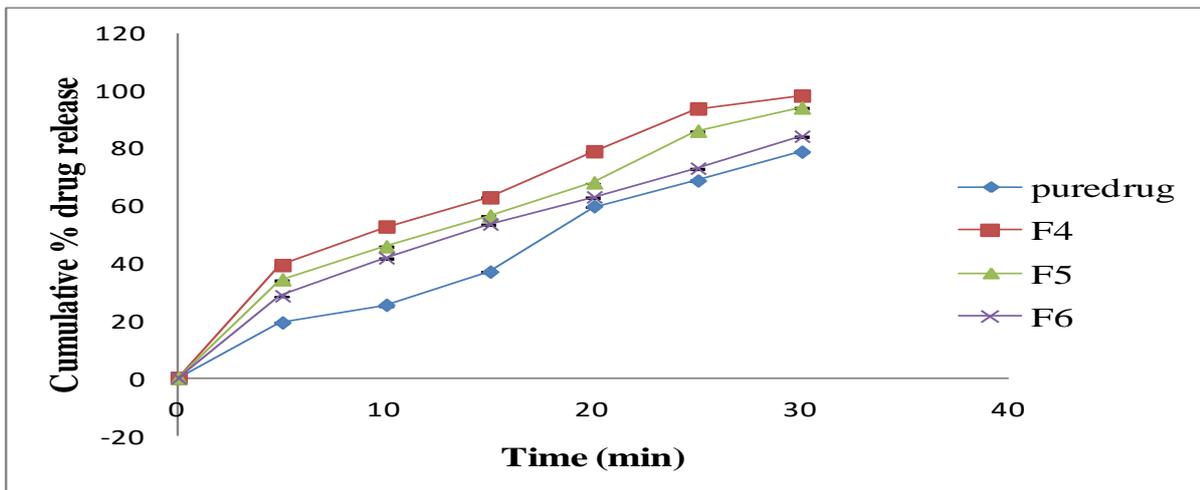


Figure 8: Drug release profile of clopidogrel bisulphate DOE formulations (F4-F6).

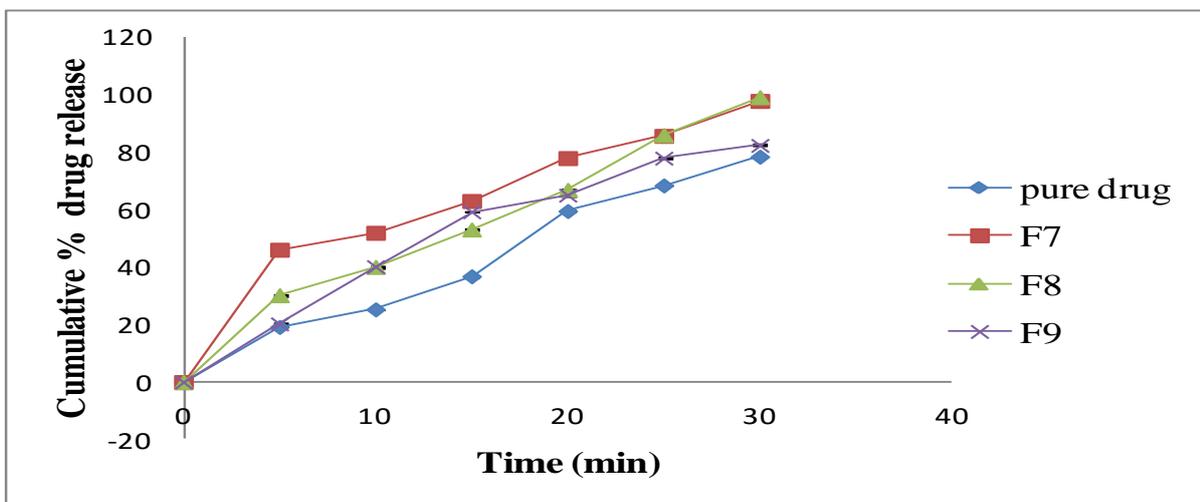


Figure 9: Drug release profile of clopidogrel bisulphate DOE formulations (F7-F9).

