WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 7, 2247-2263. Research Article ISSN 2277– 7105

ENHANCEMENT OF BIOAVAILABILITY OF VALSARTAN THROUGH NANOCRYSTAL FORMULATION

Sudha[1], Dr. Sudipta Saha[2] and Neha Katiyar*[3]

[1-2]Department of Pharmacy Babasaheb Bhimrao Ambedkar University Lucknow Uttar Pradesh (226025) India.

[3]Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh (243006) India.

Article Received on
20 April 2019,

Revised on 10 May 2019,
Accepted on 30 May 2019

DOI: 10.20959/wjpr20197-15216

***Corresponding Author**
Neha Katiyar
Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh (243006) India.

ABSTRACT

The aim of the present study was focused on the development of nanocrystals of valsartan, an antihypertensive drug to be administered through oral route. Five formulations (F1, F2, F3, F4 & F5) of nanocrystals were prepared containing valsartan. Nanocrystals was prepared by antisolvent precipitation method. Prepared valsartan containing nanocrystals were evaluated for particle size, shape, surface morphology, in-vitro drug release and solubility studies. The particle size was found to be between the range of 230nm- 950nm, the particles were uniform, spherical in shape. The drug showed better release rate in comparison to conventional dosage form. All the formulation showed better result in terms of stability. Among the five formulations the best result were found with F1 formulation of valsartan. It can be concluded that for oral preparation a better solubility and bioavailability can be achieved by use of nanocrystals formulation of drug which has poor solubility.

KEYWORDS: The aim of the present which has poor solubility.

INTRODUCTION

Solubility is a key factor for drug therapy in any route of administration. It possesses a major challenge for pharmaceutical technologists to develop new pharmaceutical products.[1] It is estimated that approximately 40% or more of the new chemical entities (NCE) generated through drug discovery programs are poorly soluble in water.[2] The drugs with low solubility lead to low oral bioavailability and erratic absorption which is particularly pertinent to drugs

within class II of the Biopharmaceutical Classification System (BCS).[3] Formulation of this class of compounds is a challenging problem faced by the pharmaceutical researcher, because typical problems associated with these drugs are a too low oral bioavailability and erratic absorption due to their too low saturation solubility and dissolution velocity.[4] For oral administration, the low concentration gradient between the gut and blood vessel due to the poor solubility of the drug leads to a limited transport consequently influences the oral absorption. Parenteral administration as microsuspensions (e.g. i.m. or i.p.) frequently cannot lead to sufficient drug levels due to the limited solute volume at the injection site. Therefore, one of the most challenging tasks in drug development is to improve the drug solubility in order to enhance the bioavailability of these drugs.[5] Several strategies have been employed to overcome these limitations. The approaches to increase the solubility and the available surface area for dissolution are classified as physical and chemical modifications. For the physical modification, the techniques include decreasing particle size (micronization, nanonization), formation of polymorphs/pseudopolymorphs (including solvates), complexation/solubilization (by means of using surfactants or cyclodextrins, conjugation to dendrimers, and addition of co-solvents) and preparation of drug dispersions in carriers. For the chemical modification, the used technique is the synthesis of soluble prodrugs and salts.[6] The micronization of drug leads to an increase in their surface area which proportionally increases in rate of dissolution and rate of diffusion (absorption). However, for very low solubility compounds the micronization fails to improve the saturation solubility and increase the bioavailability of the drug. Therefore, the further step to reduce the particle dimension to nanometer size range has been invented. The drug nanocrystals provide more benefit over microparticles. The very small particles of nanocrystals have larger surface area and possess increased saturation solubility. Therefore the dissolution velocity is increased leading to higher oral absorption and more improvement of bioavailability.[7]

Drug nanocrystals are nanoscopic crystals of parent compounds with the dimension of less than 1 μm. They are composed of 100% drug without carriers and typically stabilized with surfactants or polymeric steric stabilizers.[8]

Advantages

- Increased surface area
- Enhanced solubility
- Increased rate of dissolution

- Increased oral bioavailability
- More rapid onset of therapeutic action
- Less amount of dose required
- Decreased fed fasted variability
- Decreased patient to patient variability
- Rapid, simple and cheap formulation development
- Possibility of high amounts (30-40 %) of drug loading
- Improved stability- They are stable systems because of the use of a stabilizer that prevents reaggregation of active drug substances during preparation.[9,10]

MATERIALS AND METHODS

Materials

Chemicals

Table. 1.

S. No.	Name of Polymers	Source
1.	Valsartan	IPCA Mumbai
2.	Methanol	SDFCL, Fine chemical Ltd. Mumbai
3.	**A**cetone	SDFCL, Fine chemical Ltd. Mumbai
4.	Mannitol	Himedia Laboratories Ltd. Mumbai
5.	Isopropyl alcohol	Merck Specialties Pvt. Ltd. Mumbai

Equipments

Table. 2.

S. No	Instrument	Model/Company
1.	UV Visible spectrophotometer	Labtronics - LT 2910
2.	Electronic balance	Shimadzu AY 220
3.	Magnetic stirrer with Hot plate	Tarsons - Spinot MC 01
5.	Hot air oven	Oven-TC 544
6.	Centrifuge	Tarsons - Spinwin MC 01
8.	Vortex mixer	Remi CM 101
9.	Mechanical Stirrer	Bharat motor, Varanasi
10.	Stability chamber	Science tech India
11.	Dissolution apparatus	Veego
13.	Scanning Electron Microscope	JEOL-JSM-6490LV
14.	Microscope	Radical RXLr-4

METHODS

Preformulation Studies

Before preparation and evaluation of pharmaceutical dosage form containing active moiety, preformulation studies on various **physico-chemical properties** of procure drug was along

with the **chemical authentication** by physical appearance melting point determination and FT-IR spectra has been done.

