WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 902-928. Research Article ISSN 2277– 7105

DEVELOPMENT OF AGGLOMERATED CRYSTLAS OF IRBESARTAN BY SPHERICAL CRYSTALIZATION TECHNIQUE FOR ENHANCING THE MICROMERITIC AND SOLUBILITY PROPERTY

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Article Received on
24 Nov 2015,

Revised on 15 Dec 2015,
Accepted on 04 Jan 2016

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ABSTRACT

Irbesartan practically insoluble in water. The present aim of the work is to increase the solubility by spherical crystallization method and convert into a tablet. N, N dimethyl formamide as a good solvent, bridging solvent chloroform & bad solvent water was selected. Spherical crystals are prepared by using β-cyclodextrin and HP-β-cyclodextrin in various ratios by quasi emulsion solvent diffusion method spherical agglomerates are prepared and converted into orodispersible tablets by direct compression technique. Various super disintegrating agents (SSG, Croscarmalose and crospovidone) F36 of 1:3 rations shown high dissolution efficiency of 98.09%. ANOVA significance value of $P<0.05$ which will indicates the co-processing parameters variability within the specified limits.

KEYWORDS: Irbesartan and crospovidone.

INTRODUCTION

The first step in the formulation is often milling or granulation, in order to provide for better properties for the final tabletting or to increase bioavailability. Often very small particles are required in order to increase the dissolution rate and reach sufficient bioavailability. However, micronisation by milling is extremely inefficient, can cause physical and chemical instability and produces powders with a wide size distribution and poor flowability. The alternative is to produce quite small crystals directly in the crystallization. In some cases thin needles are produced having a high surface area to volume ratio, but likewise may be quite

difficult to handle. An interesting alternative is to manufacture larger particles in situ by agglomeration of the small crystals during the crystallization. In addition, it has been revealed that agglomerates have properties that make suitable for direct compression tabletting. Crystals could be generated employing any of the available techniques like sublimation, solvent evaporation, vapor diffusion, thermal treatment and crystallization from melt precipitation by change in pH, growth in presence of additives or the grinding.[2] Thus the novel agglomeration technique that transforms crystals themselves directly into a compacted spherical form during crystallization process has been desired.[3] the use of spherical crystallization as a technique appears to be efficient alternative for obtaining suitable particles for direct compression.[4,5]

This technique of particle design of drugs has emerged as one the areas of active research currently of interest in pharmaceutical manufacturing and recently came into the forefront of interest or gained interest due to the fact that crystal habit can be modified during crystallization process which would result in better micrometric properties like particle size those can enhance the flowability of the powder drug and prepared spherical crystals can be compress directly without performing granulation, drying and so many steps those are require in wet granulation and in dry granulation process of tablet manufacturing.

Spherical crystallization

Spherical crystallization is a particle design technique, by which crystallization and agglomeration can be carried out simultaneously in one step and which has been successfully utilized for improvement of flowability and compactability of crystalline drugs.[6]

The various parameters optimized were type, amount and mode of addition of bridging liquid, temperature and agitation speed to get maximum amount of spherical crystals. These were characterized for micromeritic properties (particle size and shape, flowability), packability (bulk density), wettability (contact angle) and compressibility. It was revealed from the studies that spherical agglomerates exhibited improved solubility, flowability, wettability and compaction behavior.[7,8]

The present study, an attempt was made to improve physicochemical properties by preparing spherically agglomeration of Irbesartan in the presence of hydrophilic carrier for the enhancement of overall physicochemical performance. Therefore, in the present study, an

attempt has been made to increase solubility of Irbesartan by spherically agglomeration technique.

IMMEDIATE RELEASE

These preparations are primarily intended to achieve faster onset of action for drugs such as analgesics, antipyretics and coronary vasodilators. Other advantages include enhanced oral bioavailability through transmucosal drug delivery and pregastric absorption, convenience in administration to dysphasic patients, especially the elderly and bedridden and new business opportunities.

Conventional IR formulations include fast disintegrating tablets and granules that use effervescent mixtures, sodium carbonate (sodium bicarbonate) and citric acid (Tartaric acid) and super disintegrants such as sodium starch glycolate, Croscarmellose sodium and Crospovidone. Current technologies in fast dispersing dosage forms include modified tabletting systems, floss or shear form technology, which employs application of centrifugal force, controlled temperature. The present aim of the work is to enhance the solubility and dissolution rate of Irbesartan by spherical agglomeration method.

EXPERIMENTAL WORK

Phase solubility studies of Irbesartan[9,10]

Phase solubility studies were performed according to method reported by Higuchi and Connors. Excess (usually more than1mg/mL concentration) of drug was added to each 25mL of different pH Buffer solutions (pH 1.2 to 7.4), distilled water alone and combination with 0.5%, 1%, 2% SLS taken in stopperred conical flasks and mixture were shaken for 24hrs in rotary flask shaker. After shaking to achieve equilibrium, 2ml aliquots were withdrawn at 1hr intervals and filtered through Whatman filter paper. The filtrate was diluted if necessary and analyzed by UV- spectrophotometer at 244 nm. Shaking was continued until three consecutive readings were same.

Table No: 1.

Solvent	Amount soluble (Irbesartan) in mg/ml
0.1N Hcl (1.2 pH)	1.71
pH 2.0	0.145
pH 3.0	0.091
pH 4.5	0.065
pH 6.8	0.181

pH 7.4	0.801
Distilled Water	0.005
Distilled Water + 0.5% SLS	1.03
Distilled Water + 1% SLS	1.45
Distilled Water + 2% SLS	0.50

Preparation of Irbesartan Spherical agglomerates[11]

All spherical agglomerates were prepared by the quasi emulsion solvent diffusion method. Irbesartan (1g) with β-Cyclodextrin /HP- β-Cyclodextrin, PVP K-90/ PVA were dissolved in good solvent N, N-dimethylformamide (12.0 mL). The bridging liquid chloroform (2.0 mL) was added to it. The resulting solution was then poured drop wise in to the poor solvent distilled water (100 mL) containing Aerosil 200 Pharma (0.1 g). The mixture was stirred continuously for a period of 0.5 h using a controlled speed mechanical stirrer (Remi motors, India) at 1000 rpm. As the good solvent diffused into the poor solvent, droplets gradually solidified. Finally the co precipitated microspheres of the drug-polymer were filtered through Whatman filter paper (No.1) and dried in desicator at room temperature. The amount of stabilizer was altered to get desired agglomerates.

Table No: 2.

