Nanocrystalisation by Anti-Solvent Precipitation Technique for Solubility and Dissolution Enhancement of Telmisartan

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ABSTRACT:

Solubility is the parameter to achieve desired concentration of drug in systemic circulation for therapeutic response to be shown. There is a need for systematic formulation approaches to make such poorly soluble drugs bioavailable. Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. Nanocrystals were prepared by antisolvent precipitation method using dimethyl formamide as solvent, water as antisolvent and PVPK30 as stabilizer. The preparations were characterized in terms of particle size, nature, Stability, dissolution. Prepared nanocrystal samples showed increased solubility than the pure sample in water and increased nearly more than two-fold higher (107.64 µg/ml) than pure Telmisartan (50 µg/ml). Telmisartan was stable in the formulation and absence of any interaction between the drug and the polymer. Increase in crystalline nature in formulation as compared to pure Telmisartan. The dissolution of Telmisartan tablets containing nanocrystal formulation exhibited improved dissolution behavior than tablets prepared by pure Telmisartan i.e., Marketed formulation of Telmisartan.

Keywords: Nanocrystals, Antisolvent, Solubility, dissolution.

INTRODUCTION:

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of a drug. Major problem to the development of highly effective formulation is the poor aqueous solubility of many drugs. Solubility is the parameter to achieve desired concentration of drug in systemic circulation for therapeutic response to be shown. It is estimated that 40% or more of drug molecules identified during combinatorial screening programs are poorly water soluble. There is a need for systematic formulation approaches to make such poorly soluble drugs bioavailable.

Making such drugs bioavailable means that they show sufficiently high absorption after oral administration, or alternatively they can be injected intravenously

The main objective of this work is to prepare and evaluate nanocrystal formulation of Drug. Present study is undertaken to provide an alternative drug delivery system with improved solubility and dissolution rate in the form of drug nanocrystal which will overcome the inherent drawbacks of existing dosage form.

The major objectives of present study are-

1) To increase the solubility and dissolution rate of selected poor water-soluble drug by formulating nanocrystal.
2) To prepare nanocrystal of Telmisartan by anti-solvent precipitation method.
3) To characterize the prepared nanocrystals.
4) To study the prepared nanocrystals for solubility and in vitro release
5) To prepare nanocrystal tablet and its evaluation

MATERIALS AND METHODS

Table No. ___ shows the list of materials used for current research work.

Sr. No.	Name of Material	Supplier
1	Telmisartan API LR	Cipla (Glenmark)
2	Polyvinylpyrrolidone (PVP K-30) LR	S D Fine Chemical Ltd. Mumbai
3	Microcrystalline Cellulose LR	S D Fine Chemical Ltd. Mumbai
4	Sodium Lauryl Sulphate LR	Rankem Pvt. Ltd. Mumbai
5	Dimethyl Formamide AR	Rankem Pvt. Ltd. Mumbai
6	Magnesium Sterate LR	S D Fine Chemical Ltd. Mumbai
7	Talc LR	Rankem Pvt. Ltd. Mumbai
8	Potassium Dihydrogen Phosphate LR	Merck Specialities Pvt. Ltd
9	Hydrochloric Acid LR	Loba chemical Pvt. Ltd.
10	Sodium Starch Glycolate LR	S D Fine Chemical Ltd. Mumbai
11	Methanol LR	Loba chemical pvt. Ltd

Methods

Determination of Solubility of Telmisartan

Solubility analysis was determined in water, Dimethyl Formamide, Dimethy Sulfoxide, Methanol, Dichloromethanol and using various pH ranges i.e pH 1.2, pH 4.5, pH 6.8, pH 7.4. An excess amount of NVP was placed in glass bottles containing 20 ml of solvent. The bottles were thoroughly shaken for 12 h and kept aside for 24 h at room temperature. At the end of this period the solution was filtered and the filtrate was after appropriate dilutions absorbance of solution taken at individual solvents analyzed by using UV spectrophotometer at 296 nm.

Melting point Determination

Melting point determination is prime confirmation of drug. In this method, drug whose analysis to be carried out was filled into capillary tube and tied to the thermometer in such a way that it remains dipped in liquid paraffin bath. The temperature range at which the drug starts melting and complete melting was noted and reading were taken in triplicate.

Infrared Spectroscopy

IR spectrum of drug was measured in the solid state as potassium bromide (KBr) mixture. The pure Tapentadol was previously ground and mixed thoroughly with KBr, an infrared transparent matrix at 1:100 (sample:KBr) ratio, respectively. The KBr pellets were prepared by applying 10-12 metric ton of pressure in a motorized pellet press (Kimaya engineers, India). The pellets were then scanned over a wave range of 4000 – 400 cm^{-1} and spectra was obtained by using a FTIR spectrometer-430 (Shimadzu 8400S, Japan).

Differential Scanning Calorimetry

Differential scanning calorimeter is used to measure the specific heat and enthalpies of transition. When a sample undergoes a thermal transition, the power to the heater is adjusted to maintain the temperature and a signed proportional to the power difference is plotted on the second axis of the recorder is known as thermogram. The area under the resulting curve is direct measure of the heat of transition. Thermo grams were obtained by using a differential scanning colorimeter at a heating rate 10°C/min over a temperature range of 20-110°C. The sample was hermetically sealed in an aluminium crucible. Nitrogen gas was purged at the rate of 40ml/min for maintaining inert atmospheres.

Drug -Excipients Interaction Study

The successful formulation of a suitable and effective solid dosage form depends upon the careful selection of the excipients. Excipients are added to facilitate administration, promote the consistent release and bioavailability of drug. It's necessary to study the compatibility of the excipients with drug. Here IR spectroscopy and Differential Scanning Calorimetry (DSC) is used to predict the compatibility of drug with the excipients

Fourier Transform-Infrared spectroscopy (FTIR)

FTIR spectra of Telmisartan, PVP K30 and physical mixture of all the excipients with Telmisartan were studied. Above samples were mixed with KBr of IR grade in the ratio of 1:100 and compressed using motorized pellet press (Kimaya Engineers, India) at 10-12 tones pressure. The pellets were then scanned using FTIR spectrophotometer (Shimadzu 8400S, Japan). The FTIR spectra of mixtures were compared with that of the FTIR Spectra of pure drug and excipients, to confirm any change occurs or not in the principle peaks of spectra of plain drug and excipients.

Differential Scanning Calorimetry Study

Thermal analysis was carried out for Telmisartan, PVP K30 and physical mixture using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland) at a heating rate of 10°C /min. The measurements were performed at a heating range of 30 to 300°C under nitrogen atmospheres.

Determination of Absorption Maximum

A Solution of Telmisartan Containing the Concentration 10 µg/ml was prepared in Methanol & UV spectrum was taken using UV-Spectrophotometer. The solution was scanned in the range of 200-800nm.

Formulation Development

Nanocrystals were prepared by antisolvent precipitation method. Telmisartan was dissolved in good solvent (Dimethyl Formamide) and then it was added dropwise to antisolvent (water) containing stabilizer (PVP K30) under constant magnetic stirring at 1000 rpm. The particles precipitated from the antisolvent and a milk like suspension was formed. Then it was filtered & dried.

