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Development of oral tablet dosage form incorporating drug nanoparticles

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ABSTRACT

Nanoparticle technology is emerging as a preferred approach to address challenges involved in the delivery of BCS class-II compounds (poorly soluble and highly permeable). The development of nanoparticle formulations for BCS class-II drugs such as Glyburide, Fenofibrate and Candesartan cilexetil would result in enhanced bioavailability, reduced systemic variability and more convenient dosing regimen. To enhance oral bioavailability and reduce variability in systemic exposure, nanoparticle formulation of these drugs were developed using a wet bead milling technique. The solid-state transitions of drug nanoparticles were evaluated before and after milling using differential scanning calorimetry (DSC) and powder X-ray diffraction (XRPD). The nanosuspensions were converted into solid intermediate or granules by layering on to a water-soluble carrier Lactose using a spray granulation processes. The granules were blended with excipients for tabletting. The saturation solubility and dissolution characteristics of nanoparticle formulations were investigated and compared with commercial tablet formulations in a discriminating dissolution media. The result indicated there was no solid-state transition upon milling. A significant enhancement in dissolution rate for tablet dosage form incorporating drug nanoparticles was observed compared to the marketed products. The manufacturing process used is relatively simple and scalable indicating viability of the approach for commercial manufacture of drug product.

Keywords: Nanoparticles; poorly soluble drugs; particle size; dissolution rate; bead milling

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INTRODUCTION

Products developed using nanotechnology approaches are expected to revolutionize modern medicine, as evidenced by recent scientific advances and global initiatives to support nanotechnology and nanomedicine research. A number of compounds with good therapeutic potential that are identified using combinatorial chemistry techniques have poor aqueous solubility due to their complex structure often resulting in low and variable systemic blood levels resulting in suboptimal therapeutic response [1-3]. Currently there are limited formulation approaches available for compounds that exhibit poor aqueous solubility.

The preferred approach to enhance solubility for compounds that have an ionizable group is salt formation. However, these approaches have resulted in limited success because identification of a crystalline salt with adequate aqueous solubility requires screening of various counter-ions, solvents/crystallization conditions and at times isolation of a crystalline material is difficult task. In some instances the salt formed is extremely hygroscopic posing product development and manufacturing challenges.

For compound that does not have an ionizable group, solubility concerns are generally addressed using micronisation and / or solid dispersion technology [4,5]. Micronisation often results in particles that are < 5 µm with very small fraction that is in the sub-micron range. The decrease in particle size results in a modest increase in surface area that may not change the dissolution rate or saturation solubility to significantly impact bioavailability.

Solid dispersion compositions comprise of molecular dispersion of the drug and lipid-based surface-active carriers that can emulsify upon contact with dissolution medium. Formation of molecular dispersions (solid solution) provides a means of reducing particle size of the compounds to nearly molecular levels. As the carrier dissolves, the compound is exposed to the dissolution media as fine amorphous particles, resulting in rapid dissolution and absorption. The major drawback with this approach is to identify careers in which the drug has adequate solubility. Limited success has been achieved by formulating sparingly soluble drugs using co-solvent, surfactants or complexation agents such as cyclodextrins [6,7] and microemulsion [8]. Although these approaches have been successfully utilized, particularly for highly potent compounds requiring a low dose, there is growing need for more effective and versatile technology that can resolve formulation issues associated with dissolution and bioavailability enhancement of poorly water-soluble compounds with low or moderate potency.

In the last decade nanoparticle engineering processes have been developed and reported for pharmaceutical application [9]. There are various methodologies employed for generation of drug nanoparticle [10-12]. The report herein provides an overview of our efforts to develop a nanoparticle formulation of currently marketed formulations of BCS Class II drugs like Glyburide - an oral antihyperglycemic drug of sulphonyl urea class used for the treatment of type II diabetes, Fenofibrate - a fibric acid derivative used for the treatment of hypertriglyceridimia [13] and Candesartan cilexetil - a non-peptide angiotensin

II type 1 (AT_1) receptor antagonist used in the treatment of hypertension [14] to enhance their oral bioavailability, reduce variability and mitigate food effects.

An approach that is gaining widespread acceptance for enhancing dissolution rate and saturation solubility of BCS class-II drugs is to formulate them as nanometer-sized particles - particles less than 1 µm in diameter. For example, when the particle size of the drug is reduced from 8 µm to 200 nm there is 40-fold increase in the surface area to volume ratio. This increase in surface area can provide substantial increase in the dissolution rate if the formulation disperses into discrete particles in the gastrointestinal fluids [15].

