Research Article

Sachin Namdeo Kothawade et al. /BioMedRx 2013,1(2),188-194

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Formulation and Physical Characterization of Microcrystals for Dissolution Rate Enhancement of Telmisartan

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Received on:18-10-2012; Revised on: 22-11-2012; Accepted on:26-01-2013

ABSTRACT

Telmisartan is a potent, long-lasting, nonpeptide antagonist of the angiotensin II type-1(AT1) receptor that is indicated for the treatment of essential hypertension. Telmisartan is practically insoluble in water and its dissolution is the rate-limiting step for its absorption, which leads to variable bioavailability. The aim of this investigation was to enhance the dissolution rate of Telmisartan by formation of microcrystals using solvent change method. The in-situ micronization process was carried out using solvent change method in the presence of Polyvinylpyrrolidone (PVP) as a stabilizing agent that limits the size of the particles generated. Telmisartan was dissolved in methanol and the stabilizing agent in water (as a non-solvent). The non-solvent was poured rapidly into the drug solution under stirring by a magnetic stirrer, and the resultant was oven-dried. Microcrystals were characterized by optical microscopy, SEM, FTIR, XRD and in vitro powder dissolution study. Telmisartan microcrystals showed narrow particle size and change in crystalline shape from avicular shape. FTIR results showed no interaction between the drug and the stabilizer. XRD diffractograms of microcrystals showed smaller peak height than untreated Telmisartan indicates that crystal habit modification occurred in the microcrystals without any polymorphic changes. Negative Gibb's free energy change represented spontaneous solubility of microcrystals. Dissolution efficiency of Telmisartan microcrystals at 15 min ($DE_{15\%}$) was increased about 9 times. Microcrystals were found to have good flow property.

Key words: Telmisartan, Microcrystal, Solvent change method, Dissolution efficiency.

INTRODUCTION:

Dissolution rate in the gastro-intestinal tract is the rate limiting factor for the absorption of these drugs, and so they suffer from poor oral bioavailability[1]. For BCS class II-drugs, the dissolution rate is the limiting factor for the drug absorption rate[2]. An enhancement in the dissolution rate of these drugs can increase the blood-levels to a clinically suitable level.

In recent years, solvent change method (antisolvent precipitation method) has been used for microcrystallization of drugs in the presence of excipients for increasing the dissolution rate of poorly water soluble drugs[3]. Particle size reduction is achieved because adsorption of excipients onto the particle surface that inhibits particle growth[4]. Powder wettability can be increased through adsorption of hydrophilic stabilizing agent. Thus it is clear that precipitation in the presence of stabilizing agent can have a positive effect on dissolution rate. This technique is a rapid, easy to handle, needs only common equipment and direct process which can be performed with ease. The aim of this study is to prepare and characterize Telmisartan microcrystals and optimize the solvent ratio and stabilizing agent concentration.

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MATERIALS AND METHODS:

Telmisartan was provided as a gift sample by Matrix Labs Pvt. Ltd, Nashik. PVP K30 LR was purchased from SD Fine Chemicals Ltd, Mumbai. Potassium phosphate monobasic AR was purchased from Astron Chemicals (India) Pvt. Ltd, Ahmedabad. Sodium hydroxide (pellets) and methanol (CH3OH, HPLC–Spectra grade, B.P. = 64.7° C, MW = 32.04) were purchased from SD Fine Chemicals Ltd, Mumbai. Double distilled water was used throughout the study.

Optimization of solvent change precipitation procedure:

The solvent change precipitation [SC] was conducted by instantaneously mixing two liquids in the presence of a stabilizing agent. The organic phase (solvent phase) was a methanolic solution of Telmisartan at (8.8 gm/50ml) a PVP K30 LR was selected as a stabilizing agent in the aqueous phase.

The non-solvent (aqueous phase) was poured rapidly from a beaker into the methanolic drug solution under stirring using a magnetic stirrer. The process was carried out at room temperature. The effect of experimental variables on the yield of the precipitates was accounted[5]. Four different solvent ratios (1:2, 1:4, 1:8 and 1:16 of methanol to water) were tried to select the most appropriate ratio to achieve the smallest particles and the maximum yield of the particles. For this experiment, a high concentration of stabilizing agent (0.5%) was selected in order to avoid the stabilizing agent concentration being a

limiting factor. A second experiment was carried out using the selected solvent ratio and five different concentrations of PVP (0, 0.01, 0.05, 0.1, 0.2 and 0.5%) in order to estimate the minimum concentration of PVP necessary to obtain the smallest stable drug particle size[6].