Batch Code	Amount of drug(mg)	Amount of PVPK30 (mg)	Drug: Polymer ratio
N1	10	10	1:1
N2	10	20	1:2
N3	10	30	1:3
N4	10	40	1:4

Evaluation of Nanocrystals

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, IRA affinity-1. About 2 mg of pure drug, nanocrystal samples were used separately. Pure drug and nanocrystal samples were dispersed in KBr powder and the pellets were made by applying 6000 kg/cm2 pressure. FTIR spectra were obtained by powder diffuse reflectance on FTIR spectrometer

Scanning Electron Microscope:

The Telmisartan nanocrystal were examined morphologically by Scanning Electron Microscope (JSM-6390LV, JEOL, Japan) with 20 kV accelerating voltage. Samples were prepared by placing a droplet onto an aluminum specimen stub, dried overnight and sputter coated with gold prior to imaging.

Differential Scanning Calorimetry

Thermal analysis was performed using a differential scanning calorimetry (DSC) (Mettler-Toledo, Zurich, Switzerland). The instrument was calibrated with indium. DSC thermo-grams were recorded for bulk Telmisartan, bulk PVPK30, physical mixture and Telmisartan nanocrystals. The samples, weighing 2 mg, were analyzed in sealed and pin-holed standard 40 µl aluminium pan, with a heating rate of 10°C/min from 30°C to 300°C and during the measurement the sample cell were continuously purged with nitrogen at a flow rate of 40 ml/min.

X-ray Diffraction Studies

X-ray diffraction patterns of Telmisartan, PVPK30, & Telmisartan nanocrystal formulation were obtained using X-ray diffractometer (Brucker Axs, D8 Advance; Germany) in which Cu-Kα line used as a source of radiation by operating at the voltage 40 kV and the

current applied was 30 mA. All samples were measured in the 2θ angle range between 10° and 60° with a scanning rate of 3°/min and a step size of 0.02°.

In-Vitro dissolution studies of nanocrystals

The dissolution of pure Telmisartan and nanocrystal samples was studied by using USP dissolution apparatus type II (Electrolab). Dissolution medium (900ml) consisted of 1.2 pH was used and aliquots of dissolution medium were withdrawn at predetermined intervals. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 296nm.

Evaluation of Pre-compressional properties

Angle of repose:

Angle of repose is defined as, "the maximum angle possible between the surface of pile of powder and horizontal plane". The powder was allowed to flow out of the funnel orifice on a plane paper kept on horizontal surface. This forms a pile of angle of powder on the paper. The angle of repose was calculated by substituting the values of the base radius r and pile height h in the following equation.

$$\tan\theta = h/r$$

Therefore, $$\theta = \tan^{-1}(h/r)$$

Where, θ = angle of repose, *h* = height of the cone/pile, *r* = radius of the cone base.

Bulk density

It is the ratio of mass and bulk volume. It is required to decide the appropriate packing of dosage forms. 20gm powder was allowed to flow in a fine stream into a graduated cylinder and final volume were noted. The bulk density was obtained by dividing the weight of the sample by final volume and it was determined by following equation

$$\text{Bulk density} = \frac{\text{Bulk mass}}{\text{Bulk volume}}$$

Tapped density

20gm powder was allowed to flow in a fine stream into a graduated cylinder of a mechanical tapping device. The measuring cylinder was tapped for 100 times and final tapped volume was noted. It was calculated by using following equation

$$\text{Tapped density} = \frac{\text{Bulk mass}}{\text{Tapped volume}}$$

Carr's index

It decides the flow properties of granules or powders. The percentage compressibility of a powder was a direct measure of the potential powder arch or bridge strength and stability. Carr's index of each formulation was calculated by using following equation

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$$

Hausner's ratio

It is essential to determine the compressibility strength of powders. It was determined by using following equation

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Preparation of tablet by using Direct Compression Method

All ingredients were accurately weighed as mentioned in table. These powders were then passed through 20 mesh sieves. Nanocrystal powder, microcrystalline cellulose and sodium starch glycolate were mixed in a mortar and pestle by tumbling action. Finally, magnesium stearate and talc were added and again mixed for 5 minutes so that particle surface was coated by lubricant evenly. The blend was compressed using single punch tablet machine (Karnavati) using 10mm die.

Table 1 Formulation Design of Telmisartan tablets by Direct Compression Method

Ingredients	F_1	F_2	F_3
Telmisartan	20	20	20
Microcrystalline sodium	30	40	50
Sodium starch glycolate	45	35	25
Magnesium stereate	2.5	2.5	2.5
Talc	2.5	2.5	2.5
(All ingredients are taken in mg per tablet)			

Evaluation of the prepared nanocrystal tablet

Appearance:

The prepared tablet were visually observed for capping, chipping, lamination.

Thickness and Diameter

Thickness and diameter of tablets was determined using Vernier Caliper. Five tablets from each batch were used, and average values were calculated.

Weight Variation Test

To study weight variation, 20 tablets of each formulation were weighed using an electronic balance (AW -220, Shimadzu), and the test was performed according to the official method.

Hardness of the Tablet

Tablet should have sufficient handling during packing and transportation. It is the force required to break a tablet in a diametric compression test. For each formulation, the hardness of three tablets was determined using Pfizer hardness tester which is expressed in kg/cm^3.

Friability

Friability test is performed to assess the effect of friction and shoks, which may often cause tablet to chip, cap or break.The friability of tablet was determined by using Roche friabilator. It is expressed in percentage (%). Six tablets from each batch were selected randomly and weighed. The friabilator was operated at 25 rpm for 4 minute or run upto 100 revolutions.

Tablets were removed dedusted and weighed again. % Friability of tablet less than 1% was considered acceptable. Following formula was used to calculate the friability

$$\% F = (1 - W/W_0) \times 100$$

Where, W_0 - Weight of tablets before test; W - Weight of tablets after test.

Uniformity of content

20 tablets were weighted individually and powdered. The powder equivalents to about 0.2gm of Telmisartan transferred to a 100ml volumetric flask. Then 100ml of 0.1N HCl was added mixed and filtered. Then 1ml of filtrate diluted with 10ml of 0.1N HCl. Concentration of drug was determined by measuring absorbance at 296nm by UV-Spectrophotometer.

In vitro dissolution studies

The in vitro dissolution studies were carried out by using USP apparatus type II at 50rpm. The dissolution medium was 900ml 0.1 N HCL maintained at 37± 0.5^{0}C. Aliquots of dissolution medium were withdrawn at predetermined intervals and content of Telmisartan was determined at 296nm spectrophotometrically.

In vitro drug release kinetics

To analyze the mechanism of the drug release rate kinetics of the dosage form ,the obtained were graphed as:

Cumulative percentage drug released Vs Time(Zero order plots)
Cumulative percentage drug released Vs Square root of time (Higuchi plots)
Log cumulative percentage drug remaining Vs Time (First order plots)
Log percentage drug released Vs Log time (Peppas plots)

Zero order release rate kinetics

To study the zero-order release kinetics the release rate data are fitted to the following equation

$$F = kt$$

Where F is the fraction of drug release, K is the release rate constant,t is the release time When the data is plotted as cumulative percent drug release Vs time, if the plot is linear then the data obeys zero order release kinetics with a slope equal to K This model represents an ideal release in order to achieve prolonged pharmacological action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as Matrix tablets containing low soluble drugs.

