Department of Pharmaceutics[1], University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa; Department of Pharmaceutics[2], Shree Santkrupa College of Pharmacy, Ghogaon, Maharashtra; Department of Pharmaceutics[3], Poona College of Pharmacy, Erandwane, Pune, Maharashtra, India

Improved compressibility, flowability, dissolution and bioavailability of pioglitazone hydrochloride by emulsion solvent diffusion with additives

S. V. Patil[1,2], A. P. Pawar[3], S. K. Sahoo[1]

Received June 11, 2011, accepted July 11, 2011

Patil S. V., Department of Pharmaceutics, University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, India
sachinpatil79@rediffmail.com

Pharmazie 67: 215–223 (2012) *doi: 10.1691/ph.2012.1084*

Spherical agglomerates of pioglitazone hydrochloride were prepared by the emulsion solvent diffusion method with additives (polyethylene glycol 6000, polyvinyl pyrrolidone, β cyclodextrin, eudragit RS100, low acyl gellan gum and xanthan gum) using methanol, chloroform and water as a good solvent, bridging liquid and poor solvent respectively. Prepared agglomerates were evaluated for compressibility, solubility, dissolution rate and bioavailability, and characterized by SEM, XRPD, DSC and FTIR spectroscopy. Particle size, flowability, compactibility, packability, solubility, dissolution rate and bioavailability of plain agglomerates and agglomerates with additives (except with polyvinyl pyrrolidone) were advantageously improved compared with raw crystalline pioglitazone hydrochloride. These improved properties for direct compression were due to their large-spherical shape and enhanced fragmentation during compaction, together with increased tensile strength and reduced elastic recovery of the compacts. XRPD and DSC studies indicated polymorphic transition of pioglitazone hydrochloride from form II to I during recrystallization but this was not associated with any chemical transition, as indicated by FTIR spectra, well supported by stability studies. Thus spherical crystallization by the emulsion solvent diffusion method with selected additives is a satisfactory method for direct tableting of pioglitazone hydrochloride giving improved bioavailability.

1. Introduction

Solid dosage forms promote stability, and moreover tablets are a readily portable and easily consumed dosage form. Consequently the present focus of the industry is on better drug delivery models with maximum economy of production. One of the most economical solutions is direct tableting, as it is an efficient process involving mixing and compression of powder, saving time and cost compared with granule tableting (Shangraw 1989). However, it strongly depends on the flowability, compactibility and packability of the drug crystals used, as otherwise a lot of excipients are necessary resulting in larger tablets. The bioavailability of poorly water soluble drugs is often controlled by the rate of dissolution of the drug in the gastrointestinal tract. Several methods of enhancing the dissolution characteristics and bioavailability of slightly water-soluble drugs have been reported in the literature. These include reducing particle size to increase surface area (Nijlen et al. 2003), solubilization in surfactant systems, formation of water-soluble complexes, use of pro-drugs, drug derivatization and manipulation of the drug substance in the solid state to improve drug dissolution by decreasing the crystallinity of the drug substance (Kawashima 1984). Thus, fine crystals are preferred over large crystals of poorly soluble pharmaceuticals as they provide greater bioavailability. However, micronization of crystals frequently prevents efficient powder processing due to poor flowability, compactibility and packability. Thus a novel agglomeration technique that transforms the crystals themselves directly into a compacted spherical form during recrystallization with improved dissolution rate and bioavailability is desirable. The use of a spherical crystallization technique appears to be an efficient alternative for obtaining suitable particles for direct compression (Nokhodchi 2005). The spherical crystallization technique has already been used successfully to improve the micromeritic properties of several drugs such as acebutolol hydrochloride, mebendazole, mefenamic acid, nabumetone etc. (Patil and Sahoo 2009). Also, this method is successfully employed to enhance the bioavailability of drugs like fenbufen, aceclofenac, etc. (Martino et al. 1999; Mutalik et al. 2007).

Various methods have been reported in the literature for generating spherical agglomerates, such as spherical agglomeration (SA), emulsion solvent diffusion (ESD), ammonia diffusion and neutralization (Paradkar et al. 1994). Among these, the SA and ESD methods are widely employed. In the SA method, a nearly saturated solution of the drug in the good solvent is poured into the poor solvent, the miscibility between the poor and good solvents being stronger than the affinity between the drug and the good solvent. The bridging liquid which accelerates coalescence should be immiscible with the poor solvent and should preferentially wet the precipitated crystals (Pawar et al. 1998). In the ESD method, the drug is dissolved in the good solvent and bridging liquid and the resultant solution is dispersed into the poor solvent producing emulsion (quasi) droplets, even though the pure solvents are miscible. In this method the affinity between the drug and the good solvent is stronger than that of the good

solvent and the poor solvent. The good solvent diffuses gradually out of the emulsion droplets into the surrounding poor solvent phase and the poor solvent diffuses into the droplets, causing the drug to crystallize inside the droplets (Paradkar et al. 2002). Pioglitazone hydrochloride (PGH) is a thiazolidinedione oral antidiabetic agent used in the management of type II diabetes mellitus. It is rapidly absorbed after oral administration; peak plasma concentrations being obtained within two hours (Sweetman 2003). In the present investigation, spherical agglomerates of PGH for direct tableting were prepared by the emulsion solvent diffusion method with a range of additives: polyethylene glycol 6000, polyvinyl pyrrolidone, β cyclodextrin, Eudragit RS100, low acyl gellan gum and xanthan gum. Also, the effects of these additives on the micromeritic properties, flowability, compactibility, packability, solubility, drug release and bioavailability of PGH were studied. Thus the aim of the study was to achieve better crystals of PGH for direct compression with improved drug release and bioavailability.

2. Investigations, results and discussion

2.1. Development of spherically agglomerated crystals of PGH by ESD method

Selection of the good solvent, poor solvent and bridging liquid was done on the basis of the miscibility of the solvents and the solubility of the drug in the individual solvents. Since, PGH is soluble in methanol, slightly soluble in chloroform but insoluble in water (Maryadele 2001), methanol, chloroform and water were used as good solvent, bridging liquid and poor solvent respectively. Preliminary experiments were performed to optimize the concentrations of the solvents. In the absence of bridging liquid, the system produced agglomerates rich in needle shaped crystals. Different stirring rates were tested at optimized concentrations of the good solvent and bridging liquid (3:2) and an optimum was found to be 800 rpm. Formation of lumps and agglomerates of non-uniform size and shape was observed at lower stirring rates, while high stirring rates destroyed the agglomerates. When a solution of the drug in good solvent and bridging liquid was poured into the poor solvent, quasi-emulsion droplets of drug solution were produced initially. Subsequently, crystallization of the drug occurred at the outer surface of the droplets. The spherically agglomerated crystals were produced simultaneously after complete crystallization, the whole process being called emulsion solvent diffusion. Under stirring the agglomerates were spheronized and compacted.