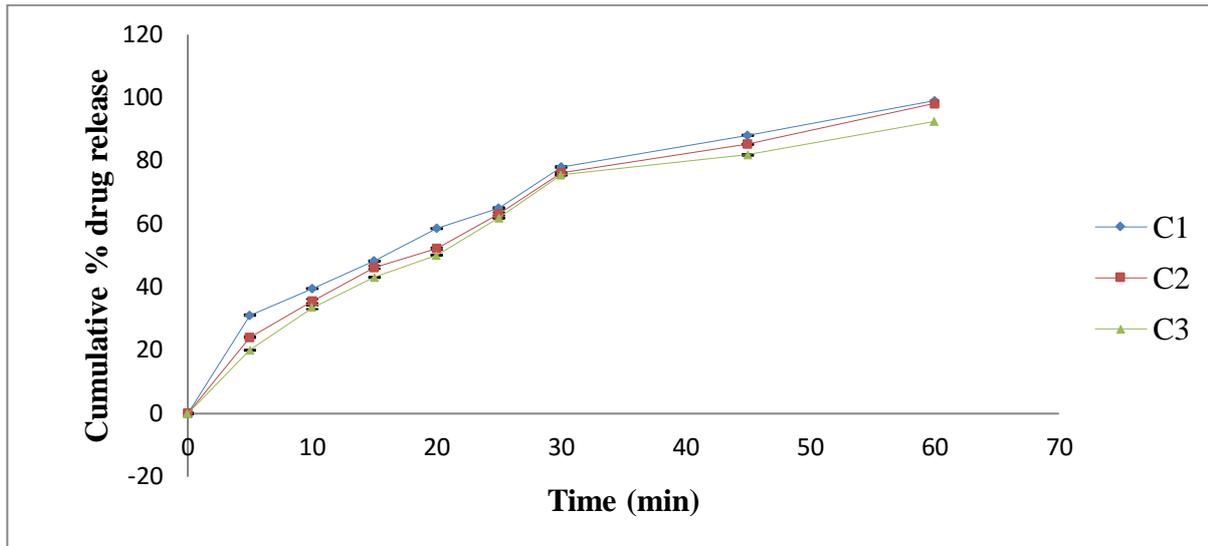


Figure 10: Drug release profile of cilostazol pellets.