Formulation Number	Irbesartan (mg)	β cyclodextrin (mg)	HP β-cyclodextrin (mg)	PVP K-90 (mg)	PVA (mg)	N,N-dimethyl Formamide (ml)	Water (ml)	Chloroform (ml)
F19	1000	500	--	--	--	25	62.5	12.5
F20	1000	750	--	--	--	25	62.5	12.5
F21	1000	1000	--	--	--	25	62.5	12.5
F22	1000	--	500	--	--	25	62.5	12.5
F23	1000	--	750	--	--	25	62.5	12.5
F24	1000	--	1000	--	--	25	62.5	12.5
F25	1000	--	--	500	--	25	62.5	12.5
F26	1000	--	--	750	--	25	62.5	12.5
F27	1000	--	--	1000	--	25	62.5	12.5
F28	1000	--	--	--	500	25	62.5	12.5
F29	1000	--	--	--	750	25	62.5	12.5
F30	1000	--	--	--	1000	25	62.5	12.5

Evaluation of spherical agglomerates[12]

a) Particle size determination

Particle size determination was carried out using optical microscopy with a calibrated eye piece micrometer and stage micrometer by taking a small quantity of formulation on slide. About 100 spherical agglomerates size was measured individually, average was taken and

their size range and mean diameter frequency was calculated. Average Particle size is calculated by the following formula, Average Particle size= £nd/ n.

b) Solubility studies

The solubility of spherical agglomerates in water was determined by taking excess quantity of spherical agglomerates and adding to screw- capped 50 ml glass vials filled with water. The vials were shaken for two hours on mechanical shaker. The solution was filtered through Whatmann filter paper No.1 and the drug concentration was determined spectrophotometrically at 244 nm.

Table No: 3.

Formulation	Particle size (µm)	Solubility (mg/ml)
Pure drug	-	0.005
F16	256	0.0636
F17	278	0.0747
F18	294	0.0866
F19	312	0.0563
F20	334	0.0649
F21	356	0.0758
F22	346	0.0441
F23	367	0.0526
F24	386	0.0652
F25	378	0.0332
F26	394	0.0428
F27	413	0.0542

c) Drug Content Estimation[13]

The percentage drug content in spherical agglomerates was estimated by dissolving 50 mg of spherical agglomerates in methanol, mixed thoroughly by shaking and the volume was made up to the mark with in 0.1N Hcl (1.2 pH). The solution was filtered and the filtrate was diluted suitably with 0.1N Hcl (1.2 pH) and absorbance was measured at 244 nm using UV/Visible spectrophotometer.[12]

Table No: 4.

Formulation	% of Drug content
F19	99.26
F20	99.54
F21	99.36
F22	99.41
F23	99.39
F24	99.24

F25	99.45
F26	99.73
F27	99.21
F28	99.22
F29	99.33
F30	99.11

d) Dissolution studies of agglomerates[13]

In-vitro dissolution studies of pure drug and spherical agglomerates were carried out for 60 minutes using USP Dissolution test apparatus type II (Lab India DISSO 2000, eight stages) at 50 rpm.Spherical agglomerates equivalent to 75 mg of pure drug (Irbesartan) used for dissolution study at 37±0.5^0 C in 900ml of 0.1N Hcl (1.2 pH) as dissolution medium. Aliquot equal to 5 ml was withdrawn at regular time intervals (10, 20, 30, 40, 50, 60 min), an equal volume of fresh dissolution medium was replaced to maintain the sink condition and aliquots were measured at 244 nm UV/Visible spectrophotometer. DE_{30}%, T_{50}, T_{90} and k^{-1} values were calculated from dissolution data.

In-vitro dissolution data of Irbesartan spherical agglomerates prepared with B-cyclodextrin in different ratios

Table No: 5.

S.No.	Sampling time (min)	Cumulative % of drug dissolved ($\overline{X}$ ± S.D.)			
		Pure Drug	F $_{19}$	F $_{20}$	F $_{21}$
1	0	0	0	0	0
2	10	4.22	33.34	37.79	40.42
3	20	6.08	42.44	48.23	50.34
4	30	8.16	61.03	65.02	67.40
5	40	10.35	70.02	75.87	79.31
6	50	12.75	81.94	86.25	88.40
7	60	15.15	89.47	93.28	95.44

Dissolution profiles of Irbesartan pure drug and spherical agglomerates prepared with B-cyclodextrin in different ratios.

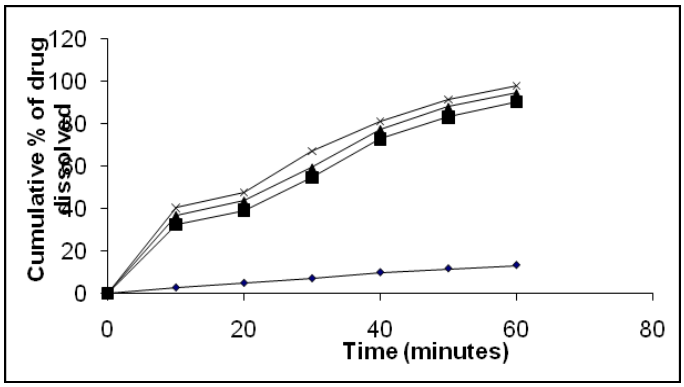

Figure No: 1.

(-♦-) Irbesartan pure drug.

(-■-) Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 0.5 ratio.

(-▲-)Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 0.75 ratio.

(-×-) Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 1 ratio.

First order plots of Irbesartan pure drug and spherical agglomerates prepared with B-cyclodextrin in different ratios

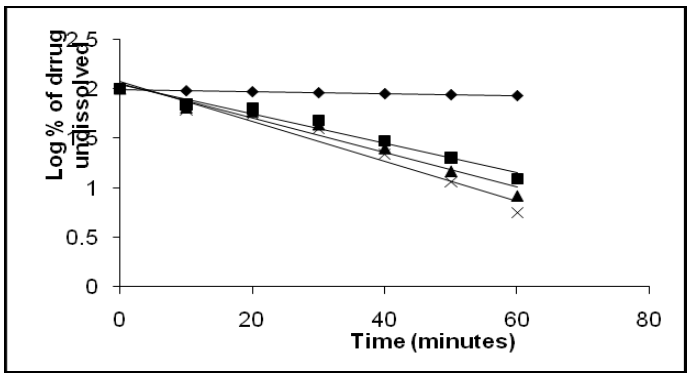

Figure No: 2.

(-♦-) Irbesartan pure drug.

(-■-) Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 0.5 ratio.

(-▲-)Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 0.75 ratio.

(-×-) Spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1: 1 ratio.

***In-vitro* dissolution kinetics of Irbesartan spherical agglomerates prepared with B-cyclodextrin in different ratios**

Table No: 6.

S.No.	Formulation	T_{50} (min)	T_{90} (min)	DE_{30} (%)	K (min^{-1})	Correlation coefficient values	
						Zero Order	First order
1	F_{19}	20.2	67.1	35.43	0.034	0.9466	0.9870
2	F_{20}	17.2	57.0	39.51	0.040	0.9250	0.9836
3	F_{21}	15.4	51.2	41.49	0.044	0.9135	0.9768

Statistical treatment for dissolution efficiencies of Irbesartan spherical agglomerates prepared with B-cyclodextrin in different ratios

Table No: 7.

Trial	Dissolution efficiencies (%) (DE_{30})			ANOVA Parameters		
	F_{19}	F_{20}	F_{21}	Calculated value (F)	Degree of freedom	Significance
1	35.54	39.67	41.84	434.02	2,6	P<0.05
2	35.11	39.45	41.23			
3	35.64	39.41	41.40			

Preparation of Irbesartan immediate release Tablets containg superdisintegrants[14]

Irbesartan containing immediate release tablets were prepared by direct compression process. All the ingredients (shown in Table No:-50&54) were properly mixed and passed through mesh no. 80. The resulting blend was lubricated with magnesium stearate and talc and compressed into tablets using the Cadmach sixteen stationary punching (round shaped, 7mm thick) machine.

Table No:-8

Ingredients (Mg)	**F_{31}**	**F_{32}**	**F_{33}**
Irbesartan agglomerates	150	150	150
Sodium Starch Glycolate(SSG)	12.5	--	-
Croscarmalose sodium	--	12.5	--
Crospovidone	--	--	12.5
Manitol	7.5	7.5	7.5
Avicel pH 102	76	76	76
Talc	2	2	2
Mg streate	2	2	2
Total weight	250	250	250

Preparation of Irbesartan immediate release Tablets containg co processed superdisintegrants[15]

Irbesartan containing immediate release tablets were prepared by direct compression process. All the ingredients (shown in Table 5.37) were properly mixed and passed through mesh no. 80. The resulting blend was lubricated with magnesium stearate and talc and compressed into tablets using the Cadmach sixteen stationary punching (round shaped, 7mm thick) machine.[111]

Table No: 9.

Co processed superdisintegrants composition ratio (Mg)	**1:1**	**1:2**	**1:3**
Ingredients	**F_{34}**	**F_{35}**	**F_{36}**
Irbesartan Agglomerates	150	150	150
Croscarmalose sodium+Crospovidone	12.5	12.5	12.5
Manitol	7.5	7.5	7.5
Avicel pH 102	76	76	76
Talc	2	2	2
Mg streate	2	2	2
Total weight	250	250	250

Evaluation of micromeritic properties of the blend[16-22]

The powder blend of immediate release tablets of Irbesartan were evaluated for bulk density, tapped density, carr's index, Hausner's ratio and angle of repose as per the procedures specified earlier for olmesartan medoxomil orodispersible tablets powder blend.

Table No: 10.

Formulation code	Bulk density (gm/cm^3)	Tapped Densitygm/cm^3)	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F_{34}	0.435	0.523	16.82	1.20	27.42
F_{35}	0.463	0.551	15.97	1.19	26.56
F_{36}	0.484	0.572	15.38	1.18	25.27

Evaluation of Irbesartan immediate release Tablets

The prepared tablets were evaluated for Weight variation test, disintegration time; friability, hardness and wetting time were as per the procedures specified earlier for olmesartan medoxomil orodispersible tablets.

a) Weight variation test

Weight variation test was done by weighing 20 tablets individually, calculating the average weight and comparing the individual tablet weight to the average weight.

b) Disintegration Time

The disintegration time was determined in distilled water at 37 ± 0.5^0C using disintegration test apparatus[11] USP ED-2L (Electro lab, Mumbai).

c) Friability

Roche Friabilator was used to determine the friability. Pre weighed tablets were placed in Friabilator and rotated at a speed of 25 rpm for 4 minutes or up to 100 revolutions. The tablets are dropped from a distance of 6 inches in each revolution. The tablets were then reweighed after removal of fines and the percentage of weight loss was calculated.

d) Hardness

Hardness of the tablet was determined using the Monsanto hardness tester. The lower plunger was placed in contact with the tablet and a zero reading was taken. The plunger was then forced against a spring by tuning threaded bolts until the tablet fractured. Then the final reading was recorded. The hardness was computed by deducting the initial pressure from the final pressure.

e) Drug content

Twenty tablets were powdered and 75 mg equivalent weight of Irbesartan in tablet powder was accurately weighed and transferred into a 100 ml volumetric flask. Initially, 5 ml methanol was added and shaken for 10 min. Then, the volume was made up to 100 ml with 0.1N Hcl (1.2 pH). The solution in the volumetric flask was filtered, diluted suitably and analyzed spectrophotometrically at 244 nm using UV-visible spectrophotometer.

Table No: 11.

S.No	Parameters	F_{34}	F_{35}	F_{36}
1	Average weight (mg)	250+0.3	250+0.1	250+0.2
2	Drug content (%)	98.54	99.8 1	99.19
3	Disintegration time (sec)	163	147	125
4	Friability (%)	0.44	0.28	0.13
5	Hardness(kg/sqcm)	4.2	4.2	3.8

f) Dissolution studies

Dissolution studies for immediate release tablets of Irbesartan were performed in 0.1N Hcl (1.2 pH) using USP dissolution test apparatus (Electrolab, Mumbai, India) with a paddle stirrer. The paddles were allowed to rotate at speed of 100 rpm. The dissolution medium was maintained at a temperature of 37+0.5^{O}C and samples were withdrawn at an interval of every 5 min the volume of the withdrawn samples were replaced by fresh dissolution medium in order to kept the volume of the dissolution medium as constant. The withdrawn samples were filtered and absorbance was measured at absorption maxima of 244 nm using UV-visible spectrophotometer. The in vitro dissolution kinetic parameters, dissolution rate constants (K^{-1}), correlation coefficient (r), the times (t_{50}) for 50% drug released (t_{50}), the times for 90% drug released (t_{90}) and dissolution efficiency [D.E.] were calculated.

Table No: 12.

