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Design and evaluation of tolvaptan solid dispersions using hot-melt extrusion and spray drying technique – A comparative study

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ABSTRACT

Tolvaptan is a selective, competitive vasopressin receptor 2 antagonist used to treat of clinically significant hypervolemic and euvolemic hyponatremia (serum sodium < 125 mEq/L or less marked hyponatremia that is symptomatic and has resisted correction with fluid restriction), including patients with heart failure and Syndrome of Inappropriate Antidiuretic Hormone. Tolvaptan belongs to BCS class IV with low solubility and low permeability, which results into poor bioavailability after oral administration. Therefore, solid dispersions (SDs) of Tolvaptan were prepared to enhance the solubility and bioavailability by two methods, Hot-melt extrusion (HME) and Spray drying technique (SDT) using various carriers like Soluplus, Copovidone (Kollidon VA64), Polyvinyl pyrrolidone (Kollidon 30) and Hypromellose 2.5cPs.to increase its aