Original Research Article

Formulation of cilostazol spherical agglomerates by crystallo-co-agglomeration technique and optimization using design of experimentation

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Abstract

Introduction: Spherical agglomeration is one of the novel techniques for improvement of flow and dissolution properties of drugs. Cilostazol is a biopharmaceutics classification system Class II drug with poor solubility resulting in limited bioavailability. The present study aims at improving the solubility and dissolution of cilostazol by crystallo-co-agglomeration technique.

Materials and Methods: Cilostazol agglomerates were prepared using various polymers with varying concentration of hydroxypropyl methylcellulose E 50 (HPMC E50), polyvinyl pyrrolidone K30 (PVP K30), and polyethylene glycol 6000. The influence of polymer concentration on spherical agglomerate formation was studied by 3^2 factorial design. Cilostazol agglomerates were evaluated for percent yield, mean particle size, drug content, aqueous solubility, and *in vitro* dissolution and further characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD).

Results: The agglomeration process resulted in optimized formulation, F3 with mean agglomerate size of 210.0 ± 0.56 µm, excellent flow properties, approximately 15-fold increase in solubility than pure cilostazol and complete drug release in 60 min. Process yield, agglomerate size, and drug release were affected by amount of PVP K 30 and HPMC E50. The presence of drug microcrystal was confirmed by SEM, whereas FTIR study indicated no chemical change. Increase in drug solubility was attributed to change of crystalline drug to amorphous form that is evident in DSC and XRD.

Conclusion: Crystallo-co-agglomeration can be adopted as an important approach for increasing the solubility and dissolution of poorly soluble drug.

Keywords: Factorial design, platelet aggregation inhibitor, solubility enhancement, spherical agglomerates, spherical crystals

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INTRODUCTION

Cardiovascular disease is a number one cause of death globally and represents 31% of the total global deaths. Out of these deaths, approximately, 40% are due to coronary heart diseases.[1,2] Now, it is well accepted that one of the major causes in the development of coronary heart disease

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is platelet hyperactivity. As a result, platelet aggregation inhibitors are gaining importance in the prevention and treatment of recurrent ischemic stroke.[3]

Cilostazol is an FDA-approved platelet aggregation inhibitor that is used in patients with peripheral arterial disease, intermittent claudication, and for the secondary prevention of brain infarction.[4] Cilostazol is cyclic adenosine monophosphate (cAMP) phosphodiesterase III inhibitors (PDE III inhibitors), inhibiting PDE activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels which leads to inhibition of platelet aggregation and vasodilation.[5] Recent studies have shown the safety and efficacy of cilostazol over aspirin for long-term prevention of recurrence of ischemic stroke and found to be associated with fewer hemorrhagic events.[6]

According to biopharmaceutics classification system, cilostazol is classified as Class II drug with poor solubility and high permeability. Poor solubility of drug results in its poor bioavailability, thus posing a challenge to formulator during dosage-form development.[7] To get the desired bioavailability, solubility enhancement of poorly soluble drug is essential before its direct compression. Few approaches of cilostazol solubility enhancement such as inclusion in cyclodextrin complex,[8] particle size reduction by nanocrystal,[9] and supercritical anti-solvent process[10] had been reported in the literature. However, these approaches are either costly or did not show significant solubility enhancement. Spherical agglomeration is a novel particle engineering technique that can satisfy the requirements for direct compression as well as solubility enhancement.[11]

The spherical agglomeration technique has been used as an effective particle design technique by Kawashima *et al*.[12] Initially, this technique was used for improving flowability, compressibility, and other flow properties of powder. Later, polymers were introduced in this system to modify the drug release by enhancing the solubility of poorly soluble drug.[13,14] In spherical crystallization process, crystal formation, growth, and agglomeration occur simultaneously within the same system. Bridging liquid is added to this system to promote formation of agglomerates. Crystallo-co-agglomeration is a modified agglomeration technique developed to overcome the limitations of spherical crystallization. It is used for size enlargement of low dose, high dose, poorly compressible drugs, and combination of drugs with or without diluents.[15] Currently, this technique is used more frequently for solubility enhancement of poorly soluble or water-insoluble drugs in addition to improving flow properties and compressibility.[16] In this technique, drug is directly crystallized and agglomerated in combination with an excipient or with another drug with the help of bridging liquid. To improve the flow properties and dissolution characteristics, crystallo-co-agglomeration technique has been used previously for ibuprofen,[15] naproxen,[17] racecadotril,[18] bromhexine hydrochloride,[19] gliclazide,[20] etc.

In the present study, an attempt has been made to enhance the solubility and dissolution of poorly soluble cilostazol by formulation of its spherical agglomerates using crystallo-co-agglomeration technique. The formulation of spherical agglomerates was optimized using 3^2 factorial design. Resulting agglomerates were evaluated for percent yield, mean particle size, flow properties, surface morphology by scanning electron microscopy, thermal behavior by differential scanning calorimetry, spectral characterization by Fourier transform infrared spectroscopy (FTIR), drug content, crystallinity by X-ray diffraction (XRD), drug content, solubility analysis in dissolution media and water, and *in vitro* drug release.

MATERIALS AND METHODS

Materials

Cilostazol was obtained as a gift sample from Amsal Chem Pvt. Ltd. Ankleshwar, Gujarat, India. Hydroxypropyl methylcellulose E 50 (HPMC E50) and polyvinyl pyrrolidone K30 (PVP K30) were gifted by Colorcon, Mumbai, India. Polyvinyl alcohol and polyethylene glycol 6000 (PEG 6000) were purchased from Loba Chemie, Mumbai, India. All the solvents used in the study were of analytical grade.