Crystallization procedure:

First, an organic solution of the drug was prepared by dissolving 8.8 gm of the drug in 50 ml of methanol. Then 200 ml of an aqueous solution containing 0.1% w/v PVP was added rapidly under stirring to the drug solution. This caused super saturation with respect to the drug and subsequent nucleation and crystal growth. The mixture was stirred for 60 min. The crystals were collected by filtration using whatman filter paper (grade 1, 90 mm diameter) followed by three consecutive washings with 10 ml of cold water to remove any non adsorbed excipient and dried in an oven at 45^0C for 2 hr.

Characterization of crystals:

Particle size analysis:

The size distribution of microcrystals and untreated Telmisartan powder was measured with an optical microscope.

Solubility study:

Excess amount of Telmisartan microcrystals and pure Telmisartan drug powder were dispersed in 20 ml of distilled water. The dispersion was shaken at 100 rpm at 37^0C for 24 h using thermostatic cabinet (Remi, RIS-24BL, Mumbai). The dispersion was filtered through a whatman filter paper (grade 1, 90 mm diameter). The filtered sample solutions analyzed using a UV-Visible spectrophotometer (Jasco V-250) at 248 nm after appropriate dilution. The mean results of triplicate measurements and the standard deviation are reported. The Gibbs-free energy of change (ΔG_{tr}^0) of Telmisartan occurred during formation of microcrystals from untreated Telmisartan powder was calculated using equation 1.

$$\Delta G^0_{tr} = -2.303\ RT\ Log\ So/Ss \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where So / Ss, is the ratio of the molar solubility of Telmisartan microcrystals in distilled water to that of the untreated Telmisartan powder. R is gas constant (8.31 KJ mol^{-1}) and T is temperature in degrees Kelvin (310.15^0 K).

Scanning electron microscopy (SEM):

Scanning electron micrographs of Telmisartan microcrystals and untreated Telmisartan drug powder were taken using a scanning electron microscope (Philips, Philips XL 30 ESEM). Samples were fixed on an aluminum stub with conductive double-sided adhesive tape and coated with gold in an argon atmosphere (50 Pa) at 50mA for 50 s.

Fourier transform infrared spectroscopy:

The FTIR spectra were recorded on a FT-IR spectrophotometer (Perkin-Elmer, Spectrum GX FTIR, USA). A blend of drug particles and KBr (about 1% w/w) was compressed into 12 mm discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded.

X-ray diffractometry:

Powder X-ray diffraction (PXRD) patterns were collected in transmission using an X-ray diffractometer with a rotating anode (Philips, X-pert-MPD) with Cu Ka1 radiation (monochromator: graphite) generated at 200 mA and 40 kV. Powder was packed into the rotating sample holder between two films (PETP).

In vitro Dissolution study: Powder Dissolution:

Powder dissolution study was carried out by using a USP apparatus II (Electrolab, TDT-08L) in 900 ml of phosphate buffer pH 7.4 at a temperature of 37 ± 1^0C at 75 rpm. A powdered sample (100 mg) was introduced directly into the dissolution medium. At regular time intervals, suitable amount of sample was withdrawn and same amount replaced by fresh medium. Drug amount released was analyzed spectrophotometrically (Jasco V-250) at wavelength of 248 nm. All studies were carried out in triplicates. Dissolution efficiency (DE %) was calculated according to equation 2.

$$D.E. = \frac{\int_0^t y \times dt}{y100 \times t} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Flow property study:

The loose bulk density (LBD) and tapped bulk densities (TBD) were determined by using density apparatus. The Carr's index (%) and the Hausner's ratio were calculated by using LBD and TBD. The angle of repose of untreated Telmisartan powder and the microcrystals were assessed by fixed funnel method.

Statistical analysis:

Paired t-test and one-way ANOVA were employed to analyze the results (Microsoft Excel and Statplus software). Difference below the probability level 0.05 was considered significant. Difference below the probability level 0.001 was considered highly significant.