Nanocrystalline dispersion comprise of drug, water and stabilizers. Stabilizers used to aid the dispersion of particles are either polymers and /or surfactants. Polymers act as the primary stabilizer whereas surfactants are used as the secondary stabilizer. To be effective the stabilizers must be capable of wetting the drug crystals and providing steric and ionic barrier to prevent agglomeration. The concentration of polymeric stabilizers can range from 1 – 10% w/v and the concentration of surfactants is generally < 2 % w/v [16]. If required, other excipients such as buffers, salts, and diluents like sugar can be added to the dispersion to enhance physical stability and further processing.

The media milling process used herein for production of drug nanoparticles is a simple procedure comprising of; attrition media, suspension and agitation. The extent of size reduction is governed by amount of grinding energy, which is determined by the intrinsic hardness of the drug, grinding media, and milling power. The nanoparticulate dispersion is subsequently converted into solid intermediates for tableting by granulating with water-soluble carriers. The dissolution characteristics of tablet formulations containing drug nanoparticles were compared with commercial formulations in physiologically relevant dissolution media.

MATERIALS AND METHODS

Materials

Glyburide (GB), Fenofibrate (FE) and Candesartan cilexetil (CA) were procured from Dr. Reddy's Laboratories (Hyderabad, India). Lactose monohydrate was purchased from Roquette (Roquette Freres, Lestrem, France). Microcrystalline cellulose (Avicel PH 102) was purchase from FMC biopolymers Ltd (USA). Polyvinyl pyrolidone [PVP (K-30)] was procured from BASF (Germany). Hydroxy propyl methylcellulose (Hypromellose, 6cps) was purchased from Colorcon (Mumbai, India). Sodium dodecyl sulphate (SDS) was purchased from Qualigen chemicals (Delhi, India). Crospovidone (Polyplasdone) was obtained from Grain processing corporation (USA). All other chemicals were of analytical reagent grade.

Preparation of nanosuspension on a laboratory scale

An in-house glass apparatus mimicking the wet bead-milling machine was fabricated for identifying appropriate stabilizer and their concentrations for preparation of nanoparticulate dispersions with adequate physical stability. The volume of the milling chamber was 250 mL thus limiting the amount of drug substance required for evaluation. A

predetermined amount of stabilizers and drug were dispersed in appropriate amount of purified water to obtain a coarse suspension. The coarse suspension was milled using a milling media that comprised of Yttrium stabilized zirconium beads (0.2 – 0.3 mm). The beads were agitated using a stirrer that rotated at a specified speed (~ 2000 rpm) to fracture the drug crystals into sub-micron sized particles. The formula composition that provided a nanosuspension with the desired particle size distribution with acceptable physical stability was scaled-up using the media mill.

Particle size analysis

Particle size and size distribution of the suspensions before, during (at different milling times) and following milling were determined using a laser diffraction method fitted with a wet sampling system (Mastersizer S, Malvern Instruments, UK). The particle diameters reported were calculated using volume distribution. The particle size obtained with different stabilizer compositions and their physical stability upon storage was evaluated. Based on particle size distributions obtained following milling and suspensions stability a suitable formula composition comprising primary stabilizer and secondary stabilizer were chosen for scale-up trials.

Scale-up of manufacturing process for production of nanosuspensions

The selected compositions were scaled-up using a bead mill (Model: Lab Star 1, Netzsch Mill, and Netzsch, Germany). The milling media comprised of 0.2 mm yttrium stabilized zirconium beads. The suspension flowed axially through the milling chamber where the shear forces generated during impaction of the milling media with the drug provided the energy input to fracture the drug crystals into nanometer-sized particles. The milling operation was performed in a re-circulation mode with the suspension fed at a rate of 150 mL per minute. The mill and pump speeds were operated at 2500 ± 50 and 120 ± 5 rpm, respectively. The temperature inside the milling chamber was controlled by circulating cooling water through the outer jacket. After milling, the suspension was collected and stored at a temperature below 25°C until further processing.