Methods

Selection of solvent

The solvents with different polarity were screened for solubility of cilostazol. An excess amount of cilostazol was added to each of selected solvent. The saturated solutions were kept in cryostat constant temperature orbital shaker (Remi, CIS 24 BL, India) at 25°C ± 0.1°C with constant shaking at 100 rpm. After 72 h, the samples were removed and centrifuged at 1000 rpm for 15 min. The supernatant was appropriately diluted with mobile phase, and the concentration of drug in each sample was determined by previously developed HPLC method. The HPLC system consisted of Agilent compact LC model 1120 and a (Photodiode Array) PDA detector set to 237 nm. Isocratic chromatography was used, and the separation was achieved using analytical column Spincotech (C18 reverse-phase,

250 mm × 4.6 mm i.d., 5 µ size, India). The mobile phase used was 0.5 M phosphate buffer: Acetonitrile: Methanol (20:40:40) with flow rate of 1.0 ml/min at room temperature. Detection was done by UV detector at 237 nm.[21]

Preparation of cilostazol spherical agglomerates

The spherical agglomerates of cilostazol were prepared by crystallo-co-agglomeration technique.[15,16] Based on the results of solubility study, dichloromethane (DCM) was selected as solvent for cilostazol. Water was used as anti-solvent, chloroform as bridging liquid, and HPMC E50, PVP K30, and PEG 60000 as polymers. PVP K30 (at different concentration, viz., 0.5, 1.0, and 1.5 g) and PEG 60000 (0.25 g) were dissolved in distilled water (100 ml) and temperature was maintained at 4°C ± 1°C. Cilostazol (1.0 g) and HPMC E50, at varying concentration (0.05, 0.075, and 0.1 g), were dissolved in DCM (5 ml). Drug solution was added to the polymer solution at 4°C ± 1°C under constant stirring at 700 rpm using a mechanical stirrer (Remi, RQ122/D, India). To avoid the drug loss during agglomerate formation, the polymer solution was previously saturated with drug. The stirring was continued until formation of agglomerates, which were then filtered and dried overnight at room temperature. The agglomerates were evaluated for percent yield, size, drug content, flow behavior, aqueous solubility, *in vitro* dissolution, and solid state characterization by scanning electron microscopy, FTIR, differential scanning calorimetry, and XRD.

Experimental design

The formulations of spherical agglomerates were optimized using two factors and three levels (3^2) full factorial design [Table 1]. Main and interaction effects of the formulation variables, amount of HPMC E50 (X_1) and amount of PVP K30 (X_2), on percent yield (Y_1) and mean particle size (Y_2) of agglomerates were studied using this experimental design.[18] Multiple linear regression analysis was used to generate mathematical relationship for factors and responses and the surface response and counterplots were obtained (Design-Expert, version 9.0, STAT-EASE, USA).

Evaluation of spherical agglomerates

Percent yield and drug content

Percent yield of prepared agglomerates was calculated using following formula.

$$\% \text{ yield} = \frac{\text{Total weight of agglomerates}}{\text{Total weight of drug and excipients}} \times 100$$

Drug content was calculated as ratio of experimentally measured amount of drug to theoretical value and expressed in percentage. The samples were randomly taken from the prepared batch from three different positions and triturated in mortar and pestle. Powder (100 mg) was weighed and dispersed into 100 ml of methanol and sonicated for 20 min. The resultant solution was then filtered through Whatman filter paper and further diluted with methanol. Drug content was spectrophotometrically determined (Shimadzu, 1700, Japan) at 258.0 nm.[13]

Micromeritic properties of agglomerates

The mean particle size and size distribution for pure drug and prepared agglomerates was determined using optical microscopy (Lawrence and Mayo, India).

Bulk density (BD) and tapped densities (TD) were determined using BD apparatus. Carr's index (CI) (%) and Hausner's ratio (HR) were then calculated using bulk and TD. Angle of repose (AR) for pure drug and prepared agglomerates was determined using fixed funnel method.[22,23]

Scanning electron microscopy

Surface morphology of agglomerates was studied using field emission scanning electron microscope (FEI, Nova Nano Scanning electron microscopy 450 [SEM 450], USA). Before estimation, sample was coated with 20-nm thin platinum layer by auto fine coater to render them electrically conductive. Afterward, the stubs containing the

Table 1: Data of dependent variables for spherical agglomerates of cilostazol

Batch number	HPMC E-50 (g) X_1	PVP K-30 (g) X_2	Yield (%) Y_1	Mean particle size (µm)* Y_2	Drug content (%)*	Solubility in 0.3% SLS solution (mg/ml)*
F1	0.050	0.5	53.8	209.2±0.87	81.56±0.74	0.81±0.15
F2	0.050	1	57.8	210.1±0.75	83.07±0.65	0.94±0.18
F3	0.050	1.5	55.2	210.0±0.56	82.04±0.60	0.98±0.10
F4	0.075	0.5	46.0	209.8±0.68	96.80±0.73	0.54±0.013
F5	0.075	1	45.6	211.0±0.49	94.22±0.87	0.53±0.16
F6	0.075	1.5	45.6	212.1±0.65	97.31±0.94	0.49±0.10
F7	0.100	0.5	36.0	198.4±0.95	80.85±1.02	0.57±0.08
F8	0.100	1	35.0	201.2±1.01	92.03±0.82	0.50±0.15
F9	0.100	1.5	34.3	195.7±0.98	95.32±0.56	0.48±0.11
Pure drug	-	-	-	46.6±75	-	0.06±0.03