RESULTS AND DISCUSSION

Optimization of solvent to antisolvent ratio:

The mean particle size of untreated Telmisartan powder was 37.5 µm while particles precipitated in the presence of 0.5 % of PVP was less than 5.5 µm. No important disparity was achieved in particle size values and cumulative drug release among the different methanol/water ratio. This was might be because of the fact that constant concentration (0.5 %) of PVP was used in optimization of methanol/water ratios. Statistical analysis of the particle size of Telmisartan microcrystals produced by using various methanol/water ratios by a one-way ANOVA test showed that methanol/water ratio had not significant effect ($p > 0.05$) on the particle size of the Telmisartan microcrystals. Methanol/water ratios had highly significant effect ($p > 0.001$) on % crystal yield of the Telmisartan microcrystals. So, selection of optimum methanol/water ratio was based on the % crystal yield of microcrystals at various ratios. Maximum crystal yield and small particle size obtained at a solvent ratio 1:4. Therefore, 1:4 was optimum methanol/water ratio for crystallization of Telmisartan. A lower % crystal yield in case of ratios of 1:2, 1:8 and 1:16 because of methanol/

water ratio of 1:2 entails a little efficient polarity change because aqueous phase was insufficient to bring out complete crystallization from organic solvent, whereas a1:8 or 1:16 ratio means the use of a high aqueous phase volume that solubilizes a Telmisartan fraction[7].

Optimization of concentration of PVP:

Table 1 that presents the mean diameter of Telmisartan particles precipitated using different concentrations of PVP after 60 min. The minimum concentration of PVP required to obtain small and stable size particles of Telmisartan was 0.1%, below this concentration the particle growth occurs. Results of statistical analysis showed that concentration of PVP had highly significant effect ($p > 0.001$) on the particle size of the Telmisartan microcrystals as compared to untreated Telmisartan powder. Telmisartan particles produced by crystallization without PVP have a bigger size and broader particle size distribution, whereas the system with the stabilizer PVP stops the molecular association and the crystal growth instantaneously at the moment of solvent change[5].

During the crystal precipitation, surface energy of the system increases. Here, PVP adsorbed onto the newly created surface of the precipitated drug in order to lower the interfacial tension. Thereby, the surface energy and consequently the enthalpy of the system are lowered. The formed small particles, which normally would aggregate in order to lower the surface energy, are stabilized sterically against crystal growth by an adsorbed layer of PVP. Micron sized particle formed and simultaneously stabilized in the formed dispersion by PVP.

Higher crystal yield 76.6% was obtained in case of Telmisartan microcrystals prepared using 0.1% PVP. Results of one-way ANOVA showed that 0.05% and 0.5% of PVP had significant effect ($p > 0.05$) and 0.1 % of PVP showed highly significant difference ($p > 0.001$) on % crystal yield of the Telmisartan microcrystals as compared to % crystal yield of Telmisartan crystals prepared using without stabilizing agent.

The tendency of the solid phase to exhibit solubility is best described by the Gibbs free energy change ($? G°_{tr}$). Negative Gibbs free energy values indicate favorable conditions. The $? G°_{tr}$ values were all negative at various concentrations, thus indicating that Telmisartan microcrystals had higher aqueous solubility. These values decreased with increasing concentration of PVP up to 0.1 %, thereby demonstrating that drug solubility increased as the concentration of PVP increased up to 0.1 %. After 0.1 % of PVP, $? G°_{tr}$ values were increased compared to its lower concentration, which showed that after 0.1 % PVP concentration, drug solubility was decreased.

Higher 58.3% dissolution efficiency after 15 min. obtained in case of 0.1 % PVP as compared to Telmisartan microcrystals prepared using other concentration of PVP. Results of One-way ANOVA test stated that highly significant difference ($p > 0.001$) in drug release was observed for Telmisartan microcrystals prepared using 0.05, 0.1, 0.2 and 0.5 % of PVP as compared to untreated Telmisartan powder. Telmisartan microcrystals prepared using 0.1% PVP as stabilizing agent showed small particle size, higher crystal yield, high water solubility, great reduction in Gibb's free energy, and higher dissolution efficiency as compared to other Telmisartan microcrystals. Therefore, 0.1% concentration was optimum concentration of PVP for microcrystallization of Telmisartan.