First order rate kinetics

The equation for first order treatment is represented as

$$\mathbf{Log\ c = Log_{co} - kt/2.303}$$

Where, c is amount of drug remaining unreleased at time t, c_o is initial amount of drug in solution, k is first order rate constant The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

Higuchi release model

To study the Higuchi release kinetics, the release rate data were fitted to the fillowing equation

$$\mathbf{F = K.\ t^{1/2}}$$

Where F is the amount of drug release, K is the release rate constant, t is the release time When the data is plotted as cumulative drug released Vs square root of time, yields a straight line, indicating that that the drug was released by diffusion mechanism. The slope is equal to K. This model is applicable to system with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

Korsmeyer and Peppas release model

The release rate data were fitted to the following equation

$$\mathbf{Mt / M\infty = Ktn}$$

Where, **Mt / M∞** is the fractional release of drug, 't' denotes the release time, 'K' represents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusional exponent and characterize the type of release mechanism during the release process.

When the data is plotted as Log time , yields a straight line equal to n and the K can be obtained from Y-intercept.

For non-Fickian release the n values falls between 0.5 and 1.0, while for Fickian (case I) diffusion n=0.5 and zero order releas (case II transport) n=1.0 The value of n indicates the drug release mechanism, For a slab the values n=0.5 indicates fickian diffusion and values of n between 0.5 and 1.0 or n=1.0 indicates non-Fickian mechanism. In case of a cylinder n=0.45 instead of 0.5 and 0.89 instead of 1.0

This model is used to analyse the release from polymeric dosage forms, when the release mechanism os not well knoen or when there is a possilbility of more than one type of release phenomenon bing involved.

RESULTS AND DISCUSSION

Preformulation study of pure drug

Solubility:

Solubility of Telmisartan using various pH ranges likes 1.2 pH, 6.8 pH, Distilled water and results was found to be as follows:

Table 2 Solubility of Telmisartan

Sr.no	Solubility	Conc in mg/ml
1	1.2 pH	1.533
2	6.8 pH	0.148
3	Distilled water	0.0067
4	Dimethy Formamide	1.553
5	Methanol	1.486

Confirmation of Drug

Identification of drug was carried out by melting point determination, IR and DSC study.

Melting Point Determination

Melting point of Telmisartan was found by glass capillary method and melting point apparatus. The observed melting point of Telmisartan was 258°C-260°C which is very close to the standard melting point of Telmisartan i.e., 264°C-267°C

Infrared Spectrum

The IR spectrum was measured in the solid state as KBr dispersion. The IR spectrum of Telmisartan observed peaks are shown in Table

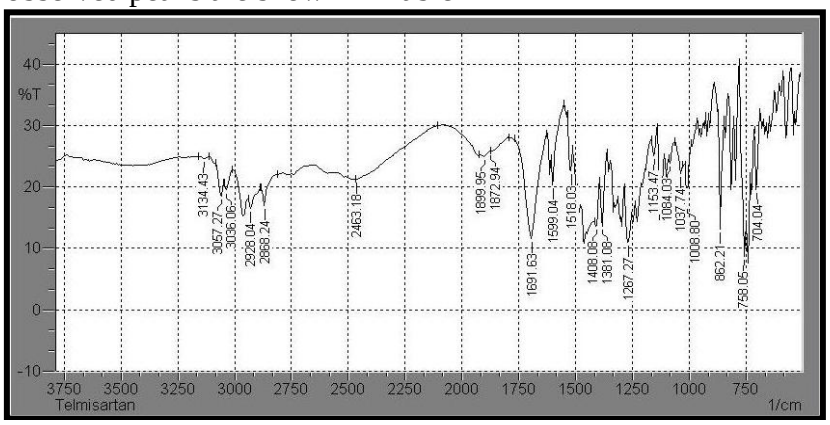


Figure 1 Infrared spectra of Telmisartan

Table 3 Principal peaks and functional group present in IR spectra of Telmisartan

Reported Peaks (cm^{-1})	Interpretation of Chemical group
3134.00	O-H strech
3360	-N-H Strech
1691.00	C=O Strech
1624.12	C=C-Strech
1244.13	C-N Strech
1545.07	NO_2 Aromatic strech
1455 & 1381	CH_3 Bending Alkanes

Melting Point Determination by DSC:

Telmisartan was confirmed by Differential Scanning Calorimetry at a scanning rate of 10°C/min. It exhibits a sharp melting endothermic peak at a temperature of 265°C which is very close to the standard melting point of Telmisartan Fig.

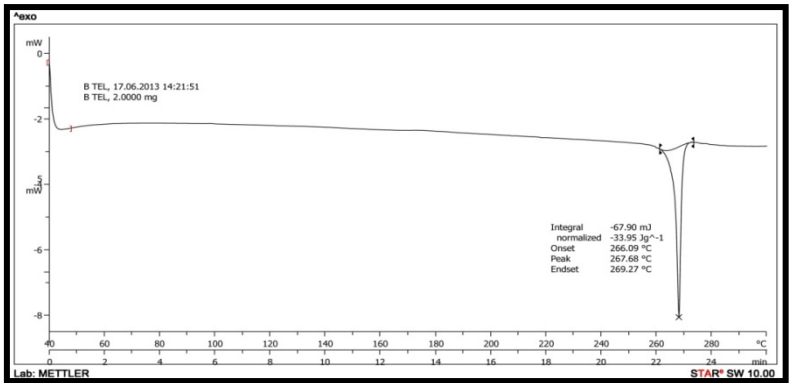


Figure 2 DSC Thermogram of pure Telmisartan

Drug -Excipients Interaction Study

Fourier Transform-Infrared spectroscopy (FTIR)

FTIR spectra of Telmisartan, PVPK30, and physical mixture are shown in Fig. and their interpretation is in Table. From interpretation it can be concluded that there is no drug polymer interaction.

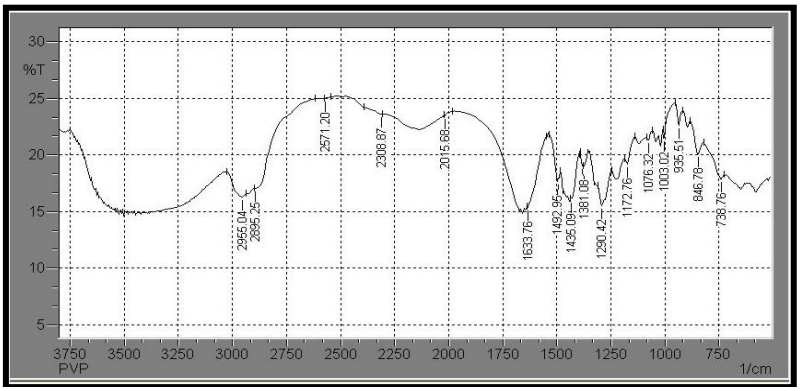


Figure 3 Infrared Spectra of PVPK30

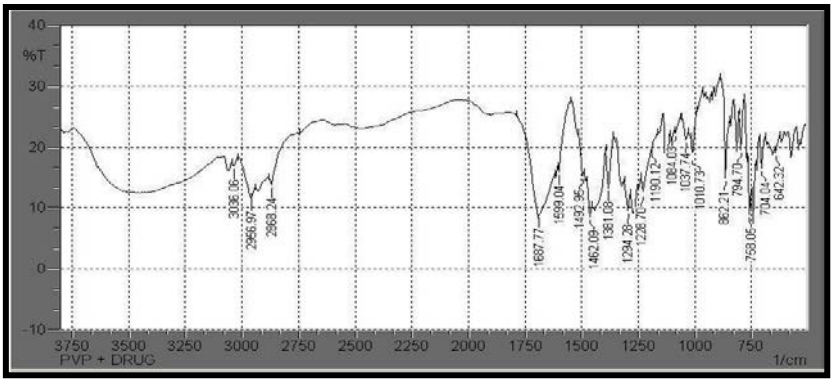


Figure 4 Infrared Spectra of Physical Mixture (Drug+PVPK30)