2.2. Thin layer chromatography (TLC)

The chemical stability of PGH was determined using TLC. The TLC of both raw crystals and spherical agglomerates of PGH showed a single spot with R_f value 0.6. This indicated that the drug had not decomposed during recrystallization.

2.3. Yield, drug content micrometric properties and solubility study

Yield, drug content, micrometric properties and solubility of the PGH agglomerates are given in Table 1. Yield and drug content was found to be satisfactory. It was found that the particle size of plain agglomerates and agglomerates with additives other than PVP was increased more than 10 times compared with the original crystals, which may be due to particle agglomeration. SEM microphotographs of drug, plain agglomerates and agglomerates with additives shown in Fig. 1 show that the agglomerates were spherical with a smooth surface with small holes, perhaps due

Table 1: Micrometric properties and solubility of raw crystals and spherical agglomerates of PGH (n = 3)

FC	Yield (%)	Drug content (%)	Diameter (μm) n = 100	Angle of repose (°)	Bulk density (g/cc)	Tap density (g/cc)	Carr's Index (%)	Hausner's ratio	Solubility (μg/mL)	
									Water	pH 2 KB
A	–	–	16.7 ± 1.05	52.23 ± 0.75	0.322 ± 0.007	0.476 ± 0.006	32.35 ± 0.5	1.42 ± 0.04	30.86 ± 1.2	109.05 ± 2.3
B	96 ± 2	92 ± 2	162.3 ± 1.13	23.14 ± 0.65	0.281 ± 0.006	0.331 ± 0.004	15.01 ± 0.4	1.18 ± 0.05	76.50 ± 1.6 **	227.84 ± 3.1 **
C	95 ± 1	94 ± 1	151.5 ± 0.81	22.23 ± 0.75	0.279 ± 0.006	0.325 ± 0.003	14.15 ± 0.6	1.16 ± 0.04	82.97 ± 2.1 **	268.27 ± 2.1 **
D	97 ± 1	91 ± 3	158.7 ± 1.19	23.23 ± 0.29	0.275 ± 0.008	0.320 ± 0.005	14.06 ± 0.7	1.16 ± 0.05	98.06 ± 1.8 **	408.96 ± 4.3 **
E	95 ± 2	92 ± 2	146.5 ± 0.70	24.13 ± 0.34	0.271 ± 0.004	0.319 ± 0.006	15.04 ± 0.5	1.17 ± 0.06	78.97 ± 1.1 **	248.13 ± 3.2 **
F	94 ± 2	93 ± 1	149.5 ± 0.83	26.12 ± 1.10	0.276 ± 0.008	0.322 ± 0.007	14.28 ± 0.7	1.16 ± 0.09	68.97 ± 2.2 **	238.31 ± 2.6 **
G	97 ± 1	91 ± 2	152.5 ± 0.53	21.21 ± 0.98	0.269 ± 0.012	0.331 ± 0.009	18.73 ± 1.1	1.23 ± 0.03	71.97 ± 1.9 **	255.37 ± 2.9 **
H	92 ± 3	90 ± 2	18.4 ± 0.96	39.23 ± 0.43	0.332 ± 0.003	0.483 ± 0.002	31.26 ± 0.5	1.45 ± 0.03	49.35 ± 1.4 *	136.12 ± 1.6 *

Significantly different from value for raw crystals of PGH at $p < 0.001$ (**) and $p < 0.01$ (*). (FC: Formulation Codes, KB: KCl Buffer)

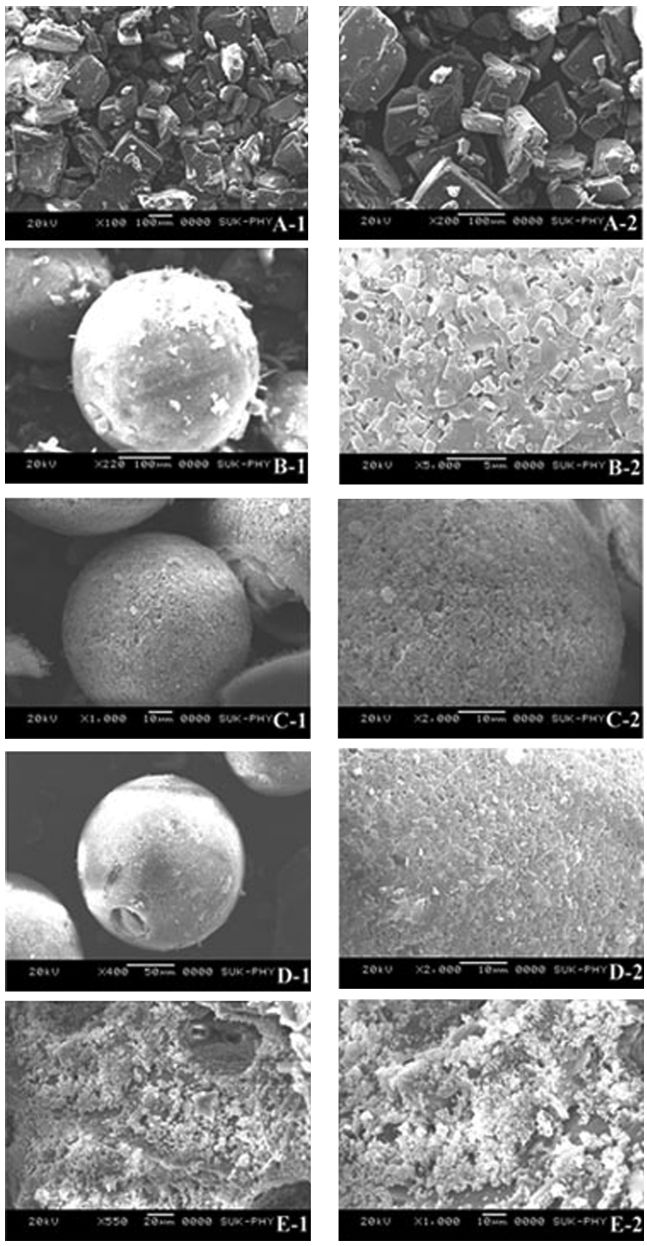

Fig. 1: SEM microphotographs of PGH and its agglomerates. A-1, A-2: Pioglitazone hydrochloride, B-1, B-2: Plane agglomerates, C-1, C-2: Agglomerates with PEG, D-1, D2: Agglomerates with β-CD, E1-E2: Agglomerates with PVP