Crystallization was carried out employing the solvent change method using PVP at 0.1% as the stabilizing agent and a solvent methanol/ water ratio 1:4. Fluffy powders were obtained.

Scanning electron microscopy (SEM):

Face specific adsorption of stabilizing agent alters the growth rates of the faces where adsorption takes place and thus changes the morphology of the crystal[6].Modification of crystal habit can improve the dissolution rate by promoting growth of more hydrophilic faces, or inhibiting growth of more hydrophobic faces. Scanning electron micrographs of Pure Telmisartan drug powder, Telmisartan crystals

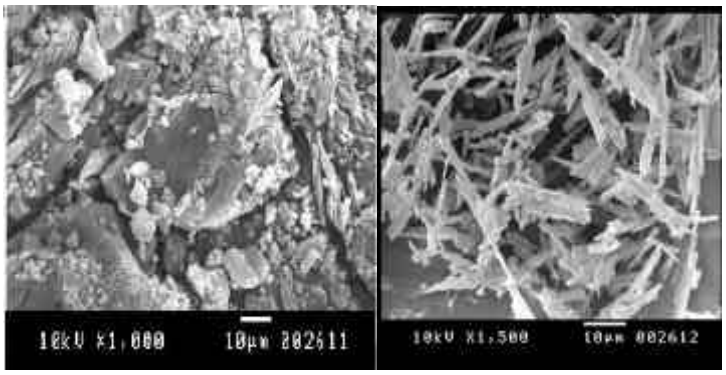
Table 1 : Percentage (%) crystal yield, Mean Particle size, solubility in distilled water, Gibb's free energy change and dissolution efficiency (DE) after 15 min of dissolution test of untreated Telmisartan powder, Telmisartan crystal prepared without PVP and its microcrystals prepared by solvent change method.

Conc. of aqueous solution of PVP	% Crystal Yield	Mean Particle Size (µm)	Solubility in water (mg/ml)	Gibbs free energy change (DG·tr)	DE15%
0.00%	69.4	42.5##	0.044 ± 0.001	-965.761	14.58
0.03%	70.6	23.25##	0.301 ±0.0023	-5893.14	49.59
0.05%	72.6#	22.5##	0.309 ±0.0018	-5965.04	53.13
0.10%	76.6##	18.25##	0.339 ± 0.002	-6203.9	58.3
0.20%	68.8	21##	0.255 ±0.0015	-5464.84	53.68
0.50%	65.3#	21.25##	0.235 ± 0.002	-5257.52	54.27
Untreated Telmisartan powder		95.25	0.031 ± 0.003		6.7

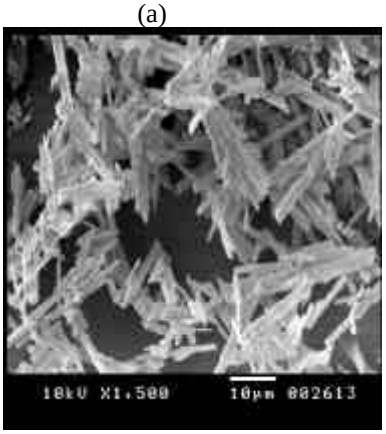
 # Significant difference ($p > 0.05$) upon application of one-way ANOVA.
Highly Significant difference ($p > 0.001$) upon application of one-way ANOVA.
DE = Dissolution efficiency

Table 2: Bulk density, tapped density, angle of repose, % Carr's index and Hausner's ratio for pure Telmisartan powder, Telmisartan crystals without PVP and its microcrystals prepared using PVP.