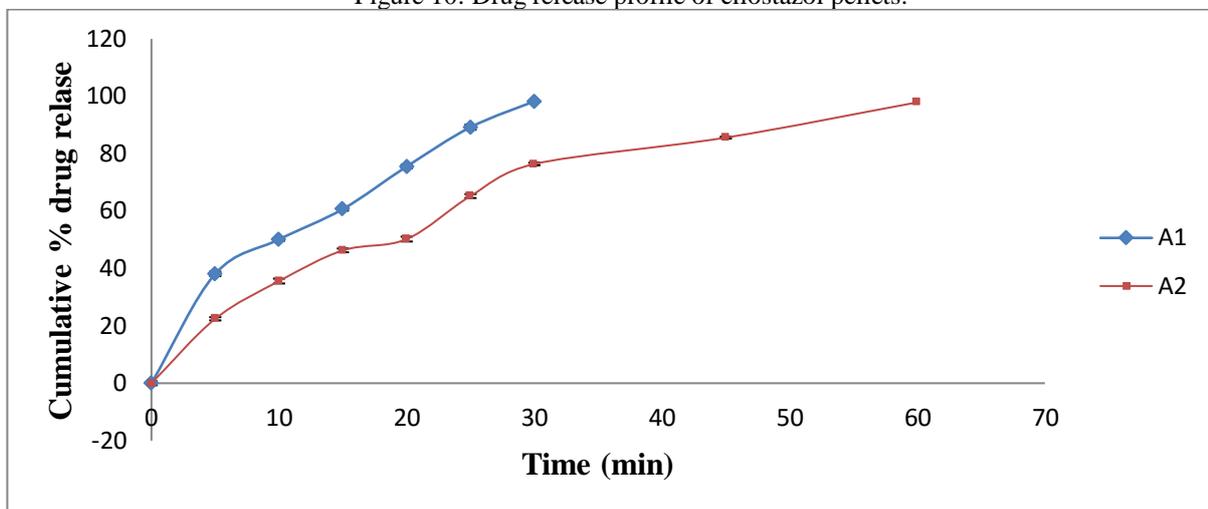


Figure 11: Drug release profile of coated clopidogrel bisulphate and cilostazol pellets in fixed dose combination.

Table 6: Physical characterization of Cilostazol pellet formulations.

Formulation code	C1	C2	C3
% yield	91	90	85
B.D	0.65±0.35	0.64±0.23	0.60±0.89
T.D	0.75±0.65	0.69±0.36	0.65±0.65
Carrs index	7.15±0.58	7.25±0.65	7.692±0.75
Hausners ratio	1.07±0.69	1.078±0.78	1.08±0.65
Angle of repose	28.30°	26.41 °	29.40 °
Hardness	0.468±0.26	0.789±0.65	0.989±0.78
Disintegration Time (sec)	49±0.65	55±0.85	58±1.02

of spheronization speed on disintegration, a slight increase in disintegration was observed with increase in spheronization speed.

Effect on drug release

The response plot and counter plot in fig 6 indicates a relative effect of increase in binder concentration and the speed on drug release after 30min of dissolution from the pellets.

Binder concentration had significant effect on drug release, as binder concentration was increased drug released was

decreased significantly. This could be attributed to higher hardness and larger pellet size obtained at higher binder concentration. Spheronizer speed did not have much significant effect on drug release. With increase in spheronization speed, size of pellets decreased that could have lead to increase in drug release. Similar results were evident from polynomial equation. The effect was found to be nonlinear as evident from the presence of quadratic term. The interactive effect of two variables was not significant on drug release.