Table 4 Drug polymer interaction study through IR spectroscopy

Functional Group	Wave numbers of observed peaks (cm^{-1})		
	Telmisartan	PVPK30	Physical mixture
O-H stretch	3134.00	1460	1460.16
-NH Stretch	3360	-	3240.52
C=O(S)	1691.00	2924.18	2935.70
C=C-(S)	1624.12	-	1387.83
C-O	-	1249-	1249.91

NH_2 Strech	-	3446.91	3446.91
C-N Strech	1244.13	-	874.75 & 713.69
NO2 Aromatic strech	1545.07	-	-
CH_3 Bending	1455 & 1381	1290.42	1294.28

Differential Scanning Calorimetry (DSC) study

The DSC Thermograms were recorded for Telmisartan, PVPK30, Physical mixture. The DSC heating and cooling curve were recorded as a plot of enthalpy (in mW) vs. the temperature in (°C). No significant change in the position ofendothermic peaks was observed after running the physical mixture (Fig. 5 & 6). Thus, physical incompatibility between the components was discarded.

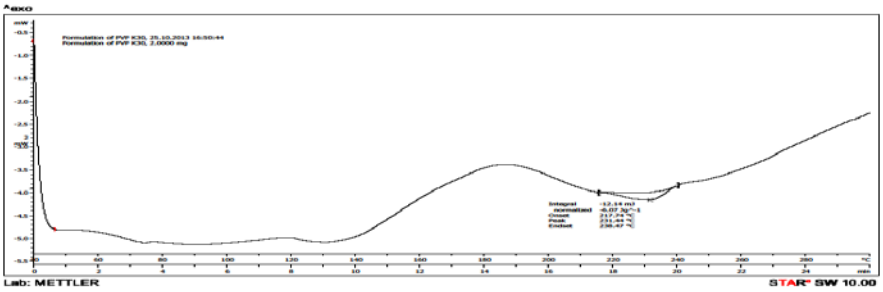


Figure 5 DSC of PVPK30

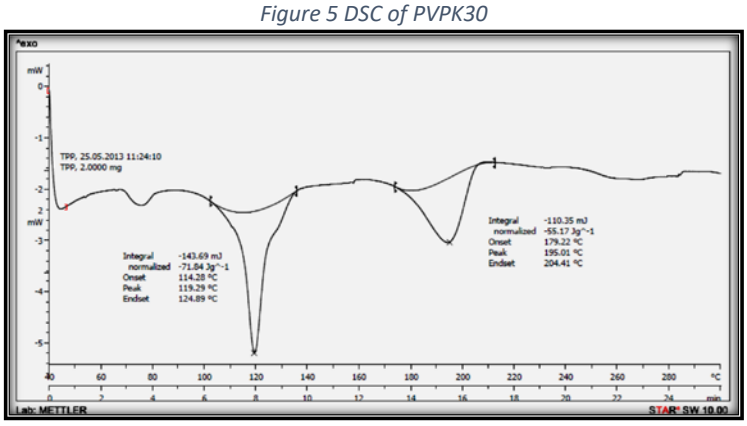


Figure 6 DSC of Physical mixture

Determination of λmax:

When Telmisartan was scanned in the wavelength rang 200-400 nm, peaks were observed at 296 nm as shown in figure

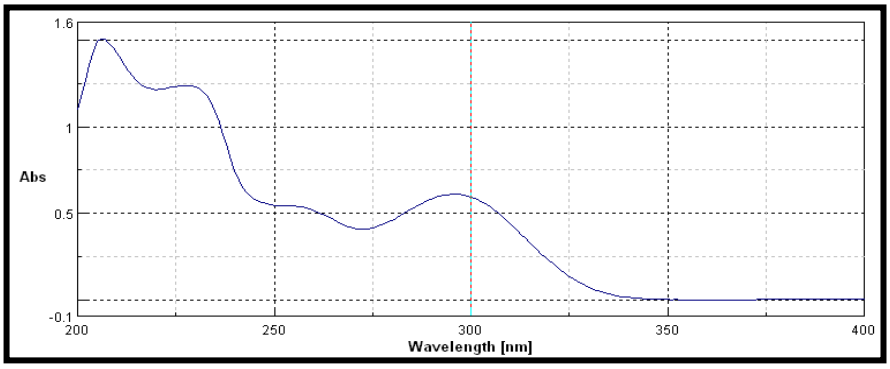


Figure 7 UV Spectra of Telmisartan

Table 5 Absorbance of Telmisartan in 0.1N HCL

Sr.No	Concentration in µg/ml	Absorbance
1	0	0
2	2	0.110
3	4	0.192
4	6	0.287
5	8	0.383
6	10	0.477
7	12	0.574

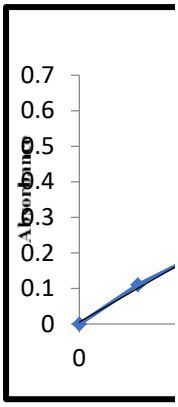


Figure 8 Standard calibration curve of Telmisartan in 0.1N HCL

Standard Calibration of Telmisartan in Water

Table 6 Absorbance of Telmisartan in water

Sr.No	Concentration in µg/ml	Absorbance
1	0	0
2	2	0.042
3	4	0.082
4	6	0.121
5	8	0.156
6	10	0.194
7	12	0.235

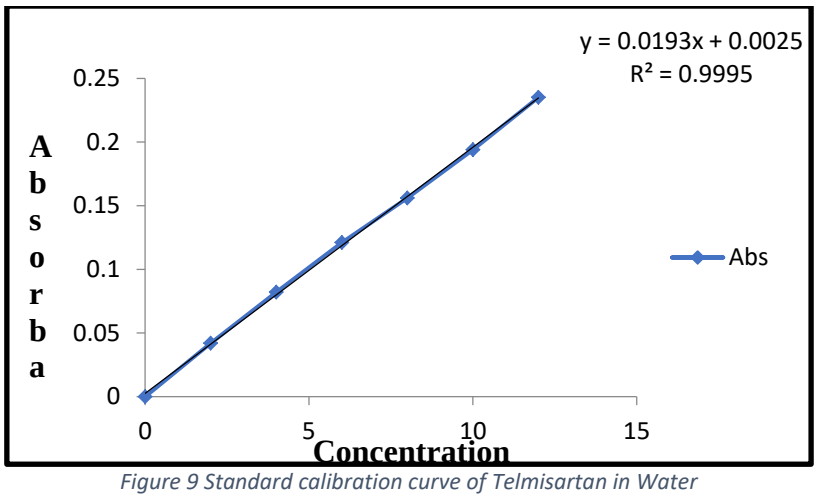


Figure 9 Standard calibration curve of Telmisartan in Water

Standard Calibration Curve in 6.8 Phosphate buffer

Table 7 Absorbance of Telmisartan in 6.8 phosphate buffer

Sr No	Concentration in µg/ml	Absorbance
1	00	00
2	10	0.075
3	20	0.137
4	30	0.202
5	40	0.269
6	50	0.346
7	60	0.418