S.No	Sampling time (min)	Cumulative % of drug dissolved ($\overline{X}$ ± S.D.)		
		F_{31}	F_{32}	F_{33}
1	0	0	0	0
2	5	19.44	23.11	25.73
3	10	41.57	45.26	51.57
4	15	52.03	55.48	62.35
5	20	62.28	66.01	75.54
6	25	72.06	75.82	84.61
7	30	78.23	81.48	90.84
8	35	85.21	89.00	95.01

9	40	88.56	92.11	98.41
10	45	92.19	95.75	--
11	50	94.78	98.10	--
12	55	96.86	--	--
13	60	98.95	--	--

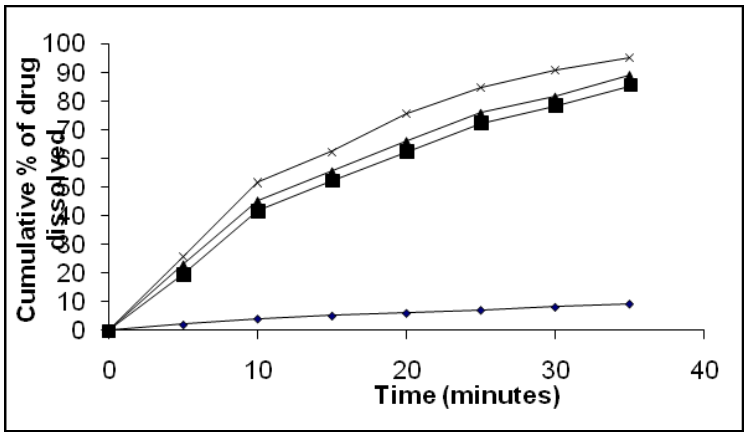

Figure No: 3.

(-♦-) Irbesartan pure drug.

(-■-) Irbesartan tablets prepared with sodium starch glycolate.

(-▲-)Irbesartan tablets prepared with croscarmalosesodium.

(-×-) Irbesartan tablets prepared with crosspovidone.

First order plots of Irbesartan Immediate Release tablets prepared with superdisintegrants

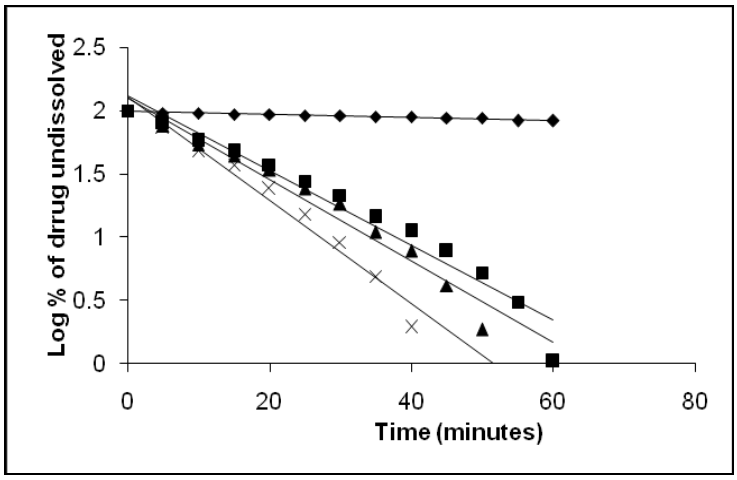

Figure No: 4.

(-♦-) Irbesartan pure drug.

(-■-) Irbesartan tablets prepared with sodium starch glycolate.

(-▲-)Irbesartan tablets prepared with croscarmalosesodium.

(-×-) Irbesartan tablets prepared with crosspovidone.

***In-vitro* dissolution kinetics of Irbesartan Immediate Release tablets prepared with various superdisintegrants**

Table No: 13.

S.No.	Formulation	T_{50} (min)	T_{90} (min)	DE_{15} (%)	K (min^{-1})	Correlation coefficient values	
						Zero Order	First order
1	F_{31}	11.3	37.6	47.75	0.061	0.8520	0.9708
2	F_{32}	10.3	34.3	51.07	0.067	0.8806	0.9764
3	F_{33}	8.0	26.6	57.54	0.086	0.8973	0.9707

Statistical treatment for dissolution efficiencies of Irbesartan Immediate Release tablets prepared with various superdisintegrants

Table No: 14.

Trial	Dissolution efficiencies (%) (DE_{15})			ANOVA Parameters		
	F_{31}	F_{32}	F_{33}	Calculated value (F)	Degree of freedom	Significance
1	47.71	51.14	57.37	278.97	2,6	P<0.05
2	47.12	51.36	57.13			
3	48.42	50.71	58.12			

Comparison for dissolution efficiencies of Irbesartan Immediate Release tablets prepared with various superdisintegrants

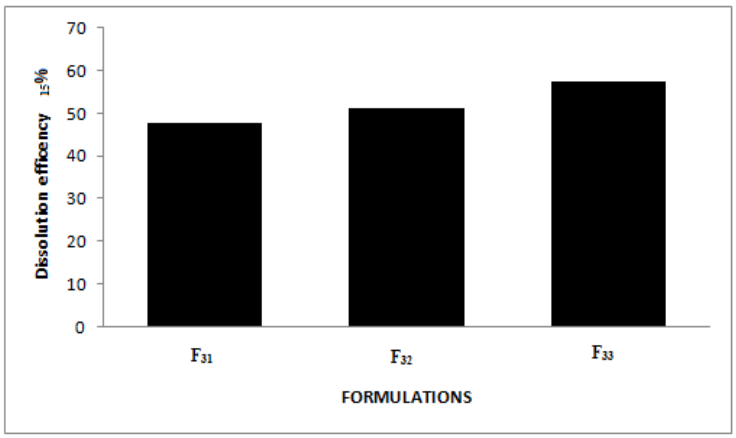

Figure No: 5.

SEM Analysis

The samples for the SEM analysis were prepared by sprinkling the spherical agglomerates on one side of the double adhesive stub.[114] The stub was then coated with fine gold dust. The spherical agglomerates were then observed with the scanning electron microscope (Leica Electron Optics, Cambridge, USA) at 10 kV.

Scanning Electron Microscope Photograph of Irbesartan agglomerates

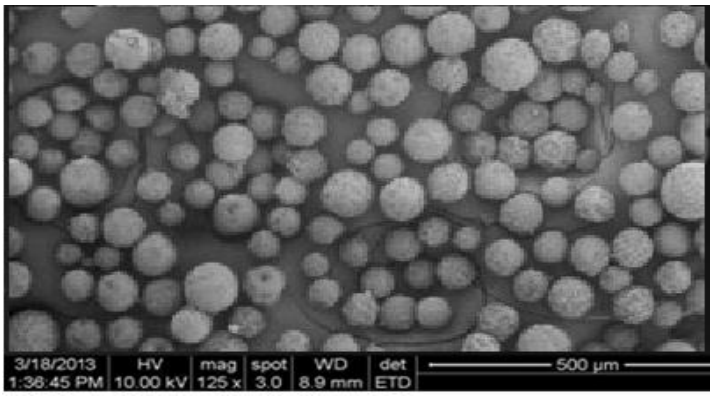

Figure No: 6.