Conversion of nanosuspensions into solid intermediates

The nanoparticulate dispersions of Glyburide, Fenofibrate and, Candesartan cilexetil were converted into solid intermediate using a spray granulation process. The suspensions were layered at a predetermined spray rate (6 ± 2 g / min) on to lactose particles using a top spray fluid bed (Model: GPCG 1.1, Fluidized Bed Coater, Glatt GmpH, Germany). The drying temperature and atomization speed were set at 40 ± 5°C and 1.2 bar, respectively. The granules were dried as they moved upward, small droplets and low viscosity of the spray medium ensured that distribution was uniform resulting in granules with a narrow size distribution.

Solid-State characterization

Differential scanning calorimetry (DSC)

The thermal properties of powder were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter / TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin- Elmer Instruments, USA). For this analysis, about 3 to 5 mg of product was placed in perforated aluminum sealed 50 µL pans and the heat runs for each sample was set from 40 to 200°C at 5°C/minute, under an inert environment using nitrogen. The apparatus was calibrated using indium.

Powder X-ray diffraction (PXRD)

The PXRD diffractograms of un-milled and milled formulations were recorded using a Panalytical Xpert Pro Diffractometer (PANalytical, The Netherlands) with a Cu line as the source of radiation. Standard runs using a 40 kV voltage, a 40mA current and a scanning rate of 0.02° min^{-1} over a 2ϑ range of 3 – 40° were used.

Solubility studies

Saturation solubility evaluations were carried out in buffer media at different pH conditions using a shake flask method. In this method excess amount (100 mg/mL) of drug substance ("as is" and dried suspension containing microparticles or nanoparticles) was added to 25 mL of each buffer maintained at 37°C and shaken for a period up to 24 hours. The samples were filtered using 0.10µm pore size Millex-VV PDVF filters (Millipore Corporation, USA) prior to analysis. Samples were diluted and concentrations were determined using an HPLC method.

HPLC method

Glyburide

Chromatographic separation for Glyburide was accomplished using an Xterra MS C8 column, 250x4.6mm, 5µ stainless steel column. The mobile phase consisted of a ixture of acetonitrile and water in the ratio of 70:30. The mobile phase was pumped isocratically at a flow rate of 2.0 mL/minute during analysis and was maintained at a column temperature of 25°C. The amount of drug dissolved at each sampling time point was monitored at a UV wavelength of 254 nm.

Fenofibrate

Chromatographic separation for Fenofibrate was accomplished using a Waters Symmetry C8 250 x 4.6 mm 5µ stainless steel column. The mobile phase consisted of a mixture of buffer and acetonitrile in ratio of 45:55, pH adjusted to 7.5 with phosphoric acid. The mobile phase was pumped isocratically at a flow rate of 1-5 mL/minute during analysis and was maintained at a column temperature of 25°C. The amount of drug dissolved at each sampling time point was monitored at a UV wavelength of 286 nm.

Candesartan Cilexetil

Chromatographic separation for candesartan cilexetil was accomplished using an Inertsil ODS-3, $C_{18,}$ 250 x 4.6mm 5µm stainless steel column (Agilent Technologies, USA). The mobile phase consisted of a mixture of buffer (0.02M monobasic potassium phosphate), acetonitrile and triethylamine in the ratio of 40:60:0.2, pH adjusted to 6.0 with phosphoric acid. The mobile phase was pumped isocratically at a flow rate of 2.0 mL/minute during analysis and was maintained at a column temperature of 25°C. The amount of drug dissolved at each sampling time point was monitored at a UV wavelength of 254 nm.

Tablet preparation

The granules containing drug nanoparticles of Glyburide, Fenofibrate and Candesartan cilexetil were blended with extra-granular excipients using a double cone blender. The blend was subsequently compressed into tablets at the desired strength. The physical properties of tablets - hardness, friability and disintegration time were measured. The tablet hardness was measured using a hardness tester (Model: 8M, Dr Schleuniger Pharmatron, USA). Each hardness value reported is an average of ten measurements. The disintegration time was measured in purified water at 37 ± 0.5°C, using a disintegration tester (Model: ED2L, Electrolab, India), using sintered disks. The disintegration time reported is an average of six measurements. Tablet friability was calculated as the percentage weight loss of 20 tablets after 100 rotations using a friabilator (Model: EF2, Electrolab, India).