	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Angle of repose (Average ± SD)	% Carr's Index (Average ± SD)	Hausner's Ratio (Average ± SD)
Untreated Telmisartan Powder	0.1724	0.37	**19.37^0**±1.1	25.49 ± 2.22%	0.89 ± 0.004
0.00%	0.1533	0.16	18.26 ± 2.15	21.33 ± 2.31%	0.9581 ± 0.004
0.1 % PVP	0.1428	0.25	9.64 ± 0.67	18.76 ± 3.06%	0.5712 ± 0.004



(a) (b)



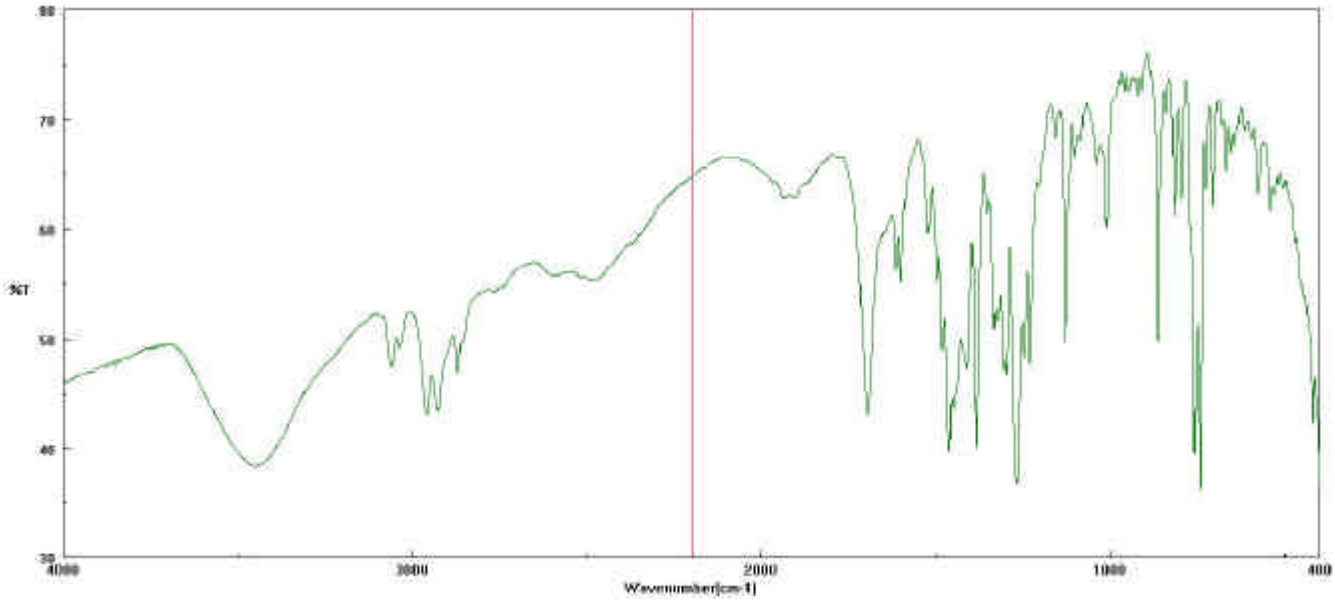
(c)

Figure 1: Scanning electron micrographs of: (a) Pure Telmisartan drug powder, (b) Telmisartan crystals without PVP (c) Telmisartan microcrystals prepared using 0.1% PVP