Stability study[22]

the optimized formulation was subjected to accelerated stability studies as per ICH (The International Conference of Harmonization) guidelines. The optimized formulations were filed into packed in the screw capped bottles and stored at 25 ± 2ºC, 60 ± 5% RH and at 40 ± 2ºC, 75 ± 5% RH for 3 months. Tablets were periodically removed and evaluated for physical characteristics and in-vitro drug release.

Table No: 15.

Time (Min)	Initial	Percentage of olmesartan medoximil orodispersible tablet Released (± sd)					
		25±2^0 C/60±5% RH			40±2^0 C/75±5% RH		
		1st month	2nd month	3rd month	1st month	2nd month	3rd month
0	0	0	0	0	0	0	0
5	63.95	63.91	63.87	63.82	63.84	63.78	63.74
10	88.39	88.27	88.24	88.19	88.22	88.17	88.13
15	94.29	94.23	94.28	94.24	94.25	94.21	94.17
20	99.00	98.72	98.67	98.61	98.64	98.59	98.54

Dissolution Kinetics of Irbesartan Immediate Release tablets stored at 25±2^{0}C/60±5% RH and 40±2^{0}C/75±5% RH.

Table No: 16.

Storage conditions	Time interval	K (min^{-1})	T $_{50}$ (min)	T $_{90}$ (min)	DE $_{15}$ (%)
25±2^0 C/ 60±5% RH	1st month	0.18	3.7	12.3	62.38
	2nd month	0.18	3.7	12.3	62.38
	3rd month	0.18	3.7	12.3	62.38
40±2^0 C/ 75±5% RH	1st month	0.18	3.7	12.3	62.38
	2nd month	0.18	3.7	12.3	62.38
	3rd month	0.18	3.7	12.3	62.38

Pharmacokinetic evaluation of Irbesartan immediate release tablets[24]

The pharmacokinetic performance of Irbesartan immediate release tablets was studied in a randomized crossover study design in rabbits. Twelve healthy rabbits with a mean age of 10±2 weeks and with a mean body weight of 3±0.2 kg were used. Two groups of rabbits with 6 in each were fasted for 12 hrs prior to study. The animal dose of pure Irbesartan and its immediate release tablets was calculated relevant to human dose. A dose of 1mg/kg of pure Irbesartan and 1mg/kg Irbesartan equalent immediate release tablets were administered orally in the form of suspension for two groups of rabbits. The rabbits were restrained in a wooden rabbit holder. The ears of the rabbits were cleaned and the hair was removed with the help of depilatory. Before withdrawal, the ear veins were dilated by swabbing with cotton or by application of warm water. The marginal ear vein of the left ear was punctured with a help of a 24 gauge needle. About 1 ml of blood samples were drawn at 0 (before drug administration), 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs after pure drug administration and at 0,1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs after administration of Irbesartan immediate release tablets.[27] Blood sample volume was replaced by administration of isotonic saline. The blood samples were collected in a micro centrifuge tube and centrifuged at 3500 rpm for 10 min. Later the plasma was collected and utilized for estimation of Irbesartan concentration.

Estimation of Irbesartan in rabbit plasma by HPLC[25]

HPLC method is a sensitive and accurate method that provides a good choice to study the pharmacokinetics of Irbesartan *in vivo*. A summary of the chromatographic conditions used in HPLC is as follows**.**

Chromatographic conditions

Chromatograph: Waters 2695 liquid chromatogram.

Mobile phase: Acetronitrile: Phosphate buffer (80:20% v/v) pH adjusted to 3.5 with orthophosphoric acid.

Internal standard: Losartan potassium

Column: XterraC_{18} Size - 100×4.60 mm. 5 µm.

Flow rate: 0.6 ml/min

Detector: UV-Visible detector-2487 Dual absorbance λ detector

Wave length: 253 nm

Injection volume: 20 µl

Temperature: Ambient

Retention time of the analyte: 7.461 min

Retention time of the Internal std: 2.363 min

Total run time: 10 min

Soft ware: Empower 2

Table No: 17.

Concentration of Irbesartan (µg/ml)	Peak Area Ratio			
	Trail – 1	Trail – 2	Trail – 3	Mean + S.E.M
0.1	0.0134	0.0156	0.0185	0.0158+ 0.0026
0.5	0.0427	0.0468	0.0396	0.0430+0.0036
1	0.1029	0.1069	0.1015	0.1037+0.0047
1.5	01585	0.1463	0.1624	0.1557+0.0081
2	0.2154	0.2298	0.2168	0.2207+0.087
2.5	0.2687	0.2764	0.2674	0.2705+0.008

Preparation of standard solutions

The stock standard solution of Irbesartan was prepared by dissolving the accurately weighed Irbesartan in methanol to give a final concentration of 1.0 mg/ml. The solution was then successively diluted with methanol to achieve 100 µg/ml and 5 µg/ml. An internal standard and working solution, of losartan potassium 10µg/ml was prepared as the former.

Preparation of plasma calibration curve samples

To 250 µl of plasma 20,100,200,300,400,500 µl of 5 µg/ml Irbesartan stock solution and 100 µl of internal standard metformin (10 µg/ml) was added. To this acetronitrile was added as precipitating agent to get a final volume of 1000 µl containing 0.1,0.5,1.0,1.5,2.0,2.5 µg/ml of Irbesartan respectively. The sample was vortexed for 10 min and then centrifuged at 5000 rpm for 10 min at room temperature. The supernatant layer was separated and filtered through 0.22 µm membrane filter and 20 µl of the filterate was injected and the calibration curve was constructed.[28] The peak area ratio was calculated and plotted against concentration of Irbesartan. From the plot peak area ratio versus concentration, the regression equation was computed and furnished below.