Dissolution studies

The dissolution characteristics of tablets containing drug nanoparticles and marketed product were determined using an USP dissolution apparatus (Model: DISSO 2000, Labindia, India) type II, fitted with a paddle that operated at 50 rpm. The dissolution media comprised of 0.1 N HCl (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8), and purified water. The volume and temperature of the dissolution medium were 500 mL and 37°C, respectively. Samples were withdrawn at predetermined time intervals, filtered in-line and assayed using an HPLC method (Waters Alliance HPLC system, USA). From these studies the discriminating dissolution media is selected based on its ability to differentiate the impact of changes in composition and particle size on dissolution rate.

RESULTS AND DISCUSSION

Production of physically stable nanosuspensions

The formula composition selected for producing nanosuspensions of Glyburide (GB), Fenofibrate (FE) and Candesartan cilexetil (CA) for scale-up trials using the media mill is summarized in Table 1. The median particle size (d50), for the milled suspensions for the four formula compositions – GB, FE and CA were, i.e. 0.134, 155 and 0.127µm, respectively. The d90, which is indicative of large particles or aggregates were 0.274, 0.546 and 0.269µm, respectively. The effect of SDS concentration on particle size distribution of nanosuspensions

indicated that the concentration of SDS required is drug specific therefore, their amount used in dispersions of GB, FE and CA were, 0.25, 0.15 and 1.00 w/v respectively.

Based on particle size distribution obtained upon milling for a predetermined time interval, Hydroxy propyl methyl cellulose (Hypromellose) 6cps was selected as the primary stabilizer and SDS as secondary stabilizer for steric stabilization of drug nanoparticles for bead milling trials. The primary and secondary stabilizers selected were able to produce stable nanosuspensions for conversion into solid intermediates for solid dosage form processing.

Conversion of nanosuspensions into solid intermediate

The nanoparticulate dispersions can be converted into solid intermediates for tabletting or capsule filling using conventional processes such as spray drying and freeze-drying [17,18]. The solid intermediate (granules) should have the ability to quickly separate into nanometer-sized drug crystals upon dispersion in water else, the surface area enhancement will not be fully utilized to achieve the desired bioavailability. Therefore, a key property of spray granules is their recovery, i.e., their ability to reconstitute into nanometer-sized particles when dispersed in an aqueous medium or physiologically relevant media. The particle size distribution of nanosuspensions and granules following re-dispersion in an aqueous medium is summarized in Table 2.

The results demonstrated the particle size distribution for nanosuspension and granules upon redispersion in water were comparable indicating complete recovery. The particle recovery on storage at room temperature for 3 months is summarized in Table 3. The result indicated that the re-dispersity behavior of granules was unaffected up on storage.

Solid-State characterization

The crystallanity of drug substance (GB, FE and CA) following milling accessed using DSC and X-ray diffraction techniques indicated no polymorphic transitions. The absence of solid-state transitions may be attributed to the fact that milling was performed under controlled temperature conditions and the aqueous phase effectively dissipated the heat generated during processing.

Solubility studies

Saturation solubility of solid intermediates containing drug nanoparticles was evaluated in 0.1N HCl, acetate buffer pH 4.5, Phosphate buffer pH 6.8 and purified water at physiological temperature (37°C) and compared with ”as is” and jet-milled drug are summarized in Table 4.

The saturation solubility of drug nanoparticles was significantly higher than jet-milled microparticles at all pH conditions. These results clearly demonstrate that reduction in

particle size to sub-micron or nanometer range affects saturation solubility that may result in enhancement in dissolution rate and concomitantly higher bioavailability

Compression into tablets

The formula compositions of tablets incorporating drug nanoparticles are summarized in Table 5. The physical properties of the tablets - hardness, friability and disintegration time are summarized in Table 6. The hardness of tablets ranged from 8 - 10 Kp based on their strengths. The friability of tablets was < 0.1% indicating good mechanical strength. The disintegration values obtained indicated that among the different disintegrants evaluated, Croscarmellose sodium showed faster disintegration for Fenofibrate and Crospovidone for Glyburidea and Candesartan cilexetil respectively.

Dissolution studies

The dissolution characteristics of tablet formulations incorporating drug nanoparticles in discriminating dissolution media are shown in Figures 1, 2 and 3. The rate and extent of drug dissolution for nanoparticle Fenofibrate formulation was comparable to the marketed product (Tricor®). The dissolution rates of tablet formulation incorporating drug nanoparticles of Glyburide and Candesartan cilexetil were significantly higher as compared to commercial formulations Daonil® and Candelong® respectively. The increase in dissolution rates may be attributed to their smaller particle size, increased surface area and solubilizing effect of surfactant (SDS). The enhanced dissolution rate of nanoparticle formulations correlates with higher absorption and improved bioavailability.