*All values are expressed as mean±SD. SD: Standard deviation, HPMC: Hydroxypropyl methylcellulose, PVP: Polyvinyl pyrrolidone, SLS: Sodium lauryl sulfate

coated samples were placed in the field emission scanning electron microscope chamber. The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 15–18 kV. To study effect on surface morphology, the agglomerates were observed at different magnification.[24]

Fourier transform infrared spectroscopy spectroscopy

To study the changes occurred during agglomeration process, FTIR spectra of cilostazol, its physical mixture with excipients and optimized spherical agglomerate formulation was recorded by KBr pellet method (Shimadzu, 8400S, Japan). FTIR spectra were recorded over the range of 400–4000 cm^{-1}.[25]

Differential scanning calorimetry

The possibility of any interaction between cilostazol and the other excipients during the spherical agglomeration process was assessed by carrying out the thermal analysis of cilostazol, physical mixture of polymer drug, and agglomerate using differential scanning calorimetry (Hitachi, DCS7020, Japan). The sample was heated from 40°C to 240°C at the rate of 10°C/min. The inert atmosphere was maintained by purging gas throughout the experiment at the rate of 40 ml/min. The sample (1–4 mg) was carefully transferred and heated in a crimped aluminum pan for accurate results. The obtained thermographs were used to decide any interaction between cilostazol and polymer.[13]

X-ray diffraction studies

XRD study was performed using X-ray diffractometer (Brucker, D8Adavnced, USA) using Cu K β-rays with a voltage of 40 kV and current of 40 mA. Samples were scanned for 2 θ from 10° to 80° using 15°/min scanning speed.[8] The diffraction patterns for cilostazol, physical mixture, and agglomerate were obtained.

Solubility analysis

To observe the change in solubility of drug after crystallo-co-agglomeration, the saturation solubility of selected agglomerate in water formulation was determined.[26] Sample was weighed and added in increments to 2 ml of dissolution medium (0.3% sodium lauryl sulfate [SLS] solution) and distilled water until saturation. The saturated solution was placed in orbital shaker (Remi, CIS 24 BL, India) for 48 h at 25°C ± 0.5°C. After 48 h, samples were centrifuged in laboratory centrifuge (Remi, R-8C, India) at 1000 rpm for 30 min. The resultant solution was then filtered, appropriately diluted and drug content in the solution was determined by UV spectrophotometry at 258.0 nm.

In vitro dissolution study

Drug release study of pure drug and prepared optimized batch of agglomerates was studied by *in vitro* dissolution using USP type II apparatus (Veego, VDA-6D, India).[26] Dissolution studies were carried out using 900 ml of 0.3% SLS as dissolution medium at 37°C ± 0.5°C, with stirring speed of 50 rpm (as mentioned in USP monograph). Aliquots of 1 ml were withdrawn after every 10 min and replaced with the same of fresh dissolution medium. Aliquots were diluted appropriately and analyzed by UV spectrophotometer at 258 nm. Cumulative percent drug release from agglomerates was calculated using PCP dissolution software (PCP Disso, v3, India).

RESULTS

Selection of solvent

To select a good solvent, solubility of cilostazol was determined in various solvents. Figure 1 shows solubility of Cilostazol in DCM, ethanol, methanol, N-methyl-2-pyrrolidone, and acetone.

Optimization by design of experiments

The evaluation of spherical agglomerates was conducted for various evaluation parameters and the results are depicted in Tables 1 and 2. The flow property of spherical agglomerates was found to be in the acceptable range (AR, 23.2–27.7; CI in the range of 12.25–22.19 and HR as 1.11–1.28). The drug content of spherical agglomerates was found to be in the range of 80.85 ± 1.022–97.31 ± 0.94%.

The results of dependent variables, % Yield (Y_1) and Mean particle size (Y_2) from nine experiments are shown in Table 1. Mathematical relationships generated for the studied response variables are expressed in the equations 1 and 2.

$$\%\text{Yield}(Y_1) = +45.53 - 10.24*X_1 - 0.13*X_2 \quad (1)$$

$$\text{Mean particle size}(Y_2) = +212.09 + 0.050*X_1 - 5.67*X_2 - 0.90*X_1X_2 - 1.56*X_1^2 - 6.89*X_2^2 \quad (2)$$

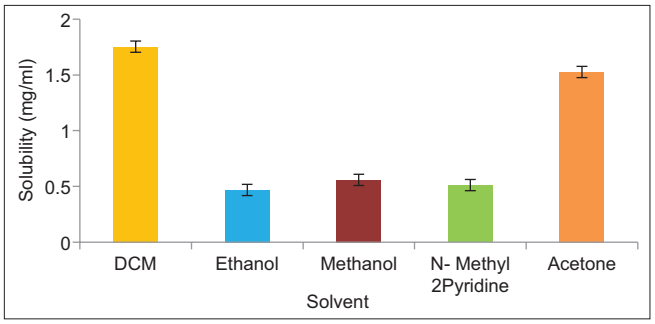

Figure 1: Solubility of cilostazol in different solvents

Table 3 indicates significance values of coefficient for different responses. Coefficients b_2 of linear equation 1 and coefficient b_1, b_{12}, and b_{11} of equation 2 showed high probe values, hence, these terms were removed from the equations and the equations were reduced to 3 and 4.