The regression equation is **y= 0.108x- 0.0035, R^2 = 0.0996.**

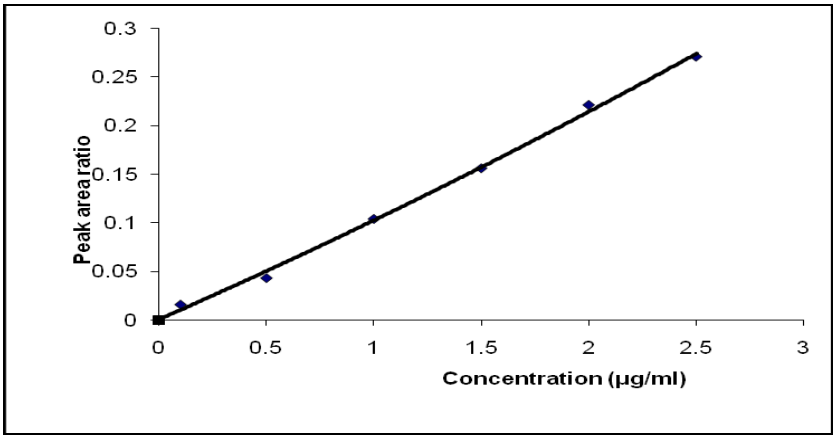

Figure No: 7.

Evaluation of pharmacokinetic parameters in rabbit serum[26,27]

The pharmacokinetic parameters of Irbesartan such as K_e (1/hr), $t_{1/2}$ (hr), K_a (1/hr), AUC $_{(0-\alpha)}$ (µg-hr/ml), MRT (hr), C_{max} (µg/ml), T_{max} (hr) were estimated by non-compartmental methods by using the formulas as describe earlier.

Table No: 18.

Time (h)	Plasma concentration (ng/ml) (Mean ± s.d)	
	Pure drug	Irbesartan Immediate Release tablets
0	0	0
0.5	27.52 ±1.32	82.34 ±0.25
1	31.24±1.45	94.83±0.15
1.5	34.67±1.53	102.36±0.51
2	39.54±1.12	113.45±0.13
3	46.75±1.43	124.83±0.72
4	48.28±1.23	135.47±0.49
5	50.46±1.24	145.43±0.55
6	54.49±1.37	158.46±0.47
8	57.45±1.68	147.17±1.52
10	60.39±1.54	137.42±1.59
12	67.56±1.24	129.52 ±1.68
14	64.35±1.26	119.64±1.26
16	59.46±1.47	105.52±1.38
18	48.72±1.39	97.45±1.15
20	35.67±1.214	83.62±1.67
24	22.43±1.28	77.34±1.38

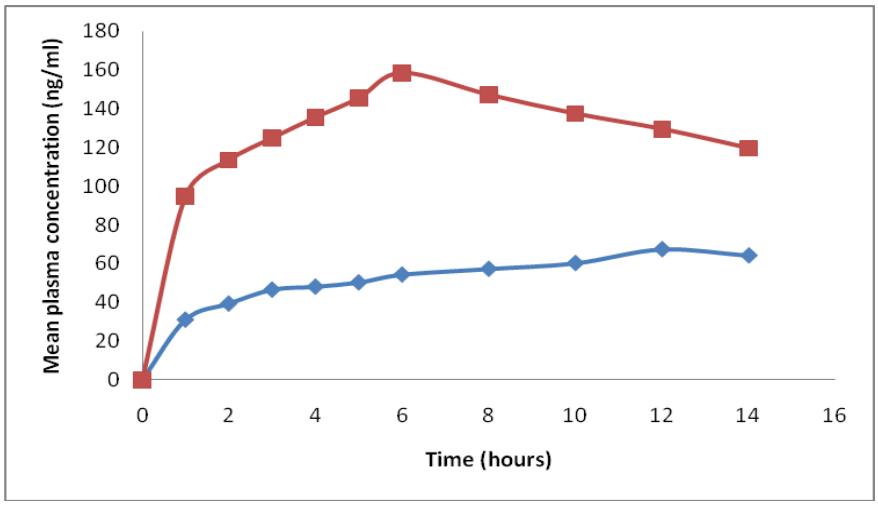

Figure No: 8.

(-♦-) Plasma Concentration-Time Curve of Irbesartan following pure drug administration.

(-■-)Plasma Concentration-Time Curve of Irbesartan following optimized Irbesartan Immediate Release tablets administration.

HPLC chromatogram showing Irbesartan and internal standard peaks

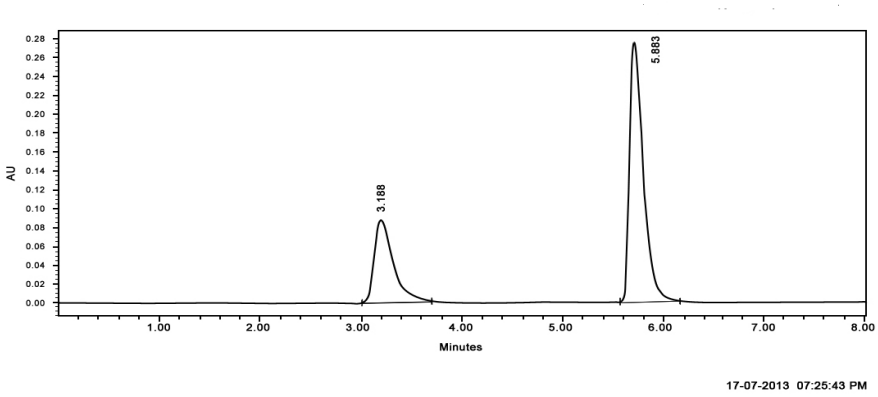

Figure No: 9.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Losartan	3.188	1216325	291018
2	Irbesartan	5.883	2502566	89101

Statistical Treatment of Pharmacokinetic Parameters (Mean ± S.D.) of Irbesartan obtained with pure drug and Irbesartan Immediate Release tablets

Table No: 19.

Pharmacokinetic parameter	Pure Drug	Irbesartan Immediate Release tablets	Calculated value of 't'
C_{max} (ng/ml)	67.56 ± 0.43	158.46 ± 0.15	12.53***
$t_{1/2}$ (h)	11.42 ± 0.32	6.15 ± 0.14	8.96***
K_{el} (h^{-1})	0.96 ± 0.004	0.82 ± 0.003	5.70***
K_a (h^{-1})	2.92 ± 0.01	7.76 ± 0.01	85.68***
$AUC_{0-\square}$ (ng h/ml)	126 ± 1.23	464.9.± 1.36	146.40***
Null hypothesis (H_o): There is no significant difference between the pharmacokinetic parameters of Irbesartan obtained with pure drug and Irbesartan Immediate Release tablets value of't' with 10 DF at the 0.001 level is 4.587.			
Result: H_o is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized Irbesartan Immediate Release tablets.			