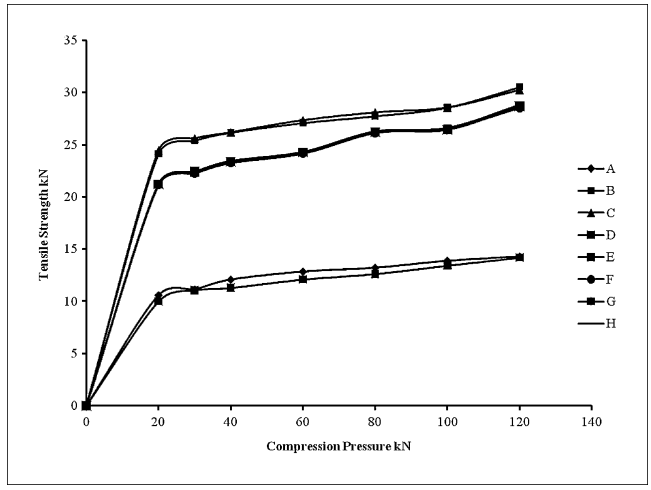

Fig. 2: Plot of tablet tensile strength as a function of compression pressure for PGH and its spherical agglomerates. A: PGH and Spherical agglomerates B: Plane, C: with PEG, D: with β-CD, E: with EU, F: with GG, G: with XG, H: with PVP

to a thin and uniform polymer coating. The bulk density of plain agglomerates and agglomerates with additives other than PVP was lower than that of raw crystals of PGH. Reduction in the bulk density of spherical agglomerates indicates greater porosity within the agglomerates (Ali et al. 2007). Angle of repose, Carr's index and Hausner's ratio values of plain agglomerates and agglomerates with additives other than PVP were lower than those of raw crystals of PGH, indicating better flowability, which might be due to large size and spherical shape of the agglomerates, clearly indicated in SEM microphotographs. The fact that PVP was highly effective in decreasing the average diameter of the resultant agglomerates might be due to its adsorption on the surface of the crystals, preventing their growth and so resulting in fine crystals (Ali et al. 2007). Angle of repose, Carr's index and Hausner's ratio values of agglomerates with PVP showed their poor flowability. In the case of agglomerates with PEG, β-CD, EU, GG and XG, average diameter was increased compared with raw crystals, but lower than that of plain PGH agglomerates. These findings suggest that these additives were poorly adsorbed at the surface, reducing the interfacial tension between bridging liquid and crystals and decreasing the adhesive force acting to agglomerate the crystals (Kawashima et al. 1986). The fact that the solubility of spherical agglomerates was higher than that of raw PGH crystals may be due to increased porosity, decreased primary particle size and polymorphic transition of the drug in agglomerates as demonstrated by DSC and XRD studies. That it was higher for agglomerates with β-CD and lower for agglomerates with PVP might be due to the fact that hardly any agglomeration occurred with PVP.

2.4. Compaction behavior of raw crystals and spherical agglomerates

The compressibility of a material is its ability to reduce in volume as a result of an applied pressure. Heckle parameters D_a, D_o, D_b, *MYP* and *ER* of raw crystals and spherical agglomerates of PGH are given in Table 2. The D_b value represents the particle rearrangement phase in the early stage of compression and tends to indicate the extent of particle fragmentation. The D_b values for plain agglomerates and agglomerates with PEG, β-CD, EU, GG and XG were higher than those for raw crystals of PGH, indicating that the agglomerates were highly fractured during early stage of compression, although fragmentation was followed by plastic deformation. The results were well supported by higher *MYP* values. The elastic recoveries of compacts of plain agglomerates and agglomerates with PEG, β-CD, EU, GG and XG were smaller than those of original drug crystals. These findings suggest that the agglomerated crystals were easily fractured, and the new crystal surface produced might contribute to promoting plastic deformation under compression. The lower D_b value of agglomerates with PVP compared with the other agglomerates might be attributed to the small particle size. Compactibility of samples was evaluated on the basis of the tensile strengths of compacts compressed under different compaction pressures. The tensile strength of tablets prepared with agglomerated crystals and raw crystals of PGH were plotted as a function of compression pressure as shown in Fig. 2. It was found that the tensile strengths of tablets with plain agglomerates and agglomerates with PEG, β-CD, EU, GG and XG were dramatically increased, indicating enhanced fragmentation during compression resulting in increased D_b. Tablets with raw crystals of PGH and agglomerates with PVP showed lower tensile strength, perhaps due to the presence of capping. The high tensile strengths of the tablets are indicative of stronger interparticulate bond-

Table 2: Heckel parameters D_a, D_o, D_b, *MYP* (mean yield pressure), *ER* (elastic recovery), Kawakita constants *a*, *b* and Kuno's constant *k* of raw crystals and spherical agglomerates of PGH (n = 3)