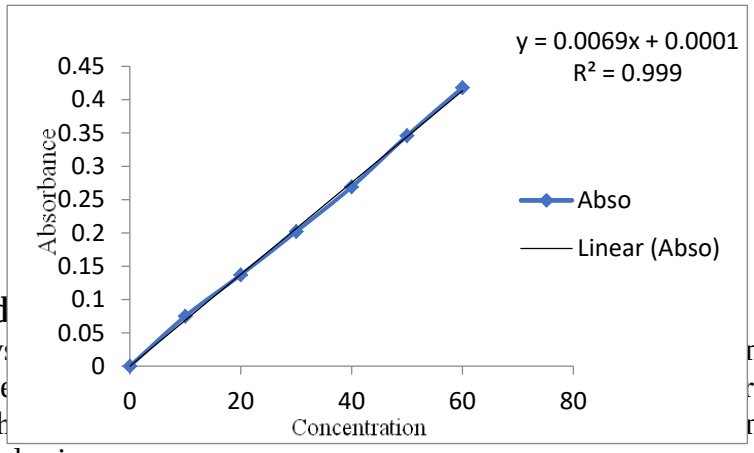


Figure 10 Calibration curve in pH 6.8 Phosphate buffer

Solubility stud

Prepared nanocrys mple in water and incre re Telmisartan (40 µg /ml). The high may be due to the reduction in partic

Table 8 Solubility study of Telmisartan Nanocrystals

Batch code	Solubility (µg /ml)	Increase in solubility (in fold)
Pure Drug	40	1.00
N1	88.35	1.607
N2	101.32	2.026
N3	103.67	2.076
N4	103.62	2.072

Characterization of Nanocrystal formulation

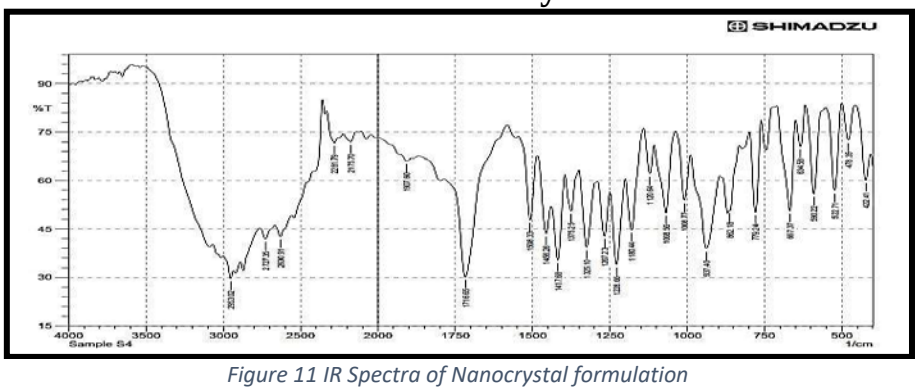


Figure 11 IR Spectra of Nanocrystal formulation

DSC of Nanocrystal Formulation

Differential scanning calorimetric analysis were carried out to examine the thermal behavior of the components used in the nanocrystal formulations. The DSC thermograms of the Telmisartan drug and nanocrystal formulations are given in Fig. Molecules in the crystal structure have a melting endotherm while molecules in the amorphous state do not exhibit a melting endotherm. Drug candidate showed a small and sharp characteristic endothermic peak at 260.1^0 whereas DSC thermograms of formulation endothermic peak at 260.1^0.

The DSC thermograms of formulation showed characteristic endothermic peaks corresponding to those pure drugs and there is no appearance of one or more new peak or disappearance of peak corresponding to those of pure drug indicating that crystalline nature remains with slight change in crystallinity due to change in melting point.

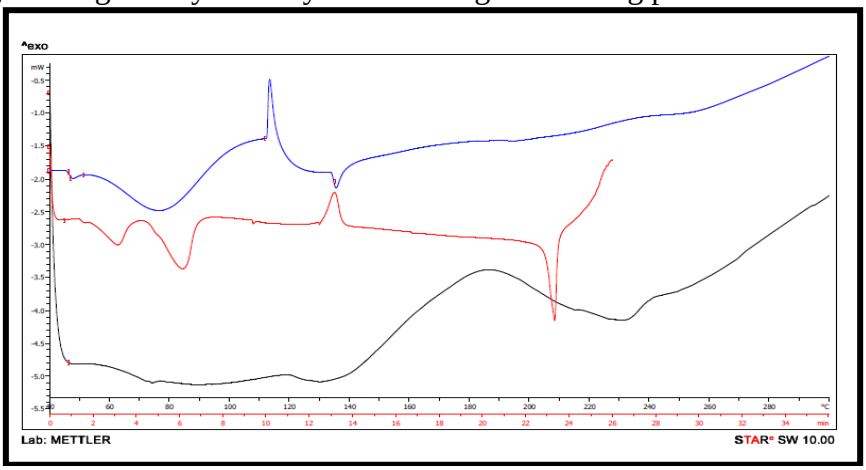


Figure 12 DSC of Nanocrystal Formulation

Morphological studies

SEM of Nanocrystal formulation

Morphological study of optimum formulation was done by taking SEM pictures of prepared Nanocrystals. It was revealed that they were spherical in shape and uniformly distributed. Studies also verified that the crystalline Telmisartan is converted to its amorphous form.

Surface morphology of the formulation was determined by using SEM and was found that particle size of formulation was below 1000 nm as compared to pure Telmisartan. The shape of formed Nanocrystals was found to be spherical and crystalline nature of all the formulations remains with slight change in crystallinity. The SEM images of pure Telmisartan, formulation shown in Figure.