RESULTS AND DISCUSSION

Phase solubility studies of Irbesartan

Phase solubility studies were done in various pH buffer solutions (pH 1.2 to 7.4), distilled water alone and combination with 0.5%, 1%, 2% SLS solutions. Phase solubility studies of Irbesartan were given in Table 5.28. From the phase solubility studies it was observed that the drug had shown more solubility in 0.1N Hcl (1.2 pH). Hence 0.1N Hcl (1.2 pH**)** was selected for the dissolution medium and standard calibration curve for the estimation of Irbesartan was carried out in 0.1N Hcl (1.2 pH). The method obeys Beer's law in the concentration range of 2-10µg/ml. Low RSD values ensured reproducibility of the method. Thus, the method was found to be suitable for the estimation of Irbesartan content in various products and *in-vitro* dissolution studies.

Preparation of Irbesartan Spherical agglomerates

All spherical agglomerates were obtained by the emulsion solvent diffusion method using distilled water as an external phase. The internal phase consisted of N, N-dimethylformamide which acts as good solvent and chloroform as a bridging liquid. The optimized parameters and procedure followed for the preparation of Irbesartan Spherical agglomerates were same as that of Olmesartan medoxomil spherical agglomeration.

The practical yield was found satisfactory and ranged from 91.22% to 95.61%. The presence of polymers in spherical agglomerates influenced the particle size of resultant agglomerates.

As the concentration of the polymers increased, the size of the agglomerates increased. The presence of surfactants/polymers on the particle surface increases particle–particle interaction, causing faster squeezing out of good solvent to the Surface, resulting in increased particle size. The primary particle size was also increased with an increase in surfactants/polymer content.

Evaluation of spherical agglomerates

Solubility study

The results of solubility study revealed that the spherical agglomerates with different surfactants/polymers showed increased solubility compared to the pure drug. This may be due to the improved porosity, decreased primary particle size and partial amorphization of drug in agglomerates. This may also be due to the improved wettability of spherical agglomerates in the presence of surfactants/polymers.

The drug content values were ranged from 96.11% to 98.39% and are shown in Table No:-38.

Dissolution studies of spherical agglomerates

Irbesartan spherical agglomerates prepared with B-cyclodextrin

The Irbesartan spherical agglomerates prepared with B-cyclodextrin exhibited better dissolution rate when compared with plain drug, which could be attributed to deposition of surfactants onto the recrystallized drug surface. The dissolution rate followed first-order kinetics as the graphs drawn between log % drug undissolved vs time were found to be linear. The dissolution rate of Irbesartan was found to be effected by the concentration of the surfactants used in the preparation of agglomerates. The *in vitro* dissolution studies of the spherical agglomerates prepared at 1:1(drug: β-cyclodextrin) ratio showed 95.44% release in 60 minutes. Based on the dissolution rate, the order of drug release from the three formulations was F_{21} (1:1) > F_{20}(1:0.75)> F_{19} (1:0.5).

A statistically significant difference between dissolution efficiencies (DE_{30}) of Irbesartan spherical agglomerates prepared with B-cyclodextrin in different ratios was calculated using a one-way analysis of variance (ANOVA). The P value was found to be less than 0.05, which indicates that there was a significant difference among F_{19}, $F_{20,}$ $F_{21,}$ with respect to dissolution efficiencies (DE_{30}).

Effect of different carriers on the dissolution

Incorporation of Hydrophilic polymer during agglomeration significantly enhanced the dissolution. Mixing of drug with a hydrophilic carrier (polymer) results in greater wetting and increase surface available for dissolution by reducing interfacial tension between the hydrophilic drug and dissolution media. It was noted that drug carrier system sink immediately, while pure drug keeps floating on the surface for a longer time interval. The cumulative percentage of drug released from different agglomerates was increased in the following order.

Agglomerates prepared with surfactants

Irbesartan spherical agglomerates prepared with β-cyclodextrin > Irbesartan spherical agglomerates prepared with HP β-cyclodextrin.

Agglomerates Irbesartan medoxomil spherical agglomerates prepared with PVP –K90> Irbesartan spherical agglomerates prepared with PVA.

Among all the formulations prepared, spherical agglomerates prepared with Irbesartan and B-cyclodextrin in 1:1 ratio showed highest drug release in 60 minutes.

B-cyclodextrin > HP β-cyclodextrin> PVP –K90> PVA

Influence of superdisintegrants on Irbesartan immediate release tablets

To study the influence of superdisintegrants on the performance of Irbesartan immediate release tablets, a set of three formulations (F_{31}, F_{32} and F_{33}) were prepared using three different superdisintegrants *viz*, Sodium starchglycolate (5%), Croscarmalose sodium (5%), Crospovidone (5%) respectively. The dissolution rate followed first-order kinetics as the graphs drawn between log % drug unreleased vs time were found to be linear. The dissolution rate of Irbesartan was found to be effected by nature of the superdisintegrants used in the preparation of tablets. Based on the dissolution rate, superdisintegrants can be rated as Crospovidone> Croscarmalose sodium> SSG.

The formulation prepared with Crospovidone was offered relatively rapid release of Irbesartan when compared with other superdisintegrants used in this investigation. A statistically significant difference between dissolution efficiencies (DE_{15}) of Irbesartan immediate release tablets prepared using three different superdisintegrants *viz*, Sodium starchglycolate (5%), Croscarmalose sodium (5%), Crospovidone (5%), was calculated using

a one-way analysis of variance (ANOVA). The P value was found to be less than 0.05, which indicates that there was a significant difference among F_{31}, F_{32} and F33 with respect to dissolution efficiencies (DE_{15}).

Influence of co processed superdisintegrants on Irbesartan immediate release tablets

To study the influence of co-processed superdisintegrants on performance of Irbesartan immediate release tablets, a set of three formulations (F_{34}, F_{35}, F_{36}) were prepared using co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) in three different ratios 1:1, 1:2, 1:3 respectively. All the tablets complied with the pharmacopoeial standards. The dissolution rate followed first-order kinetics as the graphs drawn between log % drug unreleased vs time were found to be linear. The dissolution rate of Irbesartan was found to be effected by ratio's of co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) used in the preparation of tablets. Based on the dissolution rate, the order of drug release from the three formulations was F_{36}> F_{35}> F_{34}. The formulation prepared with co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) in 1:3 ratio (F_{36}) was offered relatively rapid release of Irbesartan immediate release tablets when compared with other ratios employed in this investigation. The rate of drug release was found to be increased as the concentration of the Crospovidone increases in co-processed superdisintegrants of Croscarmalose sodium: Crospovidone.