FC	D_a	D_o	D_b	MYP	% ER	a	b	k
A	0.617 ± 0.003	0.416 ± 0.011	0.201 ± 0.007	22.54 ± 2.4	8.1 ± 1.2	0.430 ± 0.06	0.00304 ± 0.0005	0.00258 ± 0.001
B	0.411 ± 0.002 ***	0.173 ± 0.003 ***	0.238 ± 0.005 **	27.41 ± 1.8 **	4.8 ± 0.4 ***	0.274 ± 0.05	0.02546 ± 0.003	0.01077 ± 0.006
C	0.564 ± 0.003 ***	0.181 ± 0.004 ***	0.383 ± 0.003 ***	25.31 ± 1.6 **	5.1 ± 0.6 ***	0.288 ± 0.06	0.01185 ± 0.004	0.01022 ± 0.004
D	0.483 ± 0.005 ***	0.161 ± 0.008 ***	0.322 ± 0.003 ***	28.31 ± 2.3 **	5.0 ± 0.5 ***	0.288 ± 0.03	0.01044 ± 0.006	0.01046 ± 0.006
E	0.570 ± 0.002 ***	0.176 ± 0.003 ***	0.394 ± 0.004 ***	26.31 ± 1.5 **	5.8 ± 0.7 ***	0.278 ± 0.02	0.02135 ± 0.003	0.01102 ± 0.003
F	0.573 ± 0.004 ***	0.179 ± 0.007 ***	0.394 ± 0.005 ***	29.31 ± 1.2 **	6.1 ± 0.8 ***	0.281 ± 0.04	0.01947 ± 0.006	0.01075 ± 0.007
G	0.569 ± 0.005 ***	0.191 ± 0.006 ***	0.378 ± 0.003 ***	26.31 ± 1.7 **	5.5 ± 0.4 ***	0.296 ± 0.03	0.03164 ± 0.009	0.01182 ± 0.008
H	0.622 ± 0.010 *	0.411 ± 0.006 *	0.211 ± 0.006 **	21.31 ± 2.9 *	7.8 ± 0.8 *	0.435 ± 0.07	0.00313 ± 0.0006	0.00261 ± 0.003

Significantly different from value for raw crystals of PGH at $p < 0.001$ (***), $p < 0.01$ (**) and $p < 0.05$ (*).

ing between the agglomerates. The improved compactibility of agglomerates might be attributed to their characteristic structure, which is responsible for the large relative volume changes during the early stage of the compression process due to their fragmentation. It has been shown that a reduction in bulk density of agglomerates results in an increase in the tensile strength of tablets, similar results having been obtained in a study by Ali et al. (2007).

2.5. Packability determination

The packability parameters *a*, *b* and *k* obtained from Kawakita's and Kuno's equations respectively are given in Table 2. It was found that for plain agglomerates and agglomerates with PEG, β-CD, EU, GG and XG, the value of parameter *a* in Kawakita's equation was reduced, and parameters *b* and *k* in Kawakita's and Kuno's equations respectively were increased compared with those of raw crystals of PGH and its agglomerates with PVP. These findings proved that packability of plain agglomerates and agglomerates with PEG and β-CD was advantageously improved for direct tableting compared with raw crystals and agglomerates with PVP. This suggests that during tableting these agglomerates flow smoothly from the hopper into the die cavity to attain uniformity in weight, as is necessary in direct tableting. This improvement in packability and flowability is attributed to size enlargement and the spherical shape of these agglomerates.

2.6. X-ray powder diffraction (XRPD)

XRPD of raw crystals and spherical agglomerates of PGH are shown in Fig. 3. PGH has at least two polymorphic forms, that is, form I and form II (Shlomit et al. 2007). Diagnostic peaks for form I are at 2θ = 8.7, 12.7, 18.8, 20, 20.1, 22.7, 26.6, 28.3 and for form II are at 2θ = 9.2, 10.4, 15.2, 16.4, 18.6, 21.4. The XRPD of raw crystals of PGH was identical to form II and that of all spherical agglomerates of PGH were identical to form I. It showed that during spherical crystallization of PGH there was a polymorphic transition of PGH from form II to form I, the most prominent identifying feature being the absence of peaks at 8.7, 20, 22.7 and 26.6 in spherical agglomerates of PGH.

2.7. Differential Scanning Calorimetry (DSC)

DSC thermograms of raw crystals and spherical agglomerates of PGH are shown in Fig. 4. Crystals of PGH showed a melting endotherm at 192.6 °C with heat of fusion -120 J/g and all the spherical agglomerates of PGH showed a melting endotherm at 182 °C with heat of fusion -99.24 J/g. These findings indicate that the untreated sample has form II which is changed to form I during recrystallization for the spherical agglomerates (Shlomit et al. 2007). DSC is generally combined with XRPD to determine the polymorphic composition of pharmaceutical powders when the polymorphs exhibit different melting points. Thus, the DSC results were well supported by XRPD indicating polymorphic transition of PGH during recrystallization.

2.8. Fourier Transform Infrared Spectroscopy (FTIR)

Raw crystals of PGH and spherical agglomerates of PGH exhibited identical FTIR spectra as shown in Fig. 5. This indicates that the altered XRPD spectra and DSC thermograms for these samples were not associated with any changes at the molecular level (Rai 2003). It demonstrated that no chemical transition occurred during recrystallization of PGH.