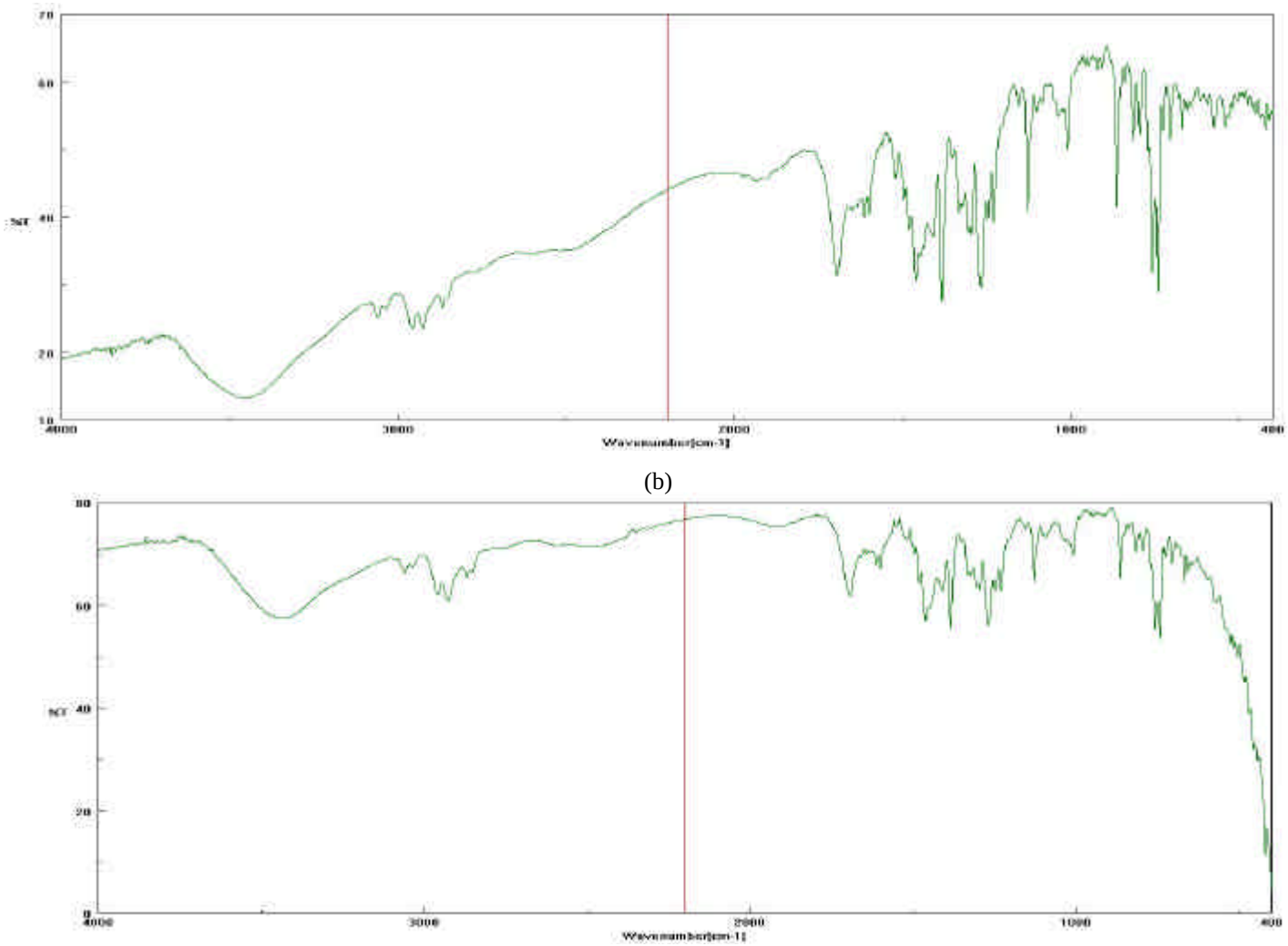
prepared without stabilizing agent and Telmisartan microcrystals shown in figure 1. Pure Telmisartan powder showed large rod like shaped crystal habit, while Telmisartan crystals without stabilizing agent plate shaped. Microcrystals prepared using 0.1 % PVP showed small platy crystals.

Fourier transforms infrared spectroscopy:

Figure 2 showed that FTIR spectras of the pure Telmisartan powder, microcrystals and Telmisartan crystals without PVP were identical and the main absorption bands of Telmisartan appeared in all the spectra.Absorption band for N-H stretching of urea group of Telmisartan ap-peared around 3330 cm-1. Similarly, the S=O stretching of sulphonamide group of Telmisartan located at 1335 cm- 1 and 1159 cm- 1 was not shifted in microcrystals. The absorption band for C=O stretching of urea group of Telmisartan located at 1702 cm- 1 and 1662 cm- 1 in pure Telmisartan drug powder, and these were not shifted in the microcrystals spectra. The FTIR spectra of all the tested samples showed the prominent characterizing peaks of pure Telmisartan which confirmed that no chemical modification of the drug had been taken place. Intensity of IR peaks of Telmisartan microcrystals were decreased as compared to untreated Telmisartan powder, implying that the change in crystal habit and particle size reduction in microcrystals is responsible for these changes.