A statistically significant difference between dissolution efficiencies (DE_{15}) of Irbesartan immediate release tablets prepared using co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) in three different ratios *viz*, 1:1, 1:2, 1:3. The resulting statistical parameters are shown in Table No:-57. The P value was found to be less than 0.05, which indicates that there was a significant difference among F_{36}, $F_{35,}$ F_{36} with respect to dissolution efficiencies (DE_{15}).

SEM Analysis

The pure olmesartan and Irbesartan powders were appeared in the form of irregular shaped crystals. This form of olmesartan and Irbesartan powders leads to very poor flow and compression difficulties. The photo-micrographs showed that the prepared agglomerates were regular in shape with a smooth and regular surface, which enabled them to flow very easily. On the basis of these findings, it was considered that good flowability and packability for agglomerates were attributed to the regular shape and smooth surface, since the area of

contacts in the powder bed for agglomerates was smaller than that for conventional irregular crystals.

Stability studies

The optimized formulations were stored at 25 ± 2ºC, 60 ± 5% RH and at 40 ± 2ºC, 75 ± 5% RH for 3 months. Drug release from optimized formulations before and after storage under varying conditions was evaluated periodically at the regular interval of every month. The results indicated that the drug release from the optimized formulations was not changed significantly when stored at varying conditions and the release data was shown no significant changes in the dissolution Kinetics of optimized formulations before and after storage under varying conditions. Thus the drug release from optimized formulations was found be quite stable.

***In vivo* evaluation**

The *in vivo* experiments were conducted as per the protocol and procedure described earlier. Pharmacokinetic parameters such as absorption rate constant, elimination rate constant, half life, AUC and AUMC were calculated from the plot of time versus plasma concentration and subjected to statistical analysis.

Pharmacokinetic evaluation of Irbesartan following pure drug administration and Irbesartan Immediate Release tablets administration

Plasma concentrations of Irbesartan at different times after pure drug administration and Irbesartan Immediate Release tablets administration were calculated. Statistical treatment of Pharmacokinetic Parameters of Irbesartan obtained with pure drug and optimized Immediate Release tablets were calculated. The results indicated that the parameters significantly differed following optimized Immediate Release tablets administration, compared to pure drug administration. The highest mean C_{max} value was observed for optimized Immediate Release tablets (158.46 ± 0.15ng/ml) compared to pure drug (67.56 ± 0.43 ng/ml). The mean time taken to peak plasma concentration for *(Tmax)* following administration of pure drug was 11.42 ± 0.32 hours, while it was 6.15 ± 0.14 hour following administration of selected optimized Immediate Release tablets. The elimination rate constant (Kel) for pure drug and optimized Immediate Release tablets were found to be 0.96 ± 0.004h^{-1} and 0.82 ± 0.003h^{-1} respectively. The absorption rate constant (Ka) for pure drug and optimized Immediate Release tablets were found to be 2.92 ± 0.01h^{-1} and 7.76 ± 0.01 h^{-1} respectively. The $AUC_{0-\alpha}$

values observed with optimized Immediate Release tablets 564.9.± 1.36 ng hr/ml in compared to pure drug values 126 ± 1.23 ng hr/ml.

Thus, the results of pharmacokinetic studies indicated rapid and higher oral absorption of Irbesartan when administered as its Immediate Release tablets. Both Ka and AUC were markedly increased by Immediate Release tablets**.**

The following conclusions were drawn from the results

1. Based on phase solubility studies 6.8 pH buffer was selected for the dissolution medium for the evaluation of olmesartan medoxomil and 0.1N Hydrochloric acid (1.2 pH**)** was selected for the dissolution medium for the evaluation of Irbesartan.
2. 25% /12.5%/62.5% proportions of N-N, dimethyl formide/chloroform/water, with stirring rate at 1000 ± 50 rpm for a period of 20 minutes were found to be suitable for the preparation of spherical agglomerates by the emulsion solvent diffusion method.
3. Incorporation of Hydrophilic polymer during agglomeration significantly enhanced the dissolution. Mixing of drug with a hydrophilic carrier (polymer) results in greater wetting and increase surface available for dissolution by reducing interfacial tension between the hydrophilic drug and dissolution media.
4. The cumulative percentage of drug (Irbesartan) released from different agglomerates was increased in the following order for agglomerates prepared with surfactants.

Spherical agglomerates prepared with B-cyclodextrin > spherical agglomerates prepared with HP β-cyclodextrin.

5. The cumulative percentage of drug (Irbesartan) released from different agglomerates was increased in the following order for agglomerates prepared with polymers.

Spherical agglomerates prepared with PVP –K90> spherical agglomerates prepared with PVA

6. The dissolution rate of drug (Irbesartan) was found to be effected by the concentration of the hydrophilic polymer used in the preparation of agglomerates. The dissolution rate followed first-order kinetics as the graphs drawn between log % drug undissolved vs time were found to be linear.
7. Among all the formulations prepared, spherical agglomerates prepared with B-cyclodextrin showed highest drug release in 60 minutes.

8. The dissolution rate of Irbesartan was found to be effected by nature of the superdisintegrants used in the preparation of tablets. Based on the dissolution rate, superdisintegrants can be rated as SSG < Croscarmalose sodium < Crospovidone.

9. The dissolution rate of Irbesartan was found to be effected by ratio's of co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) used in the preparation of tablets.

10. The formulation prepared with co-processed superdisintegrants (Croscarmalose sodium: Crospovidone) in 1:3 ratio was offered relatively rapid release of Irbesartan when compared with other ratios employed in this investigation.

11. The IR spectra of all the tested samples showed the prominent characterizing peaks of pure drugs which confirm that no chemical modification of the drug has been taken place.

12. The DSC studies result shows that the drugs retain its identity in the optimized formulation. These observations also confirmed the absence of chemical interaction of drug with additives during agglomeration process, further supporting the results of IR spectroscopy.

13. The Stability studies results indicated that the drug release from the optimized formulations was not changed significantly when stored at 25 ± 2ºC, 60 ± 5% RH and at 40 ± 2ºC, 75 ± 5% RH for 3 months.

14. The results of pharmacokinetic studies indicated rapid and higher oral absorption of olmesartan medoximil/Irbesartan when administered as its orodispersible tablets/Immediate Release tablets**.**

CONCLUSION

Present study concluded that spherical agglomerates prepared by the quasi emulsion solvent diffusion method showed an improvement in the solubility, dissolution rate, compatibility, wettability, flowability and bioavilability. These spherical agglomerates also showed excellent physico-chemical characters as compared with plain drug which indicates that the spherical agglomerates can suitable for directly compressible tablet process.

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