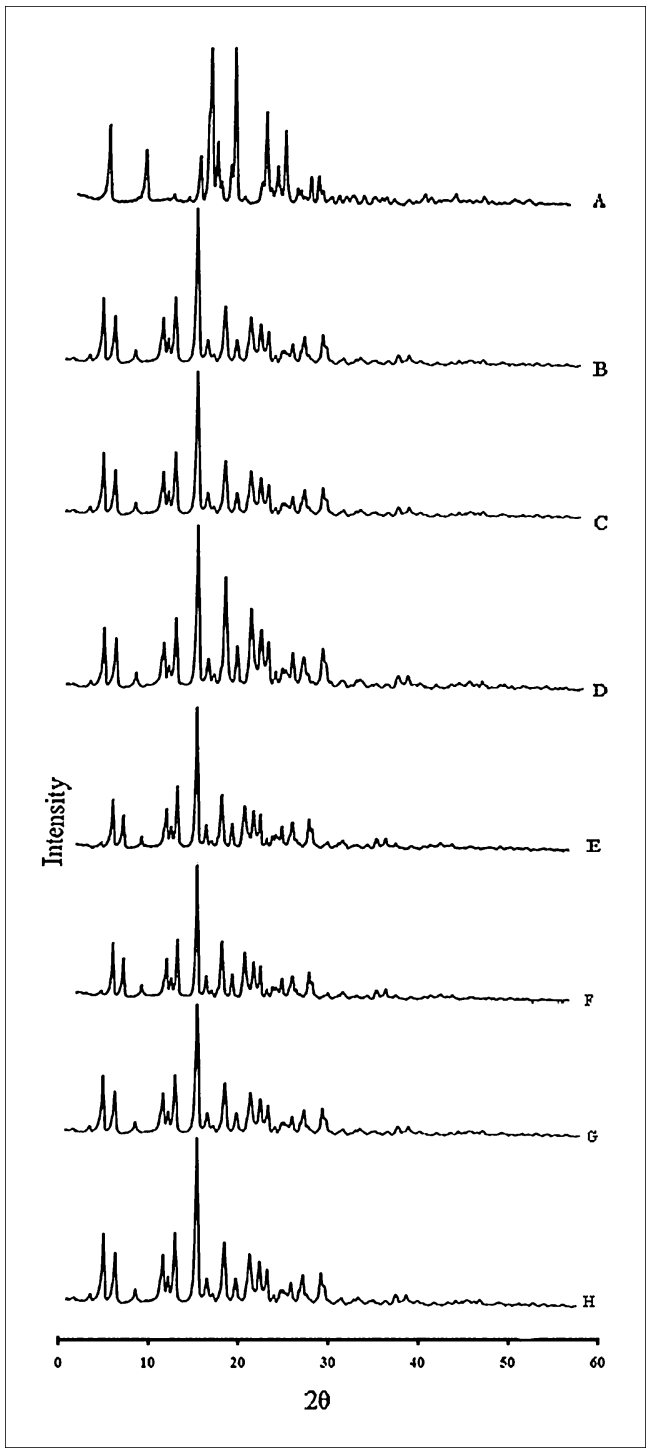

Fig. 3: X-ray powder diffraction pattern of PGH and its spherical agglomerates. A: PGH and Spherical agglomerates B: Plane, C: with PEG, D: with β-CD, E: with EU, F: with GG, G: with XG, H: with PVP

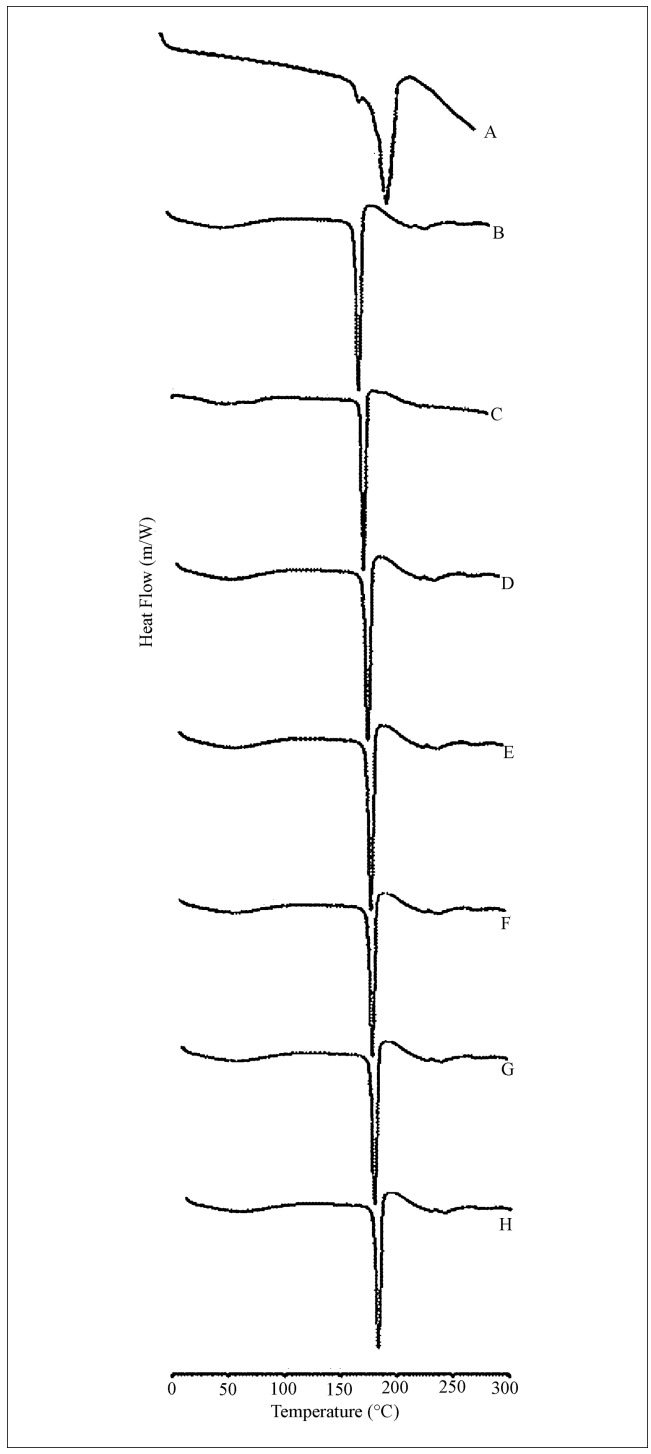

Fig. 4: DSC thermograms of PGH and its spherical agglomerates. A: PGH and Spherical agglomerates B: Plane, C: with PEG, D: with β-CD, E: with EU, F: with GG, G: with XG, H: with PVP

2.9. In-vitro dissolution studies

The rates of dissolution of raw crystals and spherical agglomerates of PGH are shown in Fig. 6. It was observed that for raw crystals of PGH up to 67% of the drug was released in 30 min, while for agglomerates of PGH drug release was increased in the order β-CD > PEG > EU > XG > GG > plain > PVP > raw crystals, which may be due to increased wettability and porosity. The presence of a bridging liquid enhances the wettability of the crystallized product which is also believed to promote the dissolution rate (Paradkar et al. 2002).

2.10. Stability studies

The agglomerates did not show any significant change in drug content and *in vitro* drug release during stability studies as given in Table 3. This indicated that the prepared agglomerates were adequately stable as specified in regulatory requirements.