(a)



(b)

(c)

Figure 2: FTIR spectra of: (a) Untreated Telmisartan powder, (b) Telmisartan crystals without PVP (c), and Telmisartan microcrystals prepared using 0.1% PVP

In vitro Dissolution study: Powder Dissolution:

The micronized Telmisartan powders showed a dramatic enhancement in dissolution rate as illustrated in Figure 3. DE15% of the studied microcrystals in comparison with the pure Telmisartan powder was presented in Table 1. Statistical analysis was performed on dissolution data using a one-way ANOVA test. Results suggested that dissolution profile of microcrystals was significantly differ ($p \pm 0.05$) from untreated Telmisartan powder. Telmisartan crystals prepared without stabilizing agent did not show significant improvement in drug release when compared with pure Telmisartan drug powder.

Dissolution enhancement effect of Telmisartan microcrystals explained by the dramatic reduction in the particle size and as a consequence the increment in the surface area, which is additionally hydrophilized by the adsorbed hydrophilic stabilizing agent. Moreover, the natural crystalline growth creates particles with no electrostatic charge and with better wettability properties. Formation of partially amorphous Telmisartan occurred during crystallization in presence of stabilizing agent also contributed for dissolution enhancement.

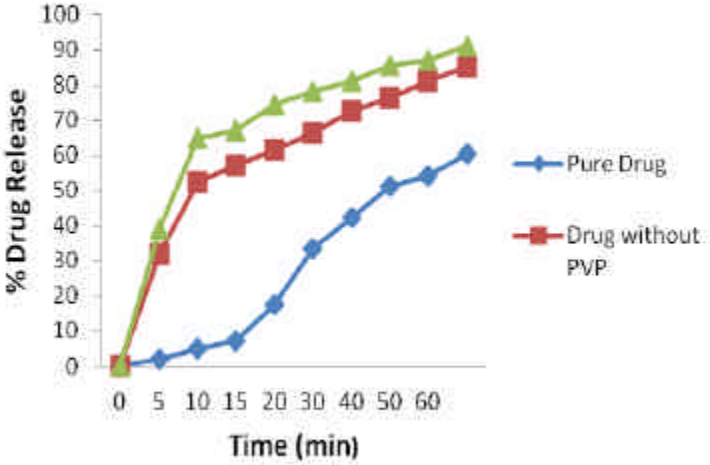


Figure 3: Dissolution profiles of (a) pure Telmisartan powder (b) Telmisartan crystal prepared without PVP (c) Telmisartan crystals prepared using various concentration of PVP