Organoleptic properties of drug

As per Indian pharmacopoeia drug sample was physically characterized on the basis of color, odor and taste. All these parameter were than compared with standard drug.

Melting point determination

Melting point of the drug sample was determined by capillary tube method. In this method small amount of drug was filled in the three separated capillaries and one end of these capillaries were sealed. The capillaries tubes were placed on the melting point apparatus (Macro scientific works). The temperature was gradually increase and noted down the temperature at which samples started to melt. Repeat this procedure at least three times to take an average.

Solubility studies

Solubility may be defined as a spontaneous interaction of or more substances to form a homogeneous dispersion. The solubility of Valsartan was studied in various aqueous and non aqueous solvents. Accurately 10mg of drug was weighed and taken in test tubes separately. Then 10 ml of solvent was added in test tubes at room temperature and shaken for 24 hours in vortex shaker. After that the solubility was observed visually for clear fluid.

FT-IR spectrum of Valsartan drug

I.R. spectrum of the drug sample (Valsartan drug) was obtained using FTIR Nicolet^{TM} 6700 Thermo scientific, USA. The sample was scanned at 4000cm^{-1} – 400cm^{-1}.

U.V. Spectrum of Valsartan in Methanol

A standard solution of Valsartan (100 µg/ml) was prepared by dissolving 10 mg drug in 10 ml of methanol from this solution 1ml was withdrawn and diluted upto 10ml and was scanned between 200 nm to 400 nm to obtained lambda maxima.

Preparation of Standard Curve for Valsartan in methanol

Standard stock solution of Valsartan having concentration 1000µg/ml was prepared by dissolving 10 mg of Valsartan in 10 ml of methanol. From the stock solution the another stock solution (100µg/ml) was prepared by taking 1 ml of this and diluted up to 10 ml. From

this stock different concentration of 2, 4, 8, 20, 40 and 60 µg/ml were prepared. The absorbance of these solutions was measured at 250 nm.

METHOD OF PREPARATION

Antisolvent Precipitation Method

Nanocrystals of valsartan drug was prepared by Bottom up precipitation method. In this method no stabilizer is used. These were prepared by controlled crystallization during freeze drying. In this method mannitol is used as a carrier. It easily crystallize during freeze drying. Two separate solutions of drug in isopropyl alcohol and mannitol in water were prepared and heated up to 60ºC. Aqueous solution was mixed in drug solution. Instantly after mixing, the solution was frozen and thereafter dried in vacuum desiccators.

Characterization of Nanocrystals

Particle size and Zeta potential

Malvern Zetasizer Nano-ZS90 was used to determine the mean particle size (z-average) and poly dispersity index. Prior to the measurement, the samples were dispersed with water in order to obtain a proper scattering intensity. The zeta potential (ZP) was commonly used as a measurement of indicating the physical stability of colloidal systems. In this study, the same Malvern Zetasizer was used to measure the ZP values by determining the particle electrophoretic mobility of the particles in an electrical field, which was transformed to the zeta potential. The zeta potential was measured by the same instrument equipped with a zeta potential analyzer. The particle size, PDI and ZP were analyzed in triplicate.

Particle Morphology

The morphology and surface characterization of nanocrystals were examined by using scanning electron microscope model JSM-6490LV (JEOL, JAPAN). Before examinations the samples were mounted on top of double sided sticky carbon tape on metal discs and coated with 80 nm gold/palladium in Balzers 120B sputtering device.

Differential Scanning calorimetry

DSC analysis was carried out using differential scanning calorimetelry model Pyris Diamond TG/DTA PerkinElmer (SINGAPORE). Samples (pure drug, mannitol and nanocrystals) of about 3 mg were weighed accurately and put in a aluminium pan and sealed in a lid. Heat runs for each sample has been set from 50 to 300° at a Scanning rate of 10° min-1, under dry nitrogen flow (150 ml/min). The apparatus is indium/cyclohexane calibrated.

X-ray powder diffraction (XRD)

Samples (bulkdrug, blank excipients, physical mixtures and) were evaluated with X-ray diffractometer: Model- Ultima-III, Rigaku make (Japan), Cu target slit 10 mm. 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.

In-Vitro Dissolution

The release rate of Valsartan nanocrystal was determined using USP Dissolution testing apparatus II (Paddle type). The dissolution test was performed using 900 ml of phosphate buffer (pH 6.8) at 37 ± 0.5° and 50 rpm. The samples (5 ml) of the solution were withdrawn from the apparatus at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min. intervals. The samples were replaced with fresh dissolution medium. The samples were filtered and suitably diluted. Absorbance values of these solutions were measured against respective buffer solutions at 250 nm using UV Spectrophotometer. The percentage drug release was calculated.