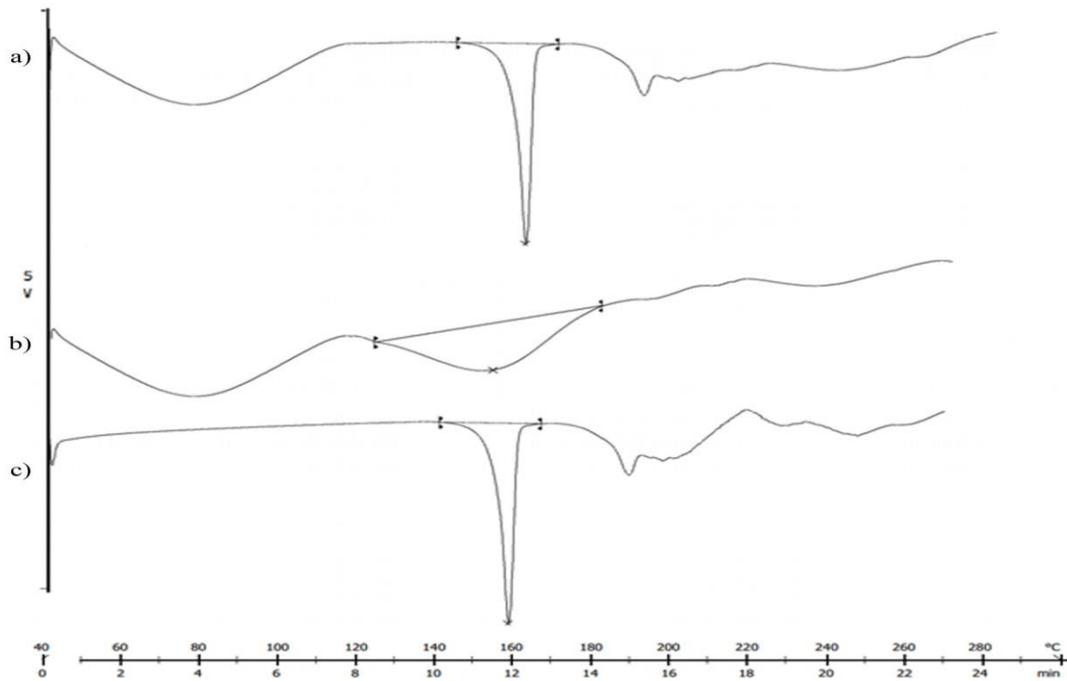


Figure 12: DSC Graph of (a) Clopidogrel bisulphate pure drug (b) Physical mixture (c) Clopidogrel bisulphate Formulation.

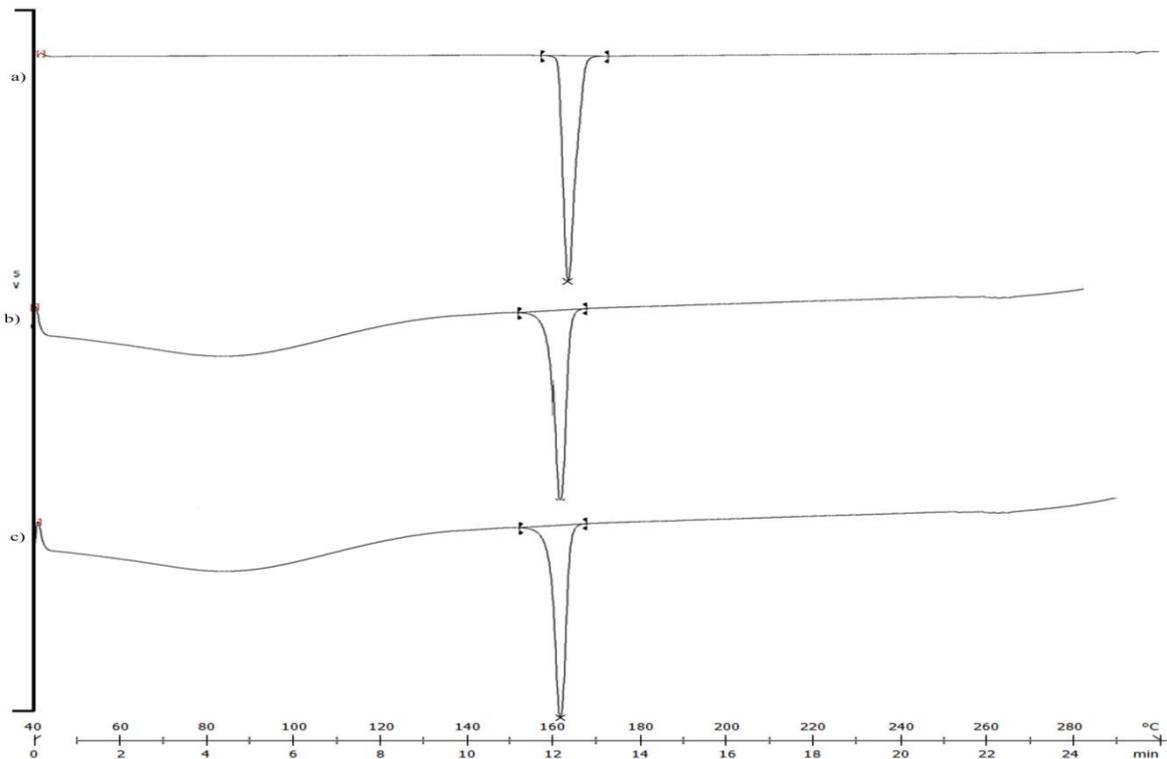


Figure 13: DSC Graph of (a) Pure Drug Cilostazol (b) Physical mixture (c) Cilostazol Formulation.