2.11. Pharmacokinetic study

The calibration curve was linear over the PGH concentration range 50–2000 ng/mL in rat plasma. The equation of the curve was y = 0.0018x–0.0096 (where y is the peak area ratio of the

Table 3: Stability study data of spherical agglomerates of PGH

FC	0 Days		30 Days		60 Days		90 Days		180 Days	
	DC	DR	DC	DR	DC	DR	DC	DR	DC	DR
B	92 ± 2	96.3 ± 1	91 ± 2	95.7 ± 1	90 ± 2	94.8 ± 1	91 ± 1	96.1 ± 2	90 ± 1	94.9 ± 3
C	94 ± 1	97.3 ± 1	93 ± 1	96.8 ± 1	92 ± 2	96.1 ± 1	92 ± 1	97.1 ± 1	91 ± 2	95.9 ± 2
D	91 ± 3	98.6 ± 1	90 ± 2	98.4 ± 1	89 ± 3	97.6 ± 1	92 ± 1	98.1 ± 1	91 ± 1	96.8 ± 2
E	92 ± 2	97.3 ± 1	91 ± 2	96.3 ± 1	92 ± 2	96.1 ± 1	92 ± 1	95.3 ± 2	90 ± 2	95.1 ± 1
F	93 ± 1	96.6 ± 1	92 ± 1	95.8 ± 1	91 ± 2	95.9 ± 1	91 ± 1	96.1 ± 1	90 ± 1	95.3 ± 1
G	91 ± 2	94.6 ± 2	90 ± 2	94.1 ± 2	89 ± 1	92.8 ± 3	89 ± 2	93.8 ± 1	88 ± 2	93.0 ± 1
H	90 ± 2	86.3 ± 3	88 ± 3	85.3 ± 2	88 ± 1	83.5 ± 2	87 ± 2	86.7 ± 1	86 ± 1	83.1 ± 2

Not significantly different from values of 0 days as $p > 0.1$ for all agglomerates. (DC: Drug content [%], DR: Drug release [%]) (n = 3)

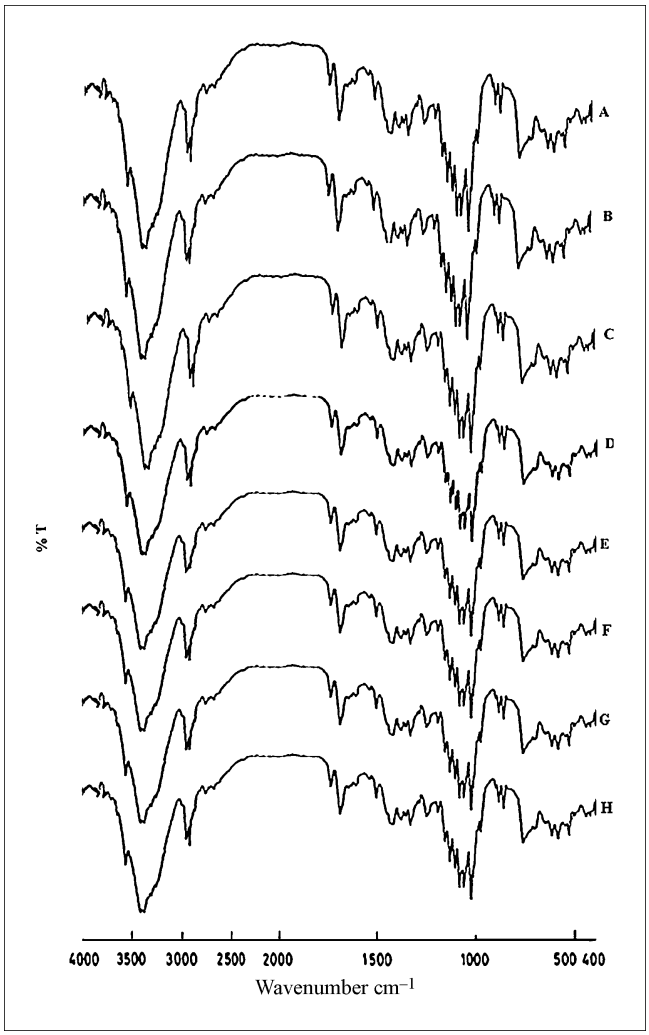

Fig. 5: IR Spectra of PGH and its spherical agglomerates. A: PGH and Spherical agglomerates B: Plane, C: with PEG, D: with β-CD, E: with EU, F: with GG, G: with XG, H: with PVP

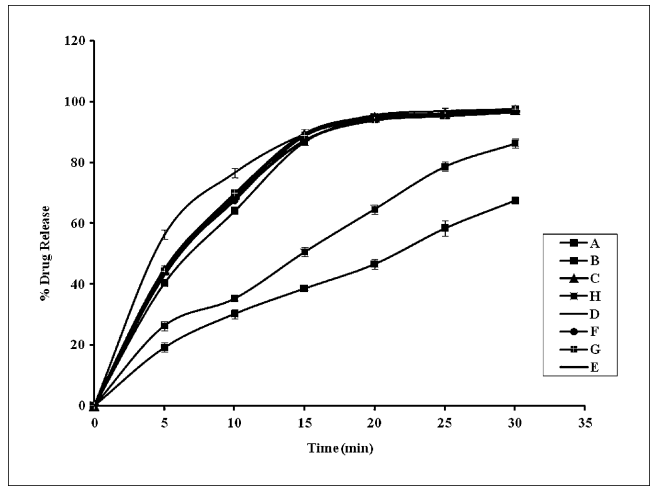

Fig. 6: Dissolution study of for PGH and its spherical agglomerates. A: PGH and Spherical agglomerates B: Plane, C: with PEG, D: with β-CD, E: with EU, F: with GG, G: with XG, H: with PVP

analyte to internal standard and x is the concentration of the analyte). The correlation coefficient (r^2) of the calibration curve generated during the validation was 0.9996 for the analyte. The lower limit of quantitation (LLOQ) of pioglitazone in plasma was verified as 50 ng/mL, as this was the lowest concentration assessed at which the accuracy was between 80 and 120%, and precision was within 20%. The lower limit of detection (LOD) was 25 ng/mL at a signal-to-noise ratio of 3. The pharmacokinetic parameters of PGH and its agglomerates are given in Table 4. C_{max} and T_{max} values of prepared agglomerates were higher than that of the pure drug, indicating an improved rate of absorption of PGH in agglomerates which is supported by the higher AUC values of the agglomerates. MRT and $t_{1/2}$ of PGH with agglomerates was lower, indicating rapid elimination of drug from the body as compared with that of the pure drug, which was well supported by the high K_e values. Thus the pharmacokinetic study indicated rapid absorption and higher bioavailability of the drug from agglomerates in comparison with the pure drug. Furthermore, the improved bioavailability achieved with spherical agglomeration of PGH may reduce the total dose of drug, which is beneficial for cost effectiveness and improved patient compliance.