Solubility Analysis

The solubility was determined by a shake-flask method. Solubility of Valsartan nanocrystal formulations were tested in different solvents such as distilled water, phosphate buffer (pH 6.8) and phosphate buffer (pH 7.4) by shake-flask method. An excess amount of valsartan nanocrystal formulation was added in 2ml of the pertinent solvents. The mixtures were stirred in a mechanical shaker for 24 hours. Visual inspection was carefully made to ensure there were excess solids in the mixture, indicating saturation had been reached. The mixtures were then centrifused filter and filtrates were diluted suitably to determine the solubility of valsartan in each solvent.

Pharmacokinetic Study

Male Sprague-Dawley (SD) rats (150-200g) in weight were housed in the laboratory with free access to food and water. SD rats were fasted overnight before the experiment, but given free access to water. For each animal, the formulation was suspended in carboxy methyl cellulose (0.025%w/v) to obtain 30mg/ml .Pure drug was also treated same as above. Each formulation and pure drug suspension was administered orally to 10 rats by oral feeding needle. The blood samples were withdrawn by retro orbital venous plexus puncture at 5min, 15min, 30min, 1hr, 2hr, 4hr, 8hr, 16hr and 24hr after oral drug and formulation administration. All the samples were collected in heparinized Eppendorf tubes and

centrifuged (10000 rpm, 15 min), and plasma was collected and stored at -20°C until analysis. Owing to the instability of valsartan in rat plasma at ambient temperature, all sample preparations were performed on an ice water bath. The drug plasma concentration was analyzed by a modification of HPLC technique.

Sample preparation

Before analysis, the plasma samples were thawed at ambient temperature. 50 µl plasma of every time interval were added to eppendorf, then 1ml methanol was added to each eppendorf. After a thorough vortex mixing for 30 min, mixtures were centrifuged at 10000 rpm for 15 min. Its supernatants were taken for HPLC analysis.

Short term Stability study

Nanocrystal Formulations was prepared and kept in a tightly sealed container at three different temperatures (4º C, 30ºC, and 45ºC). The physical and chemical stability tests of Valsartan nanocrystals were examined after 3 months of storage. Regarding the physical stability aspect samples were characterized in terms of mean particle size (z-ave), PI and ZP. Prior to the measurement, the samples were redispersed in deionized water by hand-shaking. For the chemical stability, the percent content in lyophilized nanocrystal formulations A and B was determined and reported as % label amount compared to the initial preparation.

RESULTS AND DISCUSSION

Preformulation Studies

The preformulation studies of the drug performed by evaluating various parameters as.

Physical appearance

Colour: Pure white

Odor: Odorless

Taste: Tasteless.

Appearance: Powdered form.

Melting Point: Melting point of the drug Valsartan was found to be in the range of 105 – 115 ºC.

Solubility studies: Solubility studies of drug were shown in ethanol, methyl acetate, dichloromethane, and chloroform but the drug was insoluble in water. Solubility studies of drug were performed in various solvent systems as given below table.

Table. 3: Solubility studies of drug in different solvent systems.

S. No.	Solvent	Solubility
1.	Water	Insoluble
2.	Ethanol	Freely soluble
3.	Methyl acetate	Freely soluble
4.	Dichloromethane	Freely soluble
5.	Chloroform	Freely soluble

FT-IR spectrum of Valsartan drug: I.R. spectrum of the drug sample (Valsartan drug) was obtained using FTIR Nicolet^{TM} 6700 Thermo scientific, USA. KBr pellets were prepared by grinding approximately 2% of the samples in KBr and compressed into a pellet. The sample was scanned at 4000cm^{-1} – 400cm^{-1}

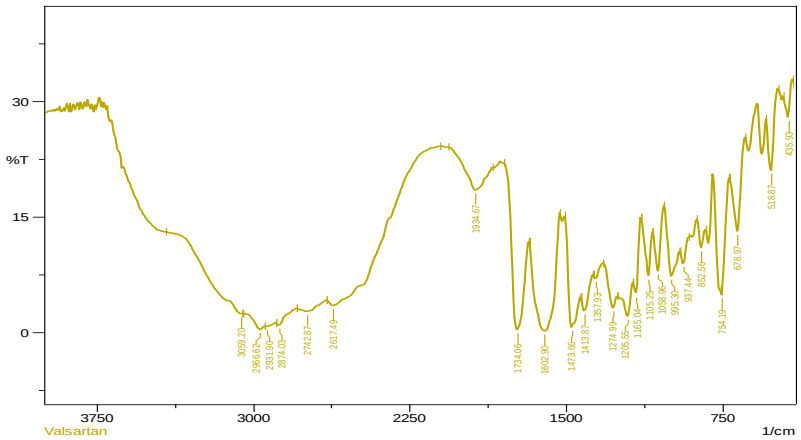


Figure. 1: FT-IR spectrum of Valsartan drug.

Table. 4: FT-IR spectrum peaks of Valsartan drug.

Compound	FTIR Peaks(cm^{-1})	Functional Groups
Valsartan drug	3059.20	-COOH
	1734.06	-C=O
	1357.93	-C-N
	1413.87	-CH3

U.V. Spectrum of Valsartan in Methanol

A standard solution of Valsartan (100 µg/ml) was prepared by dissolving 10 mg drug in 10 ml of methanol from this solution 1ml was withdrawn and diluted up to 10ml and was scanned between 200 nm to 400 nm to obtained lambda maxima. The lambda max of drug was found to be 250 nm.