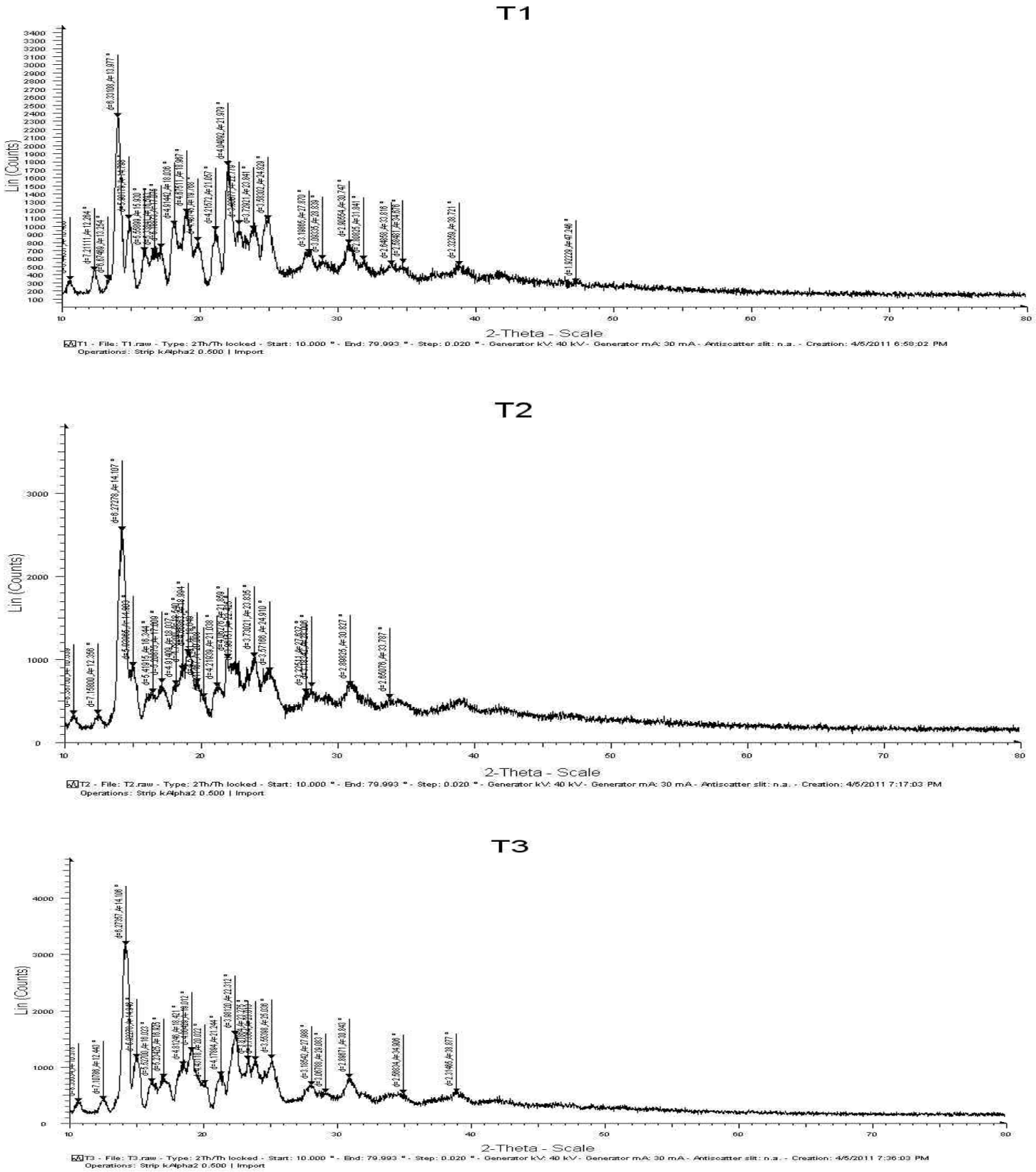


Figure 4: X-ray diffraction patterns of (T1) Pure Telmisartan powder, (T2) Telmisartan crystals without PVP (T3) Telmisartan microcrystals prepared using 0.1% PVP

Flow property study:

Bulk density, Tapped density, Angle of repose, % Carr's index and Hausner's ratio for pure Telmisartan powder, Telmisartan crystal prepared without PVP and Telmisartan microcrystals showed in Table 2. Pure Telmisartan drug powder exhibited poor flowability and compressibility as indicated by high value of Carr's index (29.49 ± 2.22%), Hausner's ratio (1.42 ± 0.004) and angle of repose (37.700 ± 1.1). This could be due to the irregular rod shape, which put hurdles in the uniform flow of powder from the funnel.

Microcrystals prepared with 0.1 % PVP showed im-proved flowability as indicated by lower value of Carr's index (13.33 ± 3.06), Hausner's ratio (1.15 ± 0.004) and angle of repose (29.090 ± 0.67). Small platy shape mi-crocrystals showed good flowability as compared to rod shaped Telmisartan powder.

CONCLUSIONS

Telmisartan microcrystals were prepared by solvent change method using PVP as a hydrophilic stabilizing agent. Solvent ratio (methanol/ water) 1:4 and 0.1% PVP were optimum parameters for microcrystallization of Telmisartan. Microcrystals produced using PVP showed narrow particle size distribution and change in the crystal habit from rod type to small plate type. The FTIR, and XRD results showed no chemical interaction between the drug and the stabilizer, and crystalline habit modification has occurred in the microcrystals without any polymorphic changes. The XRD revealed that crystallinity was reduced significantly in microcrystals. Negative Gibb's free energy change represented higher aqueous solubility of microcrystals. The enhanced dissolution rates attributed to the reduction of the particle size, change in crystal habit, formation of hydrophilic surface and the increased wettability due to adsorption of PVP and reduction in crystallinity of Telmisartan during micro-crystallization. In conclusion, the aforementioned technique is a promising tool for effective microcrystal formation during pharmaceutical development in order to increase dissolution rate of poorly water soluble active ingredient.

ACKNOWLEDGEMENT:

Authors are thankful to principal prof. D. G. Baheti for providing all necessary facilities.

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Source of support: Nil, Conflict of interest: None Declared