CONCLUSION

The results from these studies demonstrate that media milling process coupled with spray granulation is a viable approach for developing nanoparticle formulations of class-II drugs with enhanced solubility and faster dissolution. Enhancing solubility and dissolution rate of sparingly soluble compounds correlates with faster absorption and enhanced bioavailability resulting in improved therapeutic outcome. The approach described herein could be extended to BCS class IV drugs (absorption is solubility and permeability limited) if combined with agents that can enhance permeability.

Table 1: Formula composition of drug nanosuspensions

Suspension Formulation	Drug concentration (% w/v)	sodium dodecyl sulfate (% w/v)	Hypromellose (6 cps) (% w/v)	Batch size (mL)
Glyburide	10.00	0.25	1.00	5000
Fenofibrate	10.00	0.15	1.00	5000
Candesartan cilexetil	10.00	1.00	2.00	5000

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Table 2: Particle recovery from granules (in µm)

Particle Size Distribution	GB-NS	GB-SG	FE- NS	FE-SG	CA-NS	CA-SG
d (0.9)	0.274	0.274	0.546	0.935	0.269	0.331
d (0.5)	0.134	0.129	0.155	0.248	0.127	0.134
d (0.1)	0.067	0.064	0.072	0.082	0.065	0.067

GB-NS: Glyburide nanosuspension, FE-NS: Fenofibrate nanosuspension and CA-NS: Candesartan cilexetil nanosuspension.
GB-SG: Glyburide spray granulated, FE-SG: Fenofibrate spray granulated and CA- SG: Candesartan cilexetil spray granulated.

Table 3: Particle size recovery from granules stored at 25°C/60%RH for 3 months

Particle Size Distribution	GB –SG		FE-SG		CA –SG	
	initial	3 M*	initial	3 M*	initial	3 M*
d (0.9)	0.274	0.276	0.935	0.938	0.331	0.334
d (0.5)	0.129	0.131	0.248	0.251	0.134	0.136
d (0.1)	0.064	0.064	0.082	0.082	0.067	0.066

GB-NS: Glyburide nanosuspension, FE-NS: Fenofibrate nanosuspension and CA-NS: Candesartan cilexetil nanosuspension.
GB-SG: Glyburide spray granulated, FE-SG: Fenofibrate spray granulated and CA-SG: Candesartan cilexetil spray granulated.
M*: Months

Table 4: Saturation Solubility of un-milled and spray granulated nanosuspensions

Solvent	GB As-is	GB Nano	FE As-is	FE Nano	CA As-is	CA Nano
0.1 N HCl	1.46	15.41	0.011	0.134	1.22	58.53
Acetate buffer pH 4.5	2.21	14.52	0.001	0.106	0.21	0.48
Phosphate buffer pH 6.8	2.45	16.16	0.001	0.105	0.10	0.45
Purified Water	0.40	15.92	BL*	0.073	BL*	0.41

*Below Detectable Limit

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Table 5: Tablet dosage formula compositions

Ingredients (in mg)	Glyburide	Fenofibrate	Candesartan
Intermediate formulation*	140.75	496.6	78.00
Microcrystalline cellulose	-	142.7	-
Croscarmellose sodium	-	90.00	-
Crospovidone	6.25	-	10.00
Colloidal Silicon Dioxide	1.50	7.50	1.00
Magnesium Stearate	1.50	7.50	1.00
Total (mg)	150.00	750.00	90.00

* Intermediate formulation containing drug, primary and secondary stabilizers.

Table 6: Physical properties of Tablets

Formulations*	Hardness (kPa)	DT(minutes)	Friability (%)
Glyburide	7.7 ± 0.12	3.1 ± 1.6	0.02
Fenofibrate	9.4 ± 0.24	8.0 ± 2.4	0.09
Candesartan	8.8 ± 0.42	10.0 ± 1.2	0.03

* Formulations containing drug nanoparticles

Fig 1: Comparative dissolution profile of Glyburide tablets containing drug nanoparticles and commercial formulation in phosphate buffer (pH – 6.8).