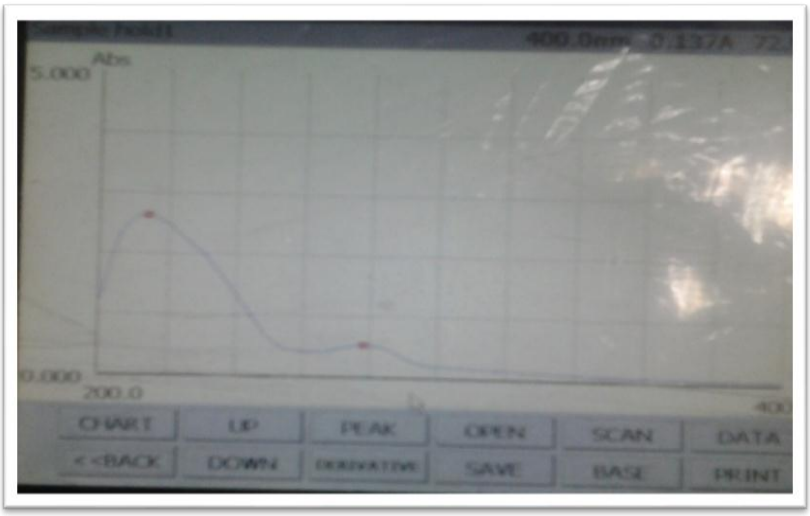


Figure. 2: U.V. Spectrum of Valsartan in Methanol.

Preparation of Standard Curve for Valsartan in methanol

Standard stock solution of Valsartan having concentration 1000µg/ml was prepared by dissolving 10 mg of Valsartan in 10 ml of methanol. From the stock solution the another stock solution (100µg/ml) was prepared by taking 1 ml of this and diluted up to 10 ml. From this stock different concentration of 2, 4, 8, 20, 40 and 60 µg/ml were prepared. The absorbance of these solutions was measured at 250 nm.

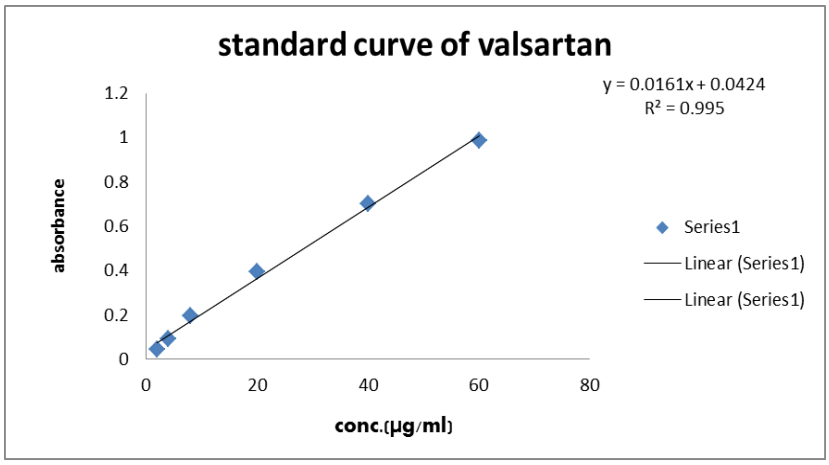


Figure. 3: Standard Curve of Valsartan in methanol.

Particle size and Poly dispersity index

The particle size and poly dispersity index of the optimized formulation (VF1) is found to be 234 nm and 1 respectively. The particle size for all formulation was found to be 230nm-950nm.The particle size is shown in table.

Table. 5: Particle size and Poly dispersity index.

Formulation	Particle size (nm)	Polydispersity index
F1	234	1
F2	284.1	0.837
F3	377.12	1
F4	828	1
F5	964.8	1

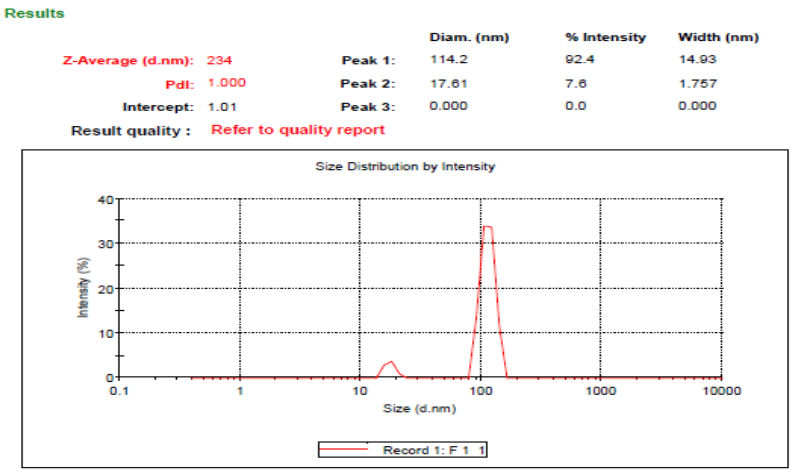


Figure. 3: Particles size and percentage intensity.

Zeta Potential

The zeta potential is a key indicator of the stability of colloidal dispersions. It is electrophoretic mobility of the particles in an electrical field. The zeta potential of optimized formulation was found to be - 41.2. F1 formulation revealed absolute ZP values higher than |30 mV| indicating long-term physically stable system. The zeta potential for all formulation was shown in table.