$$\% \text{ Yield } (Y_1) = +45.53 - 10.24 * X_1 \quad 3$$

$$\text{Mean particle size } (Y2) = +212.09 - 5.67 * X_2 - 6.89 * X_2^2 \quad 4$$

Figure 2a and b indicate counterplots and surface plots representing effect of HPMC E50 and PVP K30 on percent yield of spherical agglomerates. Figure 3a and b indicate counterplots and surface plots showing effect of PVP K30 and HPMC E50 on mean agglomerate size. Figure 4 indicates microscopy images of spherical agglomerates that reveal its spherical shape. Figure 5 indicates particle size distribution curves for formulations F1 to F9.

Scanning electron microscopy

SEM revealed the surface morphology of spherical agglomerates. SEM of cilostazol revealed needle-shaped crystals of drug. In SEM of formulated agglomerates, the small crystal of drug, clustered to exhibit spherical agglomerates, were clearly visible [Figure 6]. The spherical agglomerates were found to exhibit slightly rough surface due to the presence of small drug crystals on surface.

Fourier transform infrared spectroscopy

FTIR spectra of pure cilostazol, its physical mixture with excipients, and spherical agglomerate formulation are represented in Figure 7. For cilostazol, infrared (IR) spectrum showed characteristic absorption band for –N-N-stretch, –C=O-stretch, –C-O-stretch appeared at 3184.5, 1670, and 1043 cm^{-1}, respectively. The major peaks of the drug were also observed in the IR spectrum of spherical agglomerate. The absorption band for –N-N-stretch, –C=O-stretch, and –C-O-stretch appeared at 3184.58, 1668.48, and 1051.24 cm^{-1}, respectively.

Differential scanning calorimetry

To observe the changes occurred in cilostazol during spherical agglomeration process, differential scanning calorimetry (DSC) study was conducted. Figure 8 indicates DSC of cilostazol, its physical mixture with HPMC E50, PVP K30, and PEG 6000 and formulation of spherical agglomerate. A sharp endotherm was observed at 161.7°C representing melting point of cilostazol. DSC of physical mixture indicated decrease in intensity as well as slight shift of endotherm to 158.9°C. The melting point of PVP K30 is 160°C–165°C which is shown by a broad endotherm observed in this range. DSC of formulation indicated endotherm at 161.3°C.

Table 2: Flow properties of cilostazol spherical agglomerates

Batch number	Flow properties				
	BD (g/ml)*	TD (g/ml)*	HR	CI (%)	AR (°)
F1	0.3617±0.026	0.4657±0.032	1.28	22.19	23.38
F2	0.4345±0.031	0.5263±0.029	1.21	17.26	27.64
F3	0.3518±0.027	0.4057±0.030	1.15	13.30	23.28
F4	0.4650±0.020	0.5512±0.025	1.17	15.26	24.71
F5	0.4380±0.030	0.5099±0.026	1.11	14.58	23.13
F6	0.4676±0.020	0.5330±0.028	1.14	12.25	23.16
F7	0.4338±0.036	0.5289±0.026	1.23	18.11	26.34
F8	0.3908±0.023	0.4783±0.023	1.22	18.18	23.83
F9	0.4255±0.028	0.5131±0.025	1.21	16.83	27.77
Pure drug	0.365±0.045	0.4692±0.035	1.28	22.20	28.26

*All values are expressed as mean±SD. SD: Standard deviation, CI: Carr's index, BD: Bulk density, TD: Tapped densities, HR: Hausner's ratio, AR: Angle of repose

Table 3: Significance values (probe value) of response coefficients and results of analysis of variance

Coefficients	Significant values (probe value)	
	Percentage yield (Y_1)	Mean particle size (Y_2)
b_0	0.0001	0.0213
b_1	0.0001	0.9527
b_2	0.8177	0.0052
b_{12}	-	0.4115
b_{11}	-	0.3262
b_{22}	-	0.0140
Model	0.0001	0.0213
r^2	0.9848	0.9653
F	194.68	16.68

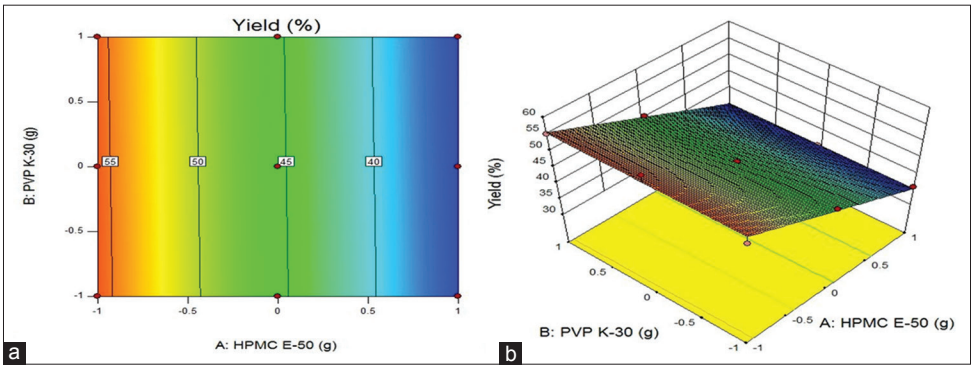

Figure 2: (a) Contour plot showing influence of concentration of hydroxypropyl methylcellulose E50 and polyvinyl pyrrolidone K30 on percent yield. (b) Response surface plot showing influence of concentration of hydroxypropyl methylcellulose E50 and polyvinyl pyrrolidone K30 on percent yield