As per USPXXIV, the immediate release formulation of clopidogrel bisulphate should release not less than 80% of the stated amount of drug within 30min. From the

dissolution of DOE batches (fig 7, 8 and 9), it is evident that all the batches released more than 80% of drug within 30 min. Thus, all the formulations complied with the

Table 7: stability study of pellet formulation of Clopidogrel bisulphate and Cilostazol.

Days	Appearance	% Drug content		% drug release	
		Clopidogrel bisulphate	Cilostazol	Clopidogrel bisulphate	Cilostazol
Before storage					
0 day	White coloured round shaped pellets	98.99±0.60	99.02±0.70	93.91±0.15	98.23±0.15
After storage					
30 days	No change in colour or shape	96.89±0.92	98.85±0.75	90.65±0.65	96.56±0.53

pharmacopoeal limits. The drug release from pellet formulations was found to be higher as compared to that of pure drug.

Formulation and evaluation of Cilostazol pellets

Based on the results of clopidogrel pellets, spheronization speed of 1100 rpm and binder concentration of 5 % was selected in order to formulate cilostazol pellets. Table 6 indicates evaluation data for cilostazol pellets. The yield and flow properties of cilostazol pellets were found to be excellent. As binder concentration was increased, hardness of pellets was increased leading to increase in disintegration time.

In vitro dissolution studies of Cilostazol were carried out in 0.3% SLS in 900ml water at rpm of 50 using type II dissolution test apparatus. The results are indicated in fig 10. With increase in binder concentration, drug release was found to be decreased. As per USPXXIV, the immediate release formulation of cilostazol should release not less than 75% of the stated amount of drug within 30min and 80% of stated amount within 60 min. From the dissolution of cilostazol batches, it is evident that all the batches released more than 80% of drug within 60 min. Thus, all the formulations comply with the pharmacopoeal limits.

In vitro drug release from Clopidogrel and cilostazol film coated pellets in a fixed dose combination

Fig 11 indicates drug release profile from pellet formulations. After film coating, no significant change was observed in drug release from Clopidogrel bisulphate and cilostazol coated pellets as compared to uncoated pellets. The drug release was complying with the USP limits.

Differential Scanning Calorimetry

In order to observe the changes occurred in the Clopidogrel bisulphate and Cilostazol formulation during pelletization, the DSC study was conducted. Fig 12 and 13 indicate DSC of drug (Clopidogrel bisulphate and Cilostazol), their physical mixture PVP K30 and formulation of pellets. The crystalline nature of Clopidogrel bisulphate and Cilostazol is evident by the sharp endotherm representing melting point at a 158°C of Clopidogrel bisulphate and 161°C of cilostazol. There was no change in the endotherm formulation which indicated no interaction.

Stability Study

Stability studies were carried out as per ICH guidelines at 40°C ± 2°C/75% ± 5% RH for the selected formulation (DOE) for 1month. After specified time intervals, parameters like physical appearance, %drug content, % Drug release, were evaluated and results are depicted in table 7. There were no major changes were observed in drug content and drug release from pellet formulation. This

indicated stability of the formulations for the period of testing.

CONCLUSION

Extrusion spheronization was found to be a suitable technique for formulation of immediate release Clopidogrel bisulphate and Cilostazol pellets. The spheronization speed and binder concentration were found to affect the average size, disintegration and dissolution of pellets. The formulation with optimized amount of binder concentration and super disintegrating agent were found to possess lower disintegration time (less than one minute) and released more than 90% of drug within 30 min of dissolution time for both the drugs and thus complied with the pharmacopoeal limits. FTIR and DSC study indicated no interaction between the drug and excipient during pellet formulation. There was no change in the drug release profile of coated pellets of both the drugs (clopidogrel bisulphate and cilostazol) as fixed dose combination (75 mg:100 mg) than when studied as a single formulation. The formulations were found to be stable after 2 months stability study. Conclusively, clopidogrel bisulphate and cilostazol pellet fixed dose combination could be successfully developed by design of experimentation and complied with pharmacopoeal limits.

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SOURCE OF SUPPORT

Nil

CONFLICT OF INTEREST

None

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