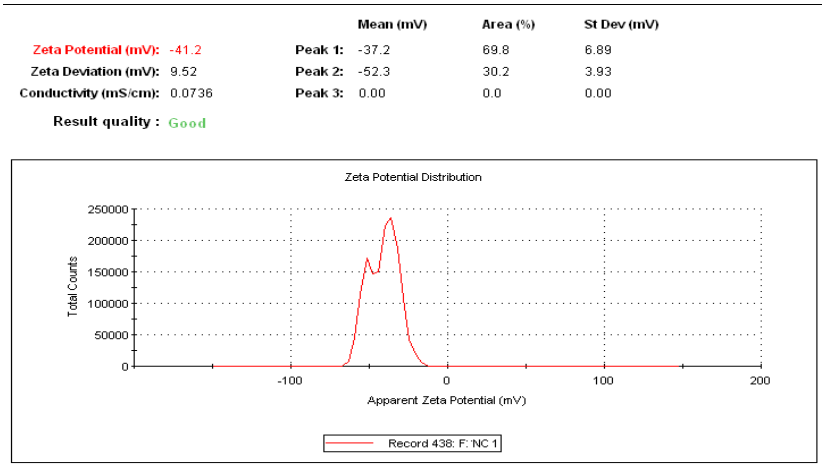


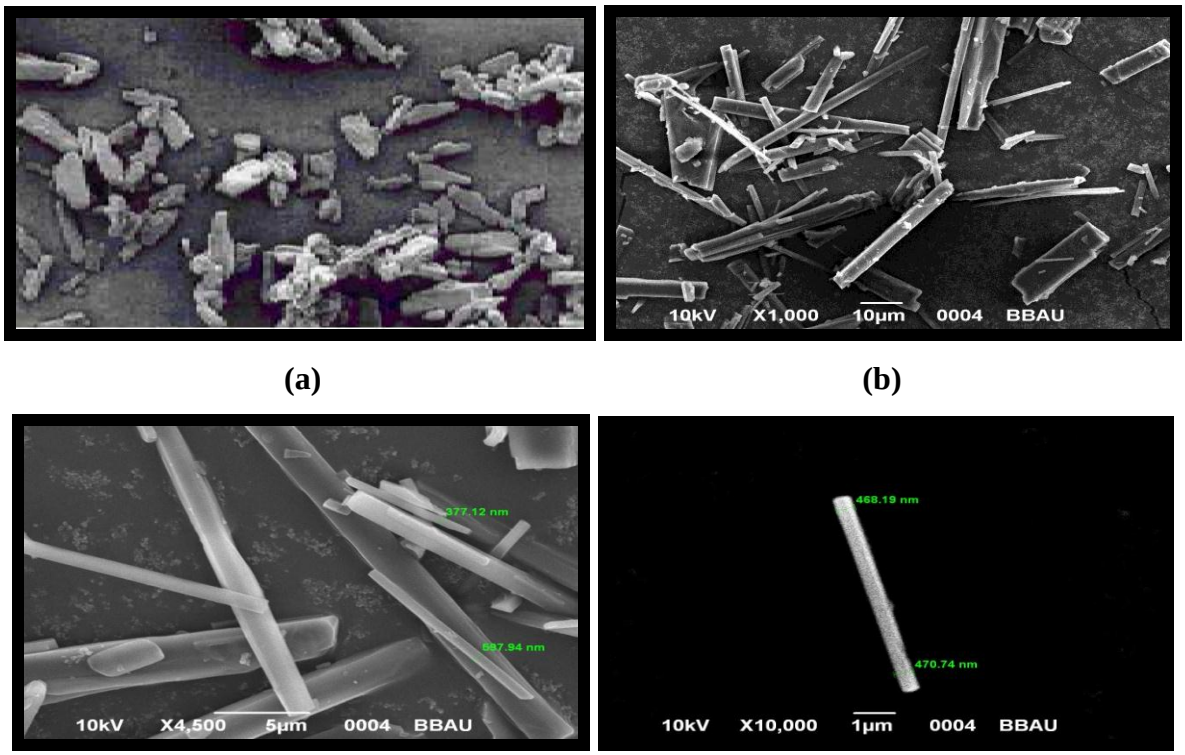
Figure. 4: Zeta Potential.

Table. 6: Zeta Potential.

Formulation	Zeta Potential
F1	– 41.2
F2	– 39.7
F3	– 30.7
F4	– 23.8
F5	– 18.8

Shape and Surface Morphology

The morphological micrographs of valsartan nanocrystals were examined by SEM as demonstrated in Fig. It was observed that the valsartan nanocrystals were rod-like shape with smooth surface. From the SEM result it was observed that the sample is crystalline in nature.