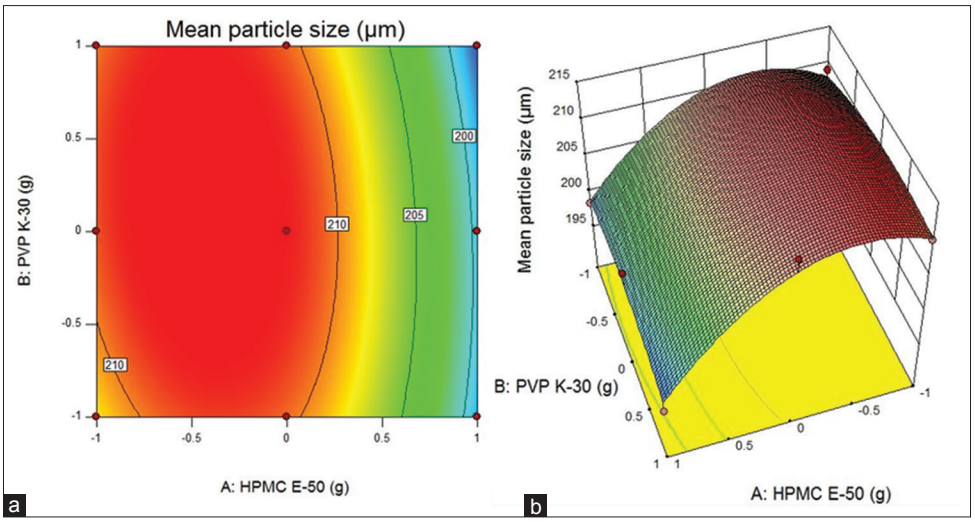

Figure 3: (a) Contour plot showing influence of concentration of hydroxypropyl methylcellulose E50 and polyvinyl pyrrolidone K30 on mean agglomerate size. (b) Response surface plot showing influence of concentration of hydroxypropyl methylcellulose E50 and polyvinyl pyrrolidone K30 on mean agglomerate size

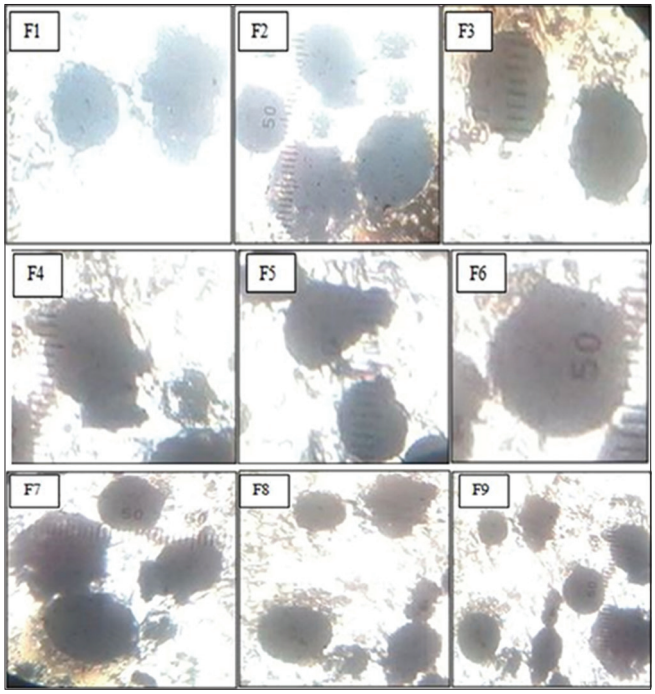

Figure 4: Microscopic image of spherical agglomerate formulations

X-ray diffraction study

To study the changes occurred in crystalline nature of drug, during spherical agglomeration process, XRD patterns of cilostazol, physical mixture of cilostazol with excipients, and agglomerate formulation were obtained [Figure 9]. XRD pattern of pure cilostazol indicated intense peak at 12.62°–13.1°, 14.18°–14.34°, 21.34°–21.58°, 23.32°–23.74°, and 31.5°–31.88°, which were characteristics peaks for cilostazol. XRD of physical mixture retained intense peak of cilostazol and also showed additional intense peaks at 13.1° to 13.4°, and 16.16° to 16.26° representing the presence of other excipients. XRD of formulation indicated reduction in the intensity of cilostazol peaks (12.68° to 13.1°, 14.18° to 14.16°). The increase in intensity for peaks at 24.9° to 25.40° was observed in spherical agglomerate formulation.

Solubility analysis

All the formulations of spherical agglomerates of cilostazol showed high solubility in dissolution medium as compared to pure cilostazol [Figure 10]. The increase in the solubility of agglomerates was found to be 10–15-fold than pure cilostazol in the presence of SLS solution. Formulation F3 indicated highest solubility (0.98 ± 0.1 mg/ml) than other agglomerate formulations.

The solubility of agglomerate formulation F3 and cilostazol was carried out in water and results are shown in Figure 11. The prepared cilostazol agglomerate showed high solubility in water (0.8541 ± 0.09 mg/ml) as compared to pure cilostazol (0.0652 ± 0.03 mg/ml). The increase was approximately 15 fold as compared to drug cilostazol.