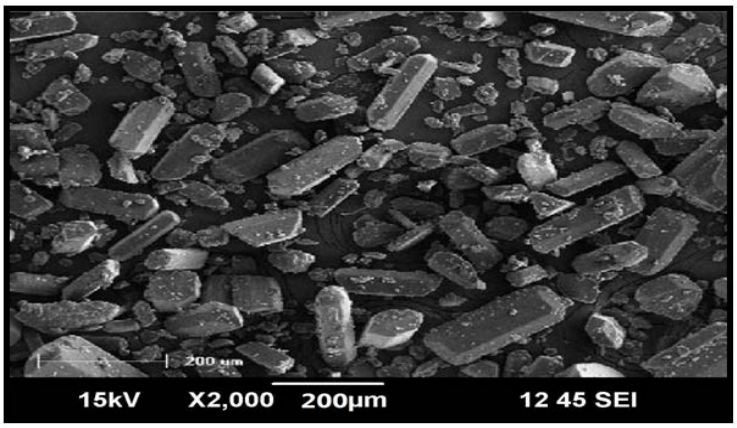
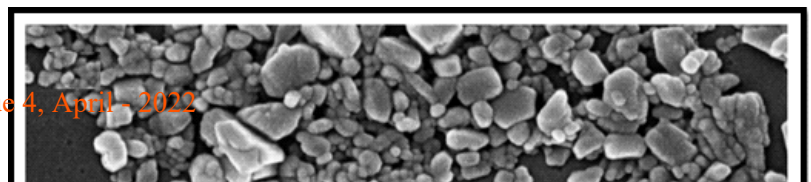


Figure 14 SEM of Pure drug



X-Ray Diffractograms:

X-ray diffractograms can provide useful information about the crystalline nature of the drug when formulated as nanocrystal. The formulations that were prepared evaluated for crystalline state to check whether there is change in nature of drug. Peaks in the X-ray diffractograms indicate the presence of crystal structure while no peaks are observed in the presence of amorphous structure. The X-ray diffractograms patterns of pure Telmisartan showed numerous sharp peaks, which are the characteristic of a crystalline compound. Nanocrystal formulation S4 showed the presence of numerous distinct peaks of 3.95o,4.36o,4.97o,5.29o,7.16o and 14.28o at 2θ whereas nanocrystal formulation showed peaks at 3.22o,4.02o,4.51o,5.39o,7.2o and 14.38o at 2θ which suggested that the drug was of a high crystalline form. The XRD results confirmed crystalline nature and showed that there is increase in crystalline nature in formulation as compared to pure drug. Following figure shows XRD patterns of pure drug and Nanocrystal's formulations.

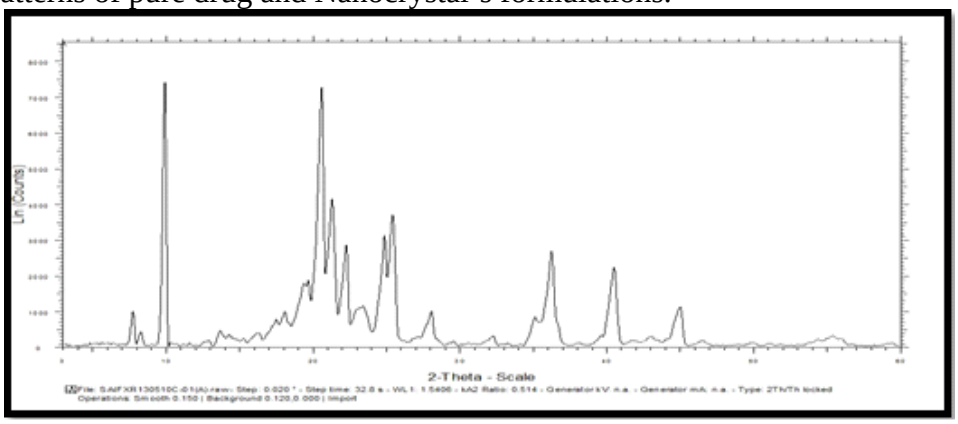
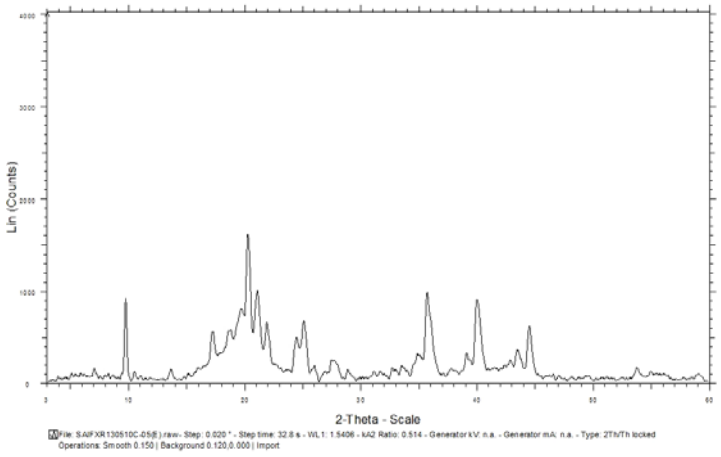


Figure 15 XRD of Pure drug



XRD of Nanocrystal formulation

In Vitro Dissolution Studies for Nanocrystal formulations

The in vitro dissolution studies were carried out by using USP apparatus type II at 50 rpm. The dissolution medium was 900ml 0.1 N HCL maintained at 37± 0.50C. Aliquots of dissolution medium were withdrawn at predetermined intervals and content of Telmisartan was determined at 296 nm spectrophotometrically.

Table 9 Dissolution data of Nanocrystals formulations

Sr. No	Time (min)	% Cumulative drug release				
		Pure drug	N1	N2	N3	N4
1	00	2.50±0.01	30.45±0.15	34.86±0.15	28.93±0.22	31.53±0.13
2	20	2.80±0.01	41.80±0.07	43.55±0.30	44.72±0.19	38.01±0.327
3	40	3.12±0.02	50.63±0.19	55.13±0.13	51.00±0.20	48.66±0.19
4	60	2.94±0.01	60.81±0.15	63.69±0.20	66.74±0.07	56.10±0.32
5	80	2.65±0.02	66.85±0.19	74.18±0.08	76.02±0.15	64.10±0.19
6	100	2.52±0.01	78.25±0.15	88.28±042	82.01±0.13	74.72±0.075
7	120	2.50±0.01	30.45±0.15	34.86±0.15	28.93±0.22	31.53±0.13
(All values are represented as mean ±standard deviation n=3)						

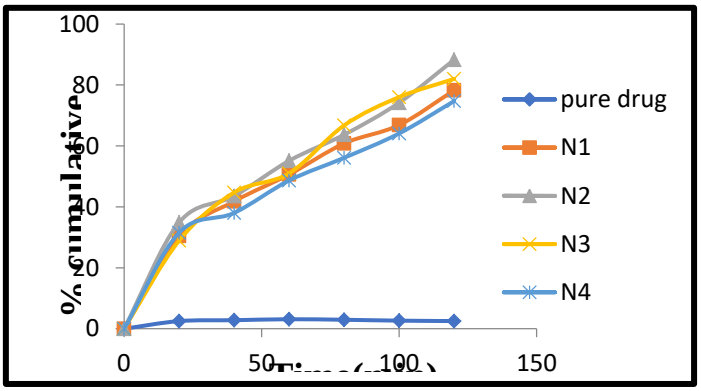


Figure 16 In vitro drug release profile

Release kinetics of Telmisartan Nanocrystals

Zero order release kinetic data

Table 10 Zero order release kinetic data

Sr. No	Time	% Cumulative drug release			
		N1	N2	N3	N4
1	00	00	00	00	00
2	20	30.45±0.15	34.86±0.15	28.93±0.22	31.53±0.13
3	40	41.80±0.07	43.55±0.30	44.72±0.19	38.01±0.327
4	60	50.63±0.19	55.13±0.13	51.00±0.20	48.66±0.19
5	80	60.81±0.15	63.69±0.20	66.74±0.07	56.10±0.32
6	100	66.85±0.19	74.18±0.08	76.02±0.15	64.10±0.19
7	120	78.25±0.15	88.28±042	82.01±0.13	74.72±0.075
(All values are represented as mean ±standard deviation n=3)					