2.12. Conclusion

Stable spherical crystals of PGH were successfully prepared by emulsion solvent diffusion. Flowability, compactibility and packability were dramatically improved for both plain agglomerates and agglomerates with PEG and β-CD compared with raw crystals of PGH, resulting in successful direct tableting without capping, whereas agglomerates of PGH with PVP as additive showed poor flowability, compactibility and packability. Also the remarkable fragmentation and increased tensile strength of plain agglomerates and agglomerates with additives other than PVP indicate improved compactibility. The improved solubility, dissolution rate and bioavailability of plain agglomerates and agglomerates with PEG, β-CD, EU, GG and XG, compared with raw crystals and PVP-containing agglomerates of PGH, shows their improved wettability. During agglomeration, polymeric transition occurred but was not associated with changes at the molecular level. It is concluded that spherical crystalliza-

Table 4: Pharmacokinetic parameters of raw crystals and all agglomerates of PGH. (n = 6)

Parameters	A	B	C	D	E	F	G	H
C_{max} (μg/mL)	9.8 ± 1.1	11.3 ± 2.1*	13.8 ± 1.8*	15.3 ± 2.7*	12.6 ± 2.2 *	11.9 ± 18 *	12.4 ± 2.3 *	10.9 ± 1.3 *
T_{max} (h)	1.5 ± 0.07	1.5 ± 0.06	1.5 ± 0.05	1.5 ± 0.08	1.5 ± 0.07	1.5 ± 0.03	1.5 ± 0.04	1.5 ± 0.06
AUC_{0-8} (μgh mL^{-1})	96.7 ± 8.6	122.5 ± 11.7 **	163.3 ± 0.04 **	172.8 ± 12.7 **	143.7 ± 9.9 **	139.4 ± 15.2 **	148.4 ± 13.5**	119.8 ± 13.4**
$T_{1/2}$ (h)	3.1 ± 0.08	2.8 ± 0.08*	2.6 ± 0.06*	2.5 ± 0.07*	2.6 ± 0.04*	2.7 ± 0.08*	2.6 ± 0.07*	3.0 ± 0.06
MRT (h)	5.2 ± 0.08	4.9 ± 0.06*	4.7 ± 0.04*	4.8 ± 0.08*	4.7 ± 0.02*	4.8 ± 0.05*	4.8 ± 0.03*	5.1 ± 0.04
K_e (h^-)	0.248 ± 0.00	0.259 ± 0.00	0.269 ± 0.00	0.278 ± 0.00	0.261 ± 0.00	0.264 ± 0.00	0.266 ± 0.00	0.254 ± 0.00

Significantly different from value of PGH raw crystals at $p < 0.01$ (**) and $p < 0.05$ (*)

tion of PGH with selected additives is a satisfactory method of improving the flowability, compactibility and packability for direct tableting, together with enhanced solubility, dissolution and bioavailability.

3. Experimental

3.1. Materials

Pioglitazone hydrochloride (PGH) and β cyclodextrin (β-CD) were kindly provided by Alembic Research Centre, Gujarat, India. Low acyl gellan gum (GG) and xanthan gum (XG) were kindly provided by C.P. Kelco Pvt. Ltd. Mumbai (India). Eudragit RS 100 (EU), polyethylene glycol 6000 (PEG), polyvinyl pyrrolidone (PVP), methanol and chloroform were purchased from Rajesh Chemicals, Pune, India. All chemicals used were of analytical grade. The pharmacokinetic studies were carried in male Wistar rats (weighing 200–250 g) obtained from the Krishna Institute of Medical Sciences, Karad, Maharashtra, India. They were housed in elevated wire cages, four animals per cage, with free access to food (Lipton Feed, Mumbai, India) and used according to the standards of the CPCSEA, India. The protocol was approved by the Institutional Animal Ethical Committee of Shree Santkrupa College of Pharmacy, Ghogaon, Maharashtra, India. (Approval No.: 1110/ac/07/CPCSEA, 24/09/2007).

3.2. Methods

3.2.1. Development of spherically agglomerated crystals of PGH by ESD method

PGH (10 g) was dissolved in a mixture of 60 mL methanol (good solvent) and 40 mL chloroform (bridging liquid). The resultant solution was poured into 500 mL of distilled water (poor solvent) containing 1% w/v of PEG/PVP/β-CD/EU/GG/XG, with stirring at 800 revolutions per minute (rpm) for 20 min at 25 °C. The recrystallized agglomerates obtained were collected by vacuum filtration and dried in an oven at 60° C for 4 h. The dried crystals were stored in a desiccator at room temperature before use. The above process was repeated several times to obtain enough materials for characterization and to observe repeatability. Formulation codes for drug, plain agglomerates, and agglomerates with PEG, β-CD, EU, GG, XG and PVP were allocated as A, B, C, D, E, F, G and H respectively.

3.2.2. Determination of yield, drug content, micrometric properties and solubility study

The yield of the prepared agglomerates was determined by weighing the agglomerates after drying using Eq. (1).

$$\text{Yield} = (\text{Prctical Weight}/\text{Theoretical Weight}) \times 100 \quad (1)$$

For determination of drug content, spherical agglomerates of PGH equivalent to 100 mg of PGH were triturated and dissolved in a solvent system containing methanol: water: hydrochloric acid 250:250:1 mL. Appropriately diluted samples were filtered through Whatman 41 filter paper (pore size 25 μm) and the drug content was determined spectrophotometrically at 269 nm using a Jasco V530 UV-visible spectrophotometer, (Jasco, Japan). The percentage drug content was calculated using Eq. (2).

$$\text{Drug Content} = \left(\frac{\text{Practical Drug Concentration}}{\text{Theoretical Drug Concentration}}\right) \times 100 \quad (2)$$

The mean particle size of PGH and its agglomerates was determined as the average diameter of 100 randomly selected particles, measured with an optical microscope. The bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose were determined (Lachman et al. 1986). The solubility of raw crystals and spherical agglomerates of PGH was determined in distilled water and in pH 2 potassium chloride (KCl) buffer. An excess amount of sample was added to 20 mL of distilled water/pH 2 KCl buffer and continuously shaken (300 rpm) at 25 ± 0.5 °C for 48 h, then sonicated using a sonicator (Dolphin^{TM}) for 2 h. Samples were filtered through 0.45 μm filters and assayed spectrophotometrically for drug content at 269 nm.

3.2.3. Thin layer chromatography (TLC)

PGH and its prepared agglomerates were dissolved in methanol and spotted separately on a reverse-silica gel plate, which was developed with a toluene: methanol: ammonia (7:3:0.1 v/v) solvent system and detected by iodine vapor.