In vitro dissolution study

In vitro dissolution of spherical agglomerate formulations was carried out in 0.3% SLS solution as per USP XXIII monograph. *In vitro* release behavior of drug cilostazol and its spherical agglomerate formulations is shown in Figure 12. Spherical agglomerate formulation (F3) indicated complete drug release after 60 min, whereas pure drug, cilostazol exhibited only 33.4 ± 0.28% of drug release after 60 min of dissolution.

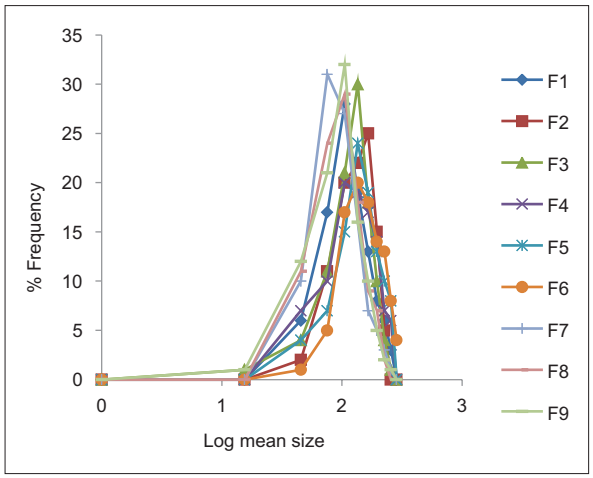

Figure 5: Size distribution curve for spherical agglomerates

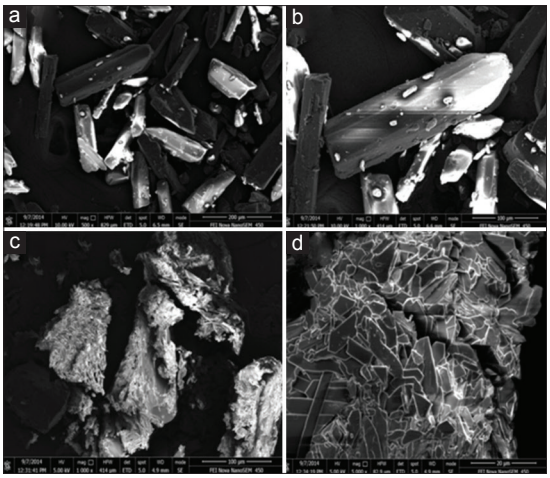

Figure 6: Scanning electron microscopy of agglomerates. (a and b) top view of drug cilostazol, (c and d) top view of spherical agglomerates

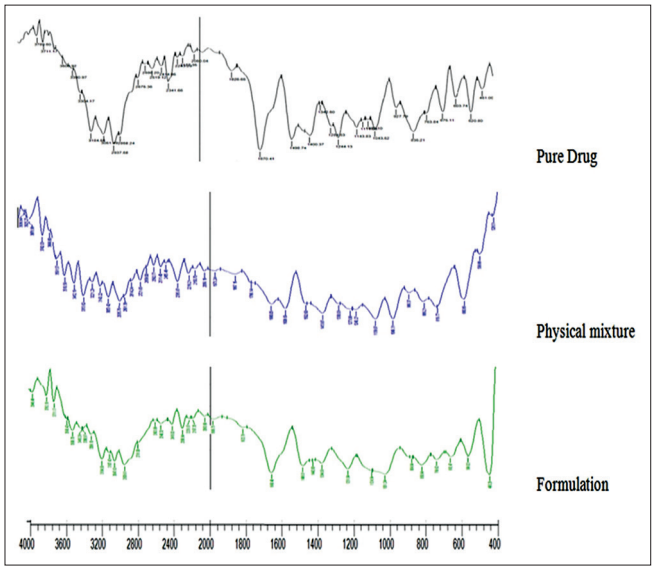

Figure 7: Infrared spectrum of cilostazol, physical mixture, and agglomerate formulation

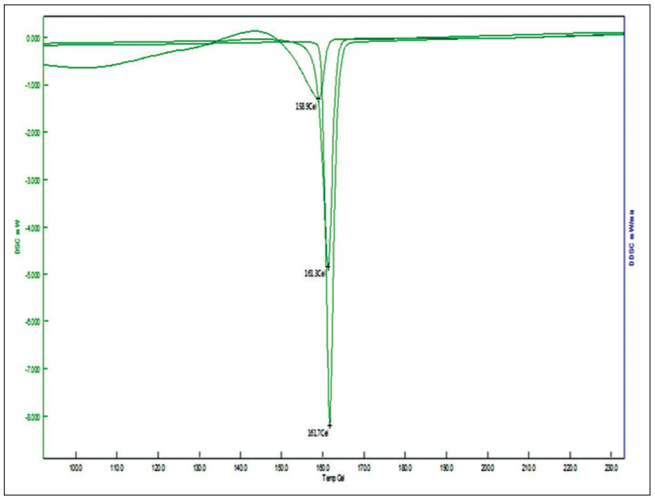

Figure 8: Differential scanning calorimetry thermogram of pure drug, physical mixture, and agglomerate formulation

DISCUSSION

Selection of solvents

Selection of good solvent, poor solvent, stabilizer, and bridging liquid is critical in formulation of spherical agglomerates. In crystallo-co-agglomeration technique, drug dissolved in good solvent is precipitated in the presence of poor solvent, and drug crystals are further agglomerated in the presence of bridging liquid. Selection of good solvent was based on the solubility of cilostazol that showed higher solubility in DCM than other solvents. DCM also acted as bridging liquid that promoted the interaction of microcrystals to give spherical agglomerates. As the main objective of study was to improve the solubility characteristics of cilostazol by co-agglomerating with polymers or excipients, selection of polymer was very critical. Considering the results from literature,[17,23] polymers, HPMC E50, PVP K30, and PEG 6000 were selected for co-agglomeration of drug. From the preliminary experiments, the concentration range for HPMC E50, PVP K30, and PEG 6000 was selected for further optimization of formulation.