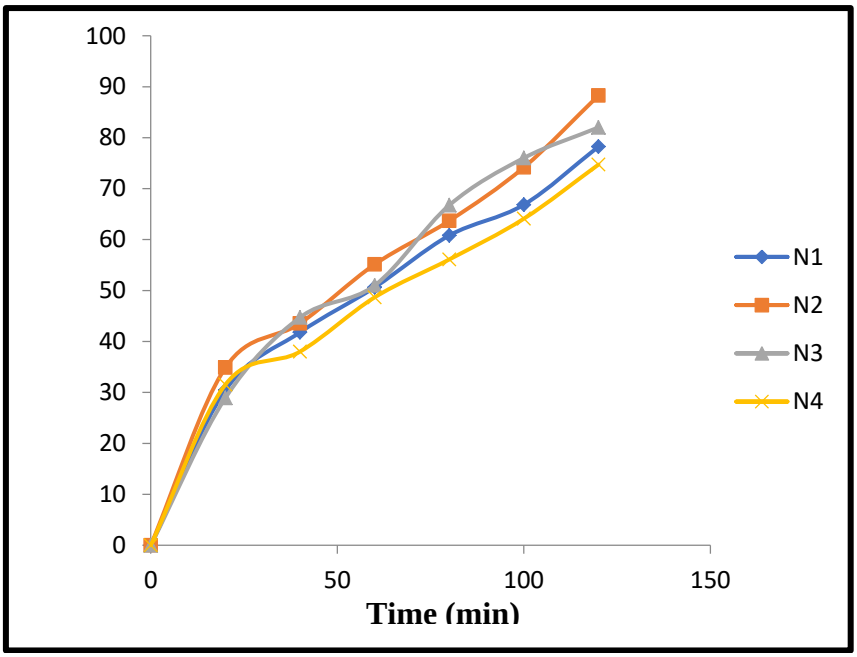


Figure 17 Zero order release kinetics

First order release kinetic data

Table 11 First order release kinetic data

Sr No	Time (min)	% Cumulative drug remain to be released			
		N1	N2	N3	N4
1	00	02±0.000	02±0.000	02±0.000	02±0.000
2	20	1.842±0.001	1.813±0.01	1.851±0.001	1.835±0.005
3	40	1.742±0.005	1.751±0.002	1.742±0.001	1.792±0.002
4	60	1.693±0.001	1.652±0.001	1.643±0.002	1.710±0.001
5	80	1.593±0.001	1.560±0.002	1.521±0.001	1.642±0.003
6	100	1.517±0.005	1.412±0.001	1.379±0.003	1.555±0.001
7	120	1.337±0.001	1.06±0.01	1.255±0.001	1.402±0.001
(All values are represented as mean ±standard deviation n=3)					

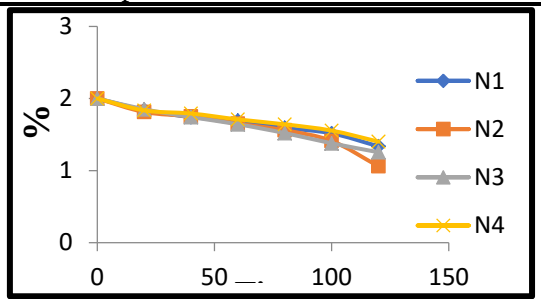


Figure 18 First order release kinetic

Higuchi matrix release kinetic data

Table 12 Higuchi matrix release kinetic data

Sr.No	√T	% Cumulative drug release			
		N1	N2	N3	N4
1	00	00	00	00	00
2	1.000	30.45±0.15	34.86±0.15	28.93±0.22	31.53±0.13
3	1.414	41.80±0.07	43.55±0.30	44.72±0.19	38.01±0.327

4	1.732	50.63±0.19	55.13±0.13	51.00±0.20	48.66±0.19
5	2.000	60.81±0.15	63.69±0.20	66.74±0.07	56.10±0.32
6	2.236	66.85±0.19	74.18±0.08	76.02±0.15	64.10±0.19
7	2.449	78.25±0.15	88.28±0.42	82.01±0.13	74.72±0.075
(All values are represented as mean ±standard deviation n=3)					

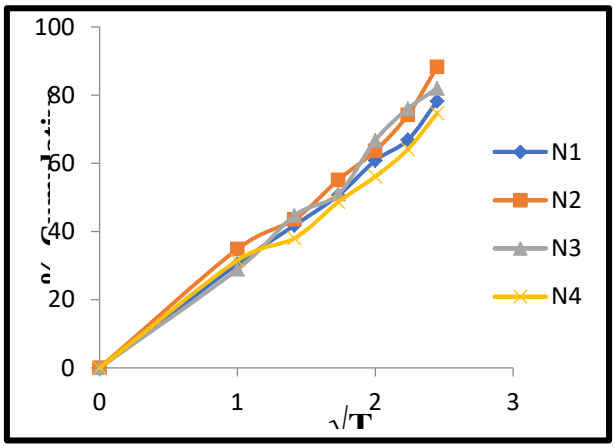


Figure 19 Higuchi matrix release kinetic

Peppa's release kinetic data

Table 13 Peppa's release kinetic data

Sr No	LogT(min)	Log % Cumulative drug release			
		N1	N2	N3	N4
1	00	00	00	00	00
2	00	1.483±0.002	1.541±0.001	1.461±0.003	1.498±0.002
3	0.301	1.621±0.001	1.638±0.002	1.650±0.002	1.579±0.003
4	0.477	1.703±0.001	1.741±0.003	1.747±0.001	1.686±0.001
5	0.602	1.783±0.001	1.803±0.001	1.823±0.005	1.748±0.002
6	0.698	1.824±0.002	1.870±0.001	1.880±0.005	1.806±0.001
7	0.778	1.893±0.001	1.945±0.002	1.913±0.005	1.873±0.005

(All values are represented as mean ±standard deviation n=3)

Table 14 Release kinetic of formulation F1-F4

Sr No	Zero order	First order	Higuchi	Peppas	
	r^2	r^2	r^2	r^2	n
Pure drug	0.815	0.942	0.985	0.960	0.489
F1	0.727	0.959	0.986	0.975	0.526
F2	0.467	0.898	0.942	0.967	0.360
F3	0.714	0.938	0.988	0.983	0.456
F4	0.714	0.951	0.968	0.930	0.312

Evaluation of Powder Characteristic of Telmisartan Tablets

The powders prepared for compression of tablets were evaluated for their flow properties. The powder characteristic indicates good flowability with an angle of repose value ranging from 31-35 i.e. (<35). The angle of repose of all formulations was found to be the

range of 31012 to 32070. The bulk density of all the formulation showed acceptable range. The bulk density of these powders was found to be in the range of 0.572 to 0.590 gm/cm3 for all formulations. The measured tapped density was in the range of 0.649 to 0.687 gm/cm3 for all formulations. Carr's index of powder was found the range of 11.86 to 14.11 for all formulations. These values indicate that the prepared powder exhibited good flow properties. The result mentioned in the table below