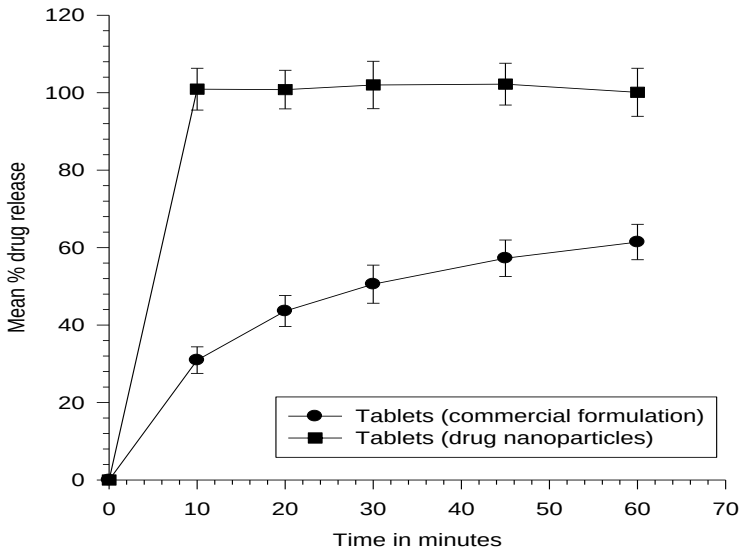

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Fig 2: Comparative dissolution profile of Fenofibrate tablets containing drug nanoparticles and commercial tablet formulation in Acetate buffer (pH 4.5).

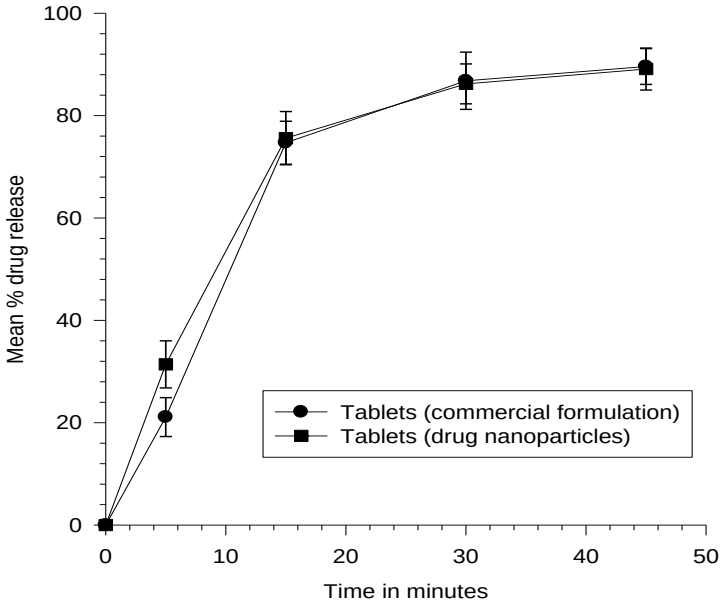
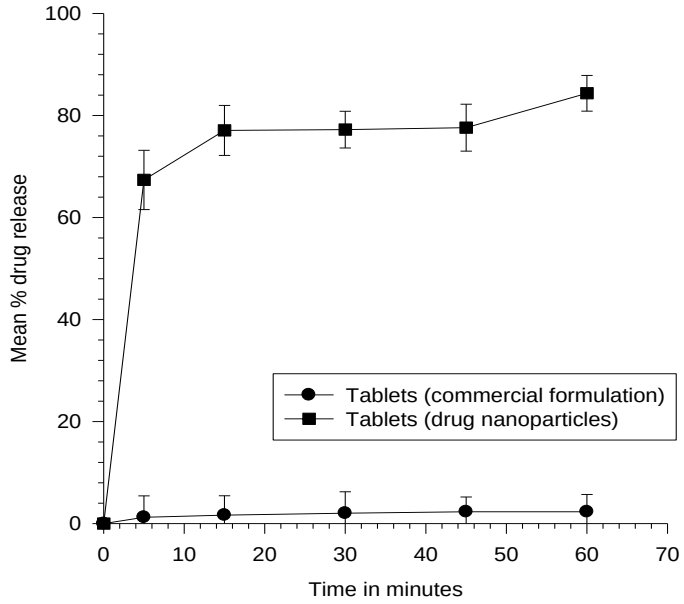


Fig 3: Comparative dissolution profile of Candesartan cilexetil tablets containing drug nanoparticles and commercial formulation in phosphate buffer (pH – 6.8).



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