Optimization by design of experiments

Effect of concentration of HPMC E50 and PVP K30 on spherical agglomeration process was studied using 3^2 full factorial design. The flow property and compressibility of cilostazol were found to be improved by spherical agglomeration process. The variation in the drug content was observed in spherical agglomerate formulations. This could be due to drug loss during agglomeration process as a result of sticking to the walls of container or incomplete agglomeration. Data from design of experiments indicated strong influence of selected variables on responses, namely,

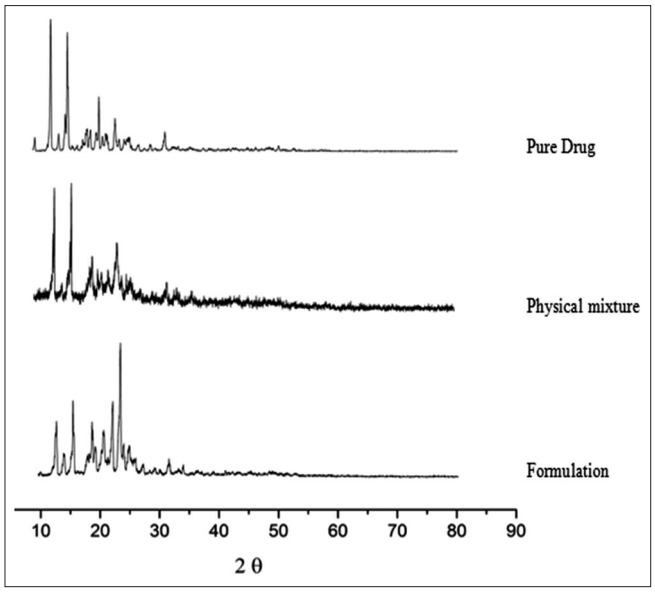

Figure 9: X-ray diffraction pattern for cilostazol, physical mixture, and agglomerates

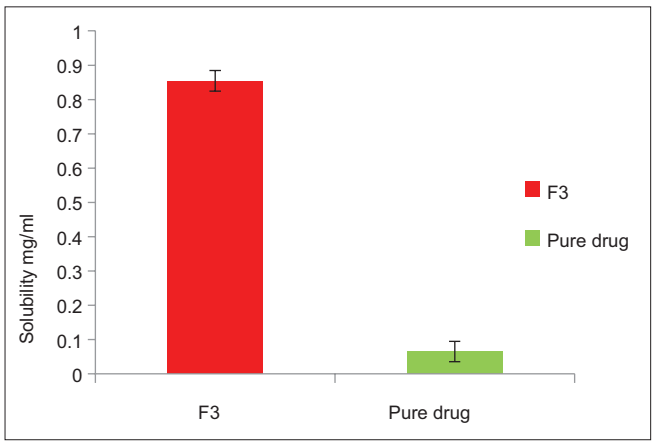

Figure 11: Solubility of pure cilostazol and spherical agglomerate formulations in water

percent yield and particle size. This was confirmed from the results of multiple linear regression analysis followed by ANOVA that indicated higher F values, 194.68 and 16.68 for responses, percent yield, and mean particle size, respectively.

Effect on percent yield

Reduced equation 3 clearly indicated that concentration of HPMC E50 influenced the yield of the spherical agglomerate, whereas concentration of PVP K 30 did not show any effect on yield. The negative coefficient of X_1 in equation indicated decrease in the percent yield with increase in concentration of HPMC E50. This could be attributed to loss of drug and excipients during agglomeration process occurred due to excessive sticking to walls of container, with increase in concentration of HPMC E50. The equation obtained was linear equation.

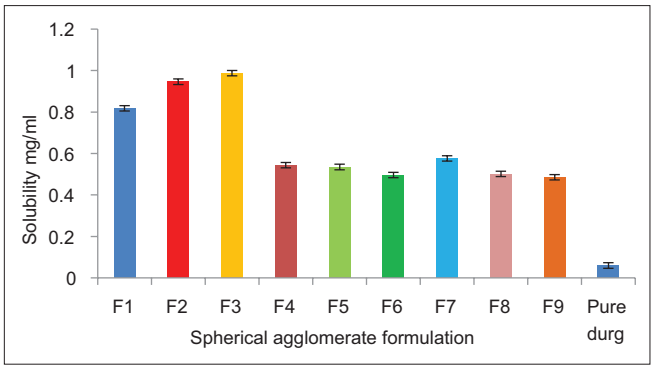

Figure 10: Solubility of pure cilostazol and spherical agglomerate formulations in 0.3% sodium lauryl sulfate solution