Table 15 Comparative study of various powder characteristics for formulation

Batch Code	Angle of repose(θ)	Bulk density (gm/cm3)	Tapped density(g/cc)	Carr's index(IC)
F1	$32^0$70	0.572	0.649	11.86
F2	$32^0$14	0.586	0.669	13.80
F3	$31^0$12	0.590	0.687	14.11

Characterization of Telmisartan Tablets

The weights of the tablets of all formulations were low standard deviation values, representing uniformity of weight. The difference in weight was within the range of 5% complying with Pharmacopeial specification (Indian Pharmacopoeia). The weight variation deviation of different formulations was found to be 1.28 to 1.60. The hardness for different formulations was found to be between 5.83 to 6.46 kg/cm2. It was indicate satisfactory mechanical strength. The diameter and thickness of all the formulations were found in the range of 9.0 to 9.4 mm and 4.28 to 4.32 mm respectively. The friability of all formulation was found to be between 0.49 to 0.62%. The tablets compressed were stable and having good physical characteristics. The percentage drug content for different tablets formulation varied from 96.47 to 98.32% was found to be within limits which indicate uniform drug distribution in all formulations, the limit (85% to 115%) of % drug content allowed by I.P.

Table 32: Physicochemical Characterization of Telmisartan Tablets

Batch Code	Weight		Hardness (kg/cm2)	Diameter (mm)	Thickness (mm)	Friability (%)	Drug Content (%)
	Average Highest (%)	Weight (mg) deviation					
F1	402.1	1.28	5.83	9.0	4.30	0.62	98.32
F2	397.5	1.85	6.26	9.5	4.32	0.53	97.27
F3	403.15	1.60	6.46	9.4	4.28	0.49	96.47

In Vitro Dissolution Studies for Telmisartan Tablet

The in vitro dissolution studies were carried out by using USP apparatus type II at 50 rpm. The dissolution medium was 900ml 0.1N HCL maintained at 37± 0.50C. Aliquots of dissolution medium were withdrawn at predetermined intervals and content of Telmisartan was determined at 296 nm spectrophotometrically.

From in vitro drug release data F2 batch shows maximum release of drug in 100 min

Table 16 Dissolution data of Telmisartan Nanocrystals tablet

Time	% Cumulative drug release

min	F1	F2	F3	Marketed
00	35.107	48.124	40.221	14.56
20	46.768	61.473	59.791	26.477
40	60.488	72.042	68.597	40.081
60	73.107	80.309	74.809	63.462
80	79.417	90.625	85.212	72.365
100	96.333	99.465	97.538	90.018
120	98.142	-	-	95.460

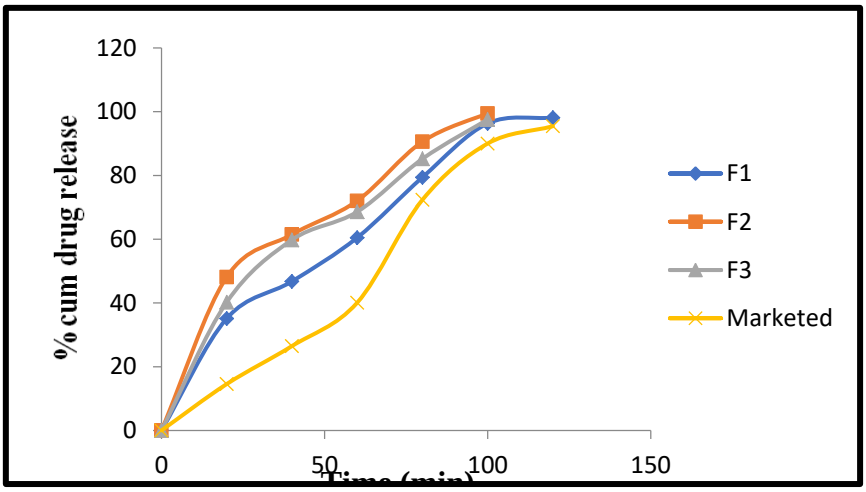


Figure 20 % cumulative drug release data of Tablet

DISCUSSION:

Telmisartan is a BCS class II drug processing a challenge in the design of dosage form due to its low aqueous solubility. Commercially It is available as tablets form.

To confirm the solubility of drug, solubility was determined at 25± 0.5^{0}C, in 0.1N HCl, pH 6.8 and distilled water. According to the results the drug was found to be poorly soluble in water when compared to 0.1N HCl and pH 6.8 buffers. As Telmisartan is a weak basic drug (pKa 7.7), an increase in solubility was releated with decrease in pH. These results revealed that the solubility of Telmisartan is pH dependant.

The λmax of Telmisartan was found to be 296nm & The calibration curve was found to be linear in the concentration range of 2-14 µg/ml with regression value of 0.994

Compatibility of drug and excipients was performed by FTIR method and DSC Thermogram.

Telmisartan nanocrystal prepared by antisolvent precipitation method.

In this method the drug should be freely soluble in solvent and insoluble in antisolvent. The antisolvent can be any solvent which is able to be mutually or partially miscible with the solution of drug, and has solubility as low as possible to the drug. So, drug was freely soluble in Dimethyl formamide, selected as a solvent and water was selected as an antisolvent.

The solubility study, particle size analysis, % drug content, in vitro drug release studt shows that batch N2 shows best result from all other batches.

Particle size result & SEM result revealed that Telmisartan nanocrystal have size below 1000nm i.e 650 nm

Nanocrystal characterization FTIR, DSC, XRD, shows that crystallinity of drug reduced towards amorphous form.

The in vitro dissolution of drug nanocrystal formulation shows increase drug release compared to pure drug

The drug release kinetic data of batch N1 to N2 shows that drug release follow korsemaye peppas model & Fickian diffusion transport mechanism.

Nanocrystal formulated into tablet by direct compression method which shows better drug release profile compared to marketed tablet of Telmisartan

CONCLUSION

Nanocrystal of Telmisartan was prepared by anti-solvent precipitation method. The prepared formulation was evaluated for FTIR, DSC and XRD and found that there is no any interaction between drug and excipients.

Surface morphology of the formulation was determined by using SEM and was found that particle size of formulation was below 1000 nm as compared to pure Telmisartan. The solubility and dissolution of the nanocrystal formulation was improved compared with pure Telmisartan and marketed formulation of Telmisartan because of reduction in particle size of formulation than pure drug. The dissolution of tablets containing nanocrystals shows more drug release compare to tablets containing pure drug. Hence this method can be used for preparation of tablets of Telmisartan by direct compression with directly compressible tablet excipients.

The above study shows that present work was a satisfactory preliminary study of improving bioavailability of Telmisartan. Further detailed study and in vivo-in vitro correlation need to be established to promise the efficiency and bioavailability of the formulation. Further studies on increasing bioavailability have to be carried out with the use of other method for the production of nanocrystals. The release kinetic shows the release by nonfickian diffusion type.

From the above studies it is evident that a promising novel conventional oral formulation of Telmisartan can be developed. Further detailed investigations are required to establish efficacy of these formulations.

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