(a) **(b)**

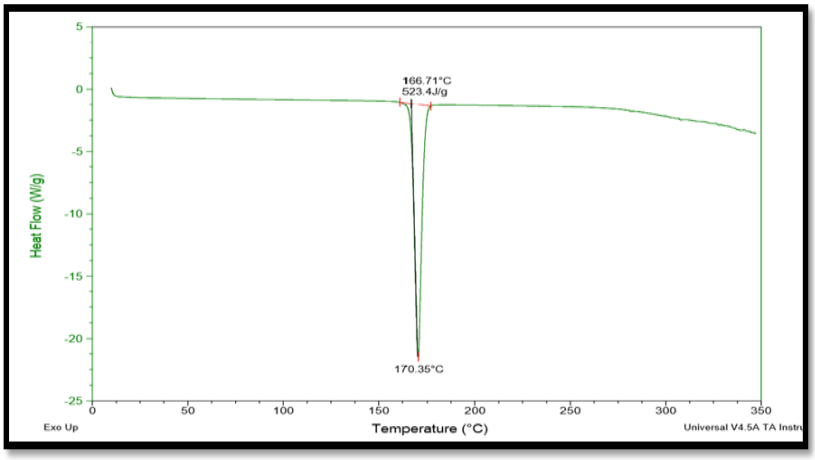
(c) **(d)**

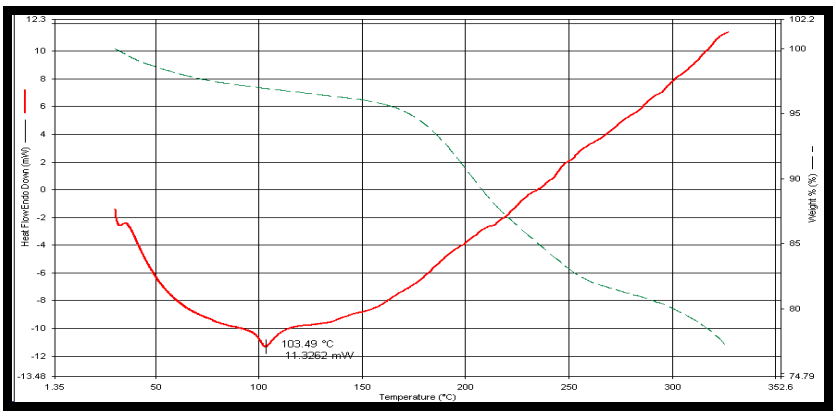
Figure. 5: Scanning Electron Photomicrograph of (a) Valsartan, (b) nanocrystal formulation F1 (c) formulation F2 (d) formulation F3.

Differential Scanning Calorimetry

DSC thermograms showed that the endothermic melting peak of valsartan nanocrystals was long and slightly shifted when compared to that of the valsartan drug. These results indicated possibility of an alteration of crystallinity of drug to other crystalline forms or to amorphous stage and presence of mannitol bound to the surface of nanocrystals. Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or

produced (i.e., endothermic and exothermic phase transformations). The thermograms for pure valsartan, mannitol and nanocrystal are presented in Fig. The valsartan showed a melting endotherm at 103.49°C whereas pure mannitol showed a melting endotherm at 170.35ºC. Thermograms of nanocrystal showed the absence of a valsartan peak, suggesting that mannitol is completely bound to the surface of drug. However, the melting peak of mannitol in nanocrystal was observed at slightly lower temperatures (between 168°C - 171°C) than that of pure mannitol (170.35°C), indicating the miscibility of the drug in carrier.





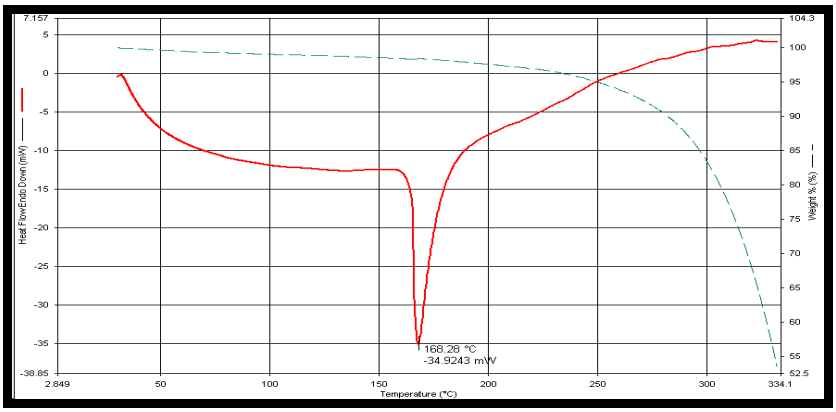
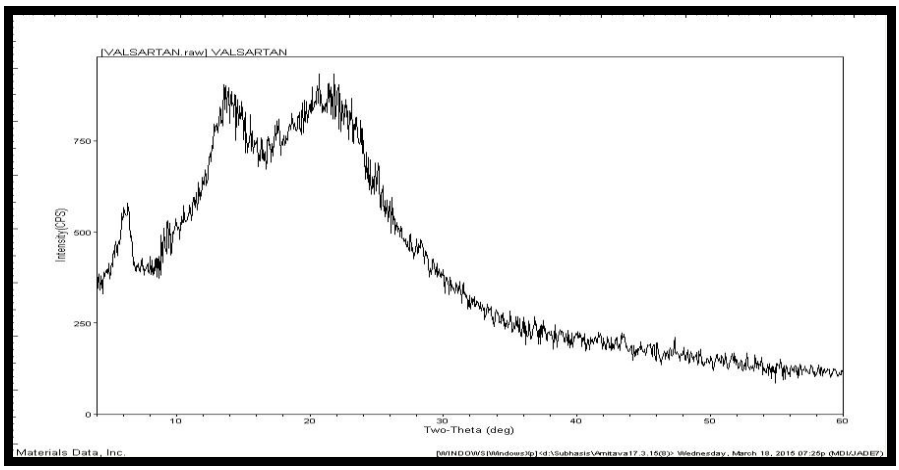
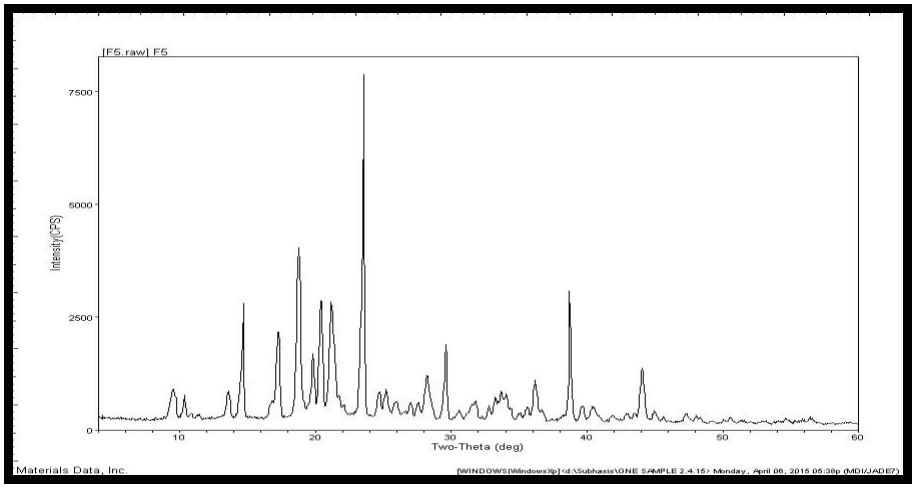