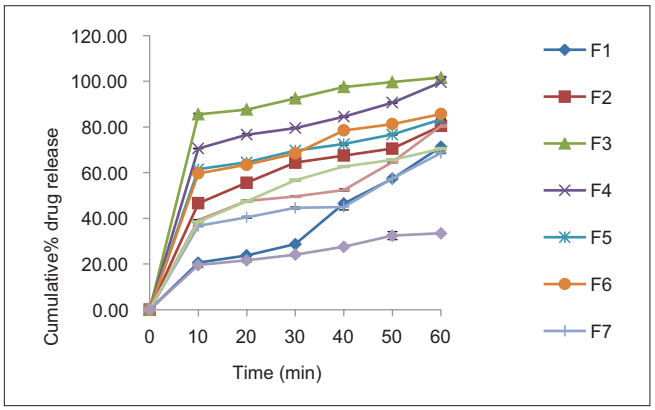

Figure 12: *In vitro* drug release profile of cilostazol and spherical agglomerate formulations

The response plot and counterplots in Figure 2 indicate that there was no interactive effect of X_1 and X_2 on percent yield.

Counterplots revealed different regions of percent yield with varying concentration of PVP K30 and HPMC E50. It can be inferred that the lower concentration of HPMC E50 (0.050 g or less) resulted in higher % yield (57% or more).

The low yield value of spherical agglomerates is indicative of loss of polymer during process. As all the polymers are water soluble (PVP K30, HPMC E50, and PEG 6000), the loss of polymer in water is unavoidable resulting in low % yield.

Effect on mean particle size

There was no significant effect observed for concentration of HPMC E50 on size of agglomerates. When amount of PVP K30 in formulation was increased from 0.5 g to 1.0 g, the resultant agglomerate size was increased. With further increase in PVP K30 concentration, there was a decrease in the mean size of agglomerates. The initial increase in agglomerate size with increase in PVP K30 concentration could be due to ability of PVP K30 to bind the crystal

during agglomeration process. Further increase in PVP K30 concentration could have increased stickiness in system which lead to improper agglomerate formation leading to wide size distribution, thus resulting into decrease in mean agglomerates size. The microscopy study revealed the spherical shape of agglomerates [Figure 4]. It also shows the presence of some smaller particle along with large particles. From the particle size distribution curves, it was observed that as concentration of HPMC E50 was increased in formulation, the particle size distribution was found to be wide, whereas at lower concentration of polymers, the narrow size distribution was obtained. Long tail on the left side of the curves indicated the presence of unagglomerated crystals in the formulations.

Scanning electron microscopy

The needle-shaped crystals of pure drug, as evident from scanning electron microscopy, contributed to poor flow property of cilostazol. The improvement in flow of agglomerates could be attributed to their spherical shape as evident from SEM.

Fourier transform infrared spectroscopy

The main characteristic peaks of cilostazol were unaffected in IR spectra of physical mixture and cilostazol agglomerates. Thus, there was no chemical change occurred in the formulation during agglomeration process.

Differential scanning calorimetry

A sharp endotherm of cilostazol indicates crystalline nature of drug. The decrease in intensity of endotherm in physical mixture could be due to the presence of other excipients. Slight shift of endotherm to 158.9°C indicated the presence of other excipient in the physical mixture. From DSC study, any interaction of drug was not observed with the excipients. However, change in the intensity of endotherm of formulation as compared to pure drug and physical mixture indicated the presence of drug in microcrystal form. Partial amorphization of drug is desirable as per the previous reports by Garala *et al*.[18] This might provide comparatively higher stability than amorphous counterparts.

X-ray diffraction study

The intense peaks observed in XRD of cilostazol indicated crystalline nature of drug. XRD of spherical agglomerate formulation indicated reduction in the intensity of cilostazol peaks. This could be due to partial amorphization of drug during agglomeration process. The increase in intensity for peaks at 24.9° to 25.40° indicates possibility of some crystalline change occurred in drug during the agglomeration process.

Solubility analysis

The spherical agglomerate formulations exhibited higher solubility in water and dissolution medium than pure cilostazol. This increase in solubility was due to partial amorphization of drug during agglomeration process. In addition, co-agglomeration with water-soluble excipients also contributed significantly to solubility enhancement.

In vitro dissolution study

There was significant improvement in the drug release from spherical agglomerate formulations as compared to pure drug. Increase in drug release may be attributed to hydrophilic nature of PVP K 30 and PEG 6000 used in co-agglomeration. In addition, change in crystalline to amorphous form of cilostazol during spherical agglomeration process could have contributed to increase in drug release. These observations were in agreement with DSC and XRD results.

Cilostazol release was found to be dependent on concentration of PVP K30. With increase in concentration of PVP K30, drug release was drastically increased (F1 to F3). In case of formulations F7, F8, and F9, as HPMC E50 concentration was higher, there was no increase in drug release with increase in PVP K30 concentration. This could be due to excessive sticking to the container leading to improper formation of agglomerates. These unagglomerated crystals did not show any improvement in drug release.

CONCLUSION

Cilostazol agglomerates were successfully formulated by crystallo-co-agglomeration technique using HPMC E50, PVP K30, and PEG 60000 as polymers. Cilostazol agglomerate formulation showed significant effect of concentration of HPMC E50 and PVP K30 on percent yield and mean particle size. The optimized formulation, F3 exhibited improved flowability, compressibility, water solubility, and drug release than pure cilostazol. The observations were supported by IR spectroscopy, DSC, and XRD. The present study indicated crystallo-co-agglomeration as a potential technique for formulation of poorly soluble drug that can improve flow behavior and dissolution profile of drug.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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