3.2.4. Compaction behavior of raw crystals and spherical agglomerates

The Heckel study was performed by compressing 500 mg of raw crystals and spherical agglomerates on a hydraulic press (Samrudhi Enterprises, Mumbai, India) using a 13 mm flat faced punch and die set, at pressures of 20, 30, 40, 60, 80, 100 and 120 kN, and the thickness, weight and diameter of the compacts were determined. Heckel parameters were determined using the Heckel equation (Heckel 1961). For determination of the parameter ER, the thickness of compacts of agglomerates and raw crystals of PGH was determined using a compression pressure of 60 kN 24 hours after releasing the tablet (Armstrong and Haines-Nutt 1974). Crushing strength was measured immediately after compression with an Erweka (Germany) type TBH 30 tablet strength tester (Fell and Newton 1970).

3.2.5. Packability determination

To determine packability, 25 g of sample was poured slowly and gently into a 25 mL measuring cylinder and tapped 100, 200, 300, 400, 500, 600, 700, 800, 1100 and 1200 times. The Stampf volumeter measurements allow calculation of the compactibility and cohesiveness values via a modified Kawakita's and Kuno's equation (Kawakita and Ludde 1971; Kuno 1979).

3.2.6. Scanning Electron Microscopy (SEM)

Agglomerates were coated with a thin gold-palladium layer with a sputter coater (VG- Microtech, United Kingdom), and the surface topography was analyzed with a Cambridge Stereoscan S120 scanning electron microscope (SEM; Cambridge, United Kingdom) operated at an acceleration voltage of 10 kV.

3.2.7. X-ray Powder Diffraction (XRPD)

Raw crystals and spherical agglomerates were analyzed by X-ray powder diffraction using a Philips PW 1729 x-ray diffractometer. Samples were irradiated with monochromatized Cu K_α –radiation (1.542 A°) and analyzed between 2–60 ° (2θ). The voltage and current used were 30 kV and 30 mA respectively. The range was 5×10^3 cycles/s and the chart speed was kept at 100 mm/2θ.

3.2.8. Differential Scanning Calorimetry (DSC)

Thermal properties of raw crystals and spherical agglomerates of PGH were analyzed by DSC (TA Instruments, USA, Model: SDT 2960). An indium standard was used to calibrate the DSC temperature and enthalpy scale. Nitrogen was used as the purge gas at a flow rate of 50 mL per min through the DSC cell and 100 mL per min through the cooling unit. The sample (5–10 mg) was heated in a hermetically sealed aluminum pan. Heat runs for each sample were set from 0 to 300 °C at a heating rate of 10 °C/min.

3.2.9. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy of raw crystals and spherical agglomerates of PGH was recorded using a Jasco V5300 (Jasco, Japan) FT-IR system using the potassium bromide (KBr) pellet method. Each spectrum was derived from single average scans collected in the region 4000 to 400 cm^{-1}.

3.2.10. In-vitro dissolution studies

Dissolution studies of raw crystals and spherical agglomerates of PGH were performed using a USP 26 type II dissolution test apparatus (Dolphin™, Mumbai, India) in 900 mL of pH 2 KCl buffer. Temperature was maintained at 37 ± 2 °C with stirring at 100 rpm for each dissolution study. PGH and its spherical agglomerates equivalent to 100 mg of PGH were used for each dissolution study. Samples were collected periodically and replaced with fresh dissolution medium. After filtration through a Whatman 41 filter paper (pore size 25 μm), the concentration of PGH was determined spectrophotometrically at 269 nm on a Jasco V30 UV-visible spectrophotometer (Jasco, Japan).

3.2.11. Stability studies

All the spherical agglomerates of PGH were subjected to accelerated stability studies according to ICH guidelines (40 ± 2 °C C and 75 ± 5% RH) for a period of 6 months in a stability chamber (Thermolab, Mumbai, India). The samples were placed in vials with bromobutyl rubber plugs and sealed with aluminum caps. The samples were withdrawn at 30, 60, 90 and 180 days and evaluated for drug content and *in vitro* drug release for 30 min.

3.2.12. Pharmacokinetic study

Rats fasted overnight were divided into 8 groups (n = 6) and treated as group 1: Pure PGH and groups 2 to 8: PGH agglomerates. Each rat was given a dose of 10 mg/kg orally as a solution in 0.1 M citric acid with a dose volume of 10 mL/kg. Subsequently, blood samples (0.3 mL) were withdrawn through the tail vein at predetermined intervals of 0.5, 1, 1.5, 2, 4, 6 and 8 h post-dose and collected into heparinized tubes from the orbital sinus. The plasma was separated immediately using cold centrifugation (Remi Centrifuge, Mumbai.) at 3000 rpm for 15 min and was stored at −72 °C until analysis. The concentration of PGH was determined by HPLC, the apparatus comprising a dual plunger pump (LC-10ATVP, Shimadzu, Kyoto, Japan), a UV-Vis detector (SPD-10AVP, Shimadzu) with a system controller (SCL-10AVP, Shimadzu) and an RP C-18 column (Hypersil BDS C18 250 cm x 4.6 mm; 5 μm), following the method described by Pattana et. al. (2006). The mobile phase was methanol–acetonitrile-mixed phosphate buffer (pH 2.6; 10 mM) (40:12:48, v/v/v). All separations were performed isocratically at a flow rate of 1.2 mL/min. Column temperature was maintained at room temperature (25 ± 2 °C). The peaks were determined using a UV detector set at a wavelength of 269 nm. Maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (T_{max}), area under the concentration– time curve (AUC), mean residence time (MRT), elimination half life ($t_{1/2}$) and elimination rate constant K_e were calculated by 'PK Solutions®', Pharmacokinetic Software for Research and Education.

3.2.13. Statistical analysis

Results are expressed as mean ± S.D for triplicate samples. The results were analyzed statistically, and significance of differences among formulation parameters were determined by one-way analysis of variance using 'Graph Pad Instate®'Version 3.05 (USA), statistical analysis program. Statistical significance was considered to be $p < 0.05$.

Acknowledgments: The authors thank Alembic Research Centre, Gujarat, India, for donating samples of pioglitazone hydrochloride and β cyclodextrin. The authors are also grateful to the Head of Department, University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, India and the Principal, Shree Santkrupa College of Pharmacy, Ghogaon, Karad, Dist. Satara, Maharashtra, India for providing research facilities.

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