Figure. 5: Differential Scanning Calorimetry.

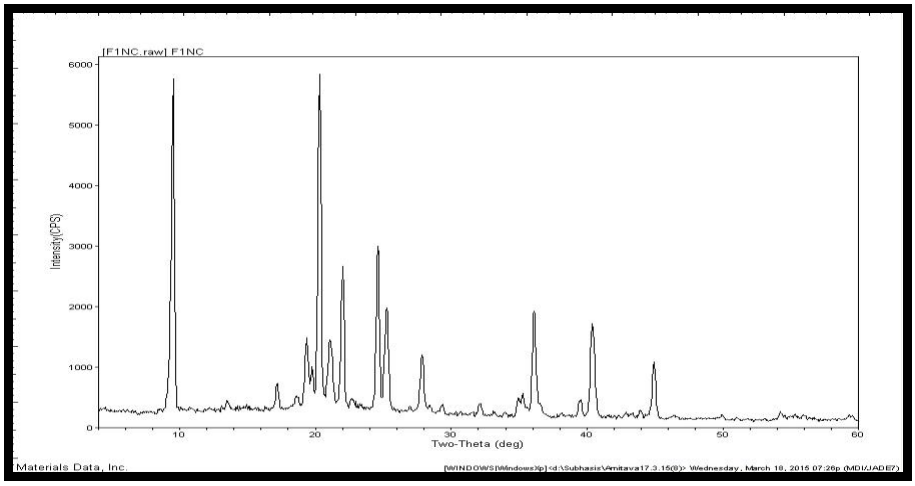
X Ray Differaction: Powder X-ray diffraction patterns of Valsartan and Valsartan Nanocrystals were also studied in order to gain insights into the crystallanity differences. The powder X-ray diffractograms are shown in fig. Valsartan showed intrinsic peaks at the diffraction angles, showing a typical crystalline pattern. The diffraction spectrum of pure valsartan showed distinct peaks at 2θ of 6.35º,20.7º,21.8º,30.0º. Mannitol showed peak at 10.0º,18.7º,20.40º,21.1º,23.35º and optimized nanocrystal formulation showed at 9.55º,19.35º,20.3,21.05º,24.6º,25.25º. The spectrum of nanocrystal showed that some peaks of pure valsartan were absent and intensity of peaks was reduced. The result indicates that the drug in nanocrystal is amorphous as compared to the pure drug. Hence, increased dissolution of the drug was observed. Our results suggest that the enhanced solubility of valsartan was not due to the transformation of the crystalline form into an amorphous state, but instead were due to the attachment of the carriers to the surface of poorly water soluble valsartan, converting the hydrophobic drug to hydrophilic form in this nanocrystal.



(a)



(b)



(c)

Figure.6:- Powder X-ray diffraction pattern of (a) Valsartan, (b) Mannitol, (c) Nanocrystal formulation.

In-Vitro Dissolution Study

Formulation F1 showed 100 % within 45 min which is quite better than other formulations. Nanocrystal formulation showed enhanced dissolution rate as compared with pure drug. In comparison with the Valsartan powder and nanocrystals dramatically increased the percent drug dissolved within 10 min.

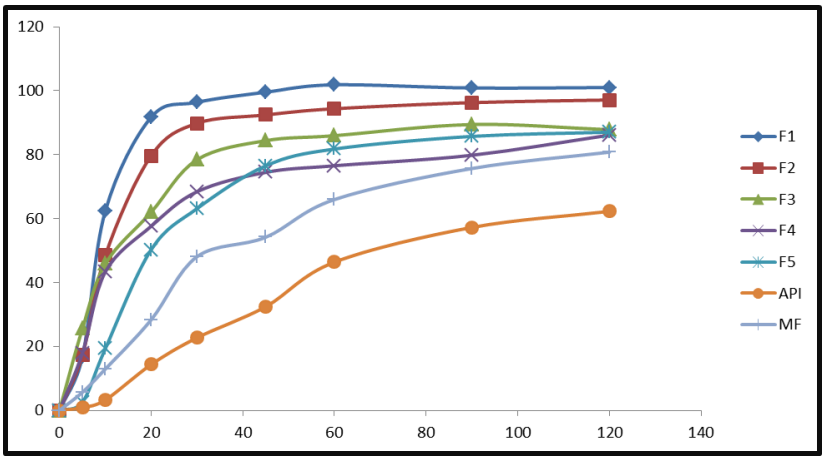


Figure. 7: In-Vitro Dissolution Study.

Table. 7: In-Vitro Dissolution Study.

Time	Formulation Code						
	VF1	VF2	VF3	VF4	VF5	API	Marketed formulation
5	16.76±10.57	17.27±11.55	25.55±1.49	17.93±3.42	2.58±2.51	0.88±0.49	5.67±0.99
10	62.40±13.75	48.52±6.07	45.87±0.62	43.35±4.66	19.32±13.32	3.25±2.05	12.94±4.94
20	91.70678±3.18	79.66±4.76	61.99±11.52	57.60±2.73	50.26±5.05	14.34±1.65	28.31±2.96
30	96.39±0.70	89.82±7.52	78.44±18.86	68.43±4.70	63.14±0.66	22.72±3.71	48.09±0.69
45	99.56±2.80	92.45±8.11	84.39±15.39	74.48±4.11	76.52±0.32	32.46±6.34	54.17±0.39
60	101.86±2.62	94.38±8.46	85.96±15.41	76.55±3.64	81.77±0.17	46.39±3.71	65.98±0.49
90	100.87±1.50	96.28±8.96	89.45±16.12	79.84±1.91	85.75±1.43	57.24±6.43	75.69±0.79
120	101.03±1.082	97.11±8.19	87.82±13.07	86.13±7.44	87.04±1.37	62.27±8.35	80.79±0.69

Solubility Studies

In this study, kinetics of saturation solubility of valsartan formulations were studied in the different dissolution media.

Table. 8: Solubility Studies.

Batch code	Distilled Water (mg/ml)	pH 6.8 (mg/ml)	pH 7.4 (mg/ml)	0.1 N HCL (mg/ml)
F1	0.592±0.0026	5.52±0.031	7.66±0.018	0.054±0.00026
F2	0.588±0.00088	5.43±0.035	6.79±0.16	0.0538±0.00017
F3	0.576±0.0026	5.15±0.079	5.93±0.035	0.0536±0.003
F4	0.058±0.00026	3.46±0.70	4.88±0.12	0.051±0.0002
F5	0.0565±0.00017	1.90±0.17	4.22±0.79	0.044±0.0005
API	0.041±0.007	0.59 ±0.0070	1.31±0.67	0.016±0.0027

CONCLUSION

Valsartan is a nonpeptide tetrazole derivative. It particularly inhibit angiotensin-II type I receptor which results in diminution of blood pressure. The drug could be successfully formulated as nanocrystals. Formulation F1 was smallest in particle size. All the formulation was stable and redispersible as PDI was less than 1 with all formulation of valsartan. The zeta potential of optimized formulation was found to be - 41.2. F1 formulation revealed absolute ZP values higher than |30 mV| indicating long-term physically stable system. In terms of release formulations F1 showed highest drug release for drugs valsartan. Nanocrystal formulation showed enhanced dissolution rate as compared with pure drug. Formulation F1 showed 100% dissolution within 45 min which is quite better than other formulations. In comparison with the valsartan powder and nanocrystals dramatically increased the percent drug dissolved within 10 min.

REFERENCES

1. Igea P.P., Bariaa R.K., Gattanib S.G., Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability, Colloids and Surfaces B: Biointerfaces, 2013; 108: 366-373.
2. Gao L., Zhang D., Chen M., Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system, J Nanopart Res., 2008; 10: 845–862.
3. Amidon G., Lennernäs H., Shah V., Crison J., A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of In Vitro Drug Product Dissolution and In Vivo Bioavailability, 2, Springer, 1995; 413–420.
4. Che E., Zheng X., Sun C., Chang D., Jiang T., Wang S., Drug nanocrystals: A state of the art formulation strategy for preparing the poorly water-soluble drugs, Asian Journal of Pharmaceutical Sciences, 2012; 7(2): 85-95.
5. Nagarwal R.C., Kumar R., Dhanawat M., Das N., Pandit J.K., Nanocrystal Technology in the Delivery of Poorly Soluble Drugs: An Overview Current Drug Delivery, 2011; 8.
6. Junyaprasert V.B., Morakul B., Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs, a j pha rm.s c i. xxx (2014) I-II.
7. Junghanns J.U.A.H., Müller R.H., Nanocrystal technology, drug delivery and clinical applications, International Journal of Nanomedicine, 2008; 3(3): 295-309.
8. Boontida Morakul , Jiraphong Suksiriworapong, Mullika Traidej Chomnawang, Peter Langguth Varaporn Burapacheep Junyaprasert Dissolution enhancement and in vitro

performance of clarithromycin nanocrystals produced by precipitation–lyophilization–homogenization method, European Journal of Pharmaceutics and Biopharmaceutics, 2014; 88: 886–896.

9. Sawant S.V., Kadam V.J., Jadhav K.R., Sankpal S.V., Drug nanocrystals : novel technique for delivery of poorly soluble drugs International Journal of Science Innovations and Discoveries, 2011; 1(3): 1-15.
10. Neethu C.S, Kavitha V.B, kumar B.D., kumar K.K., John A., Nanocrystals Drug Delivery System – A Review, Current Research in Drug Targeting, 2014; 4(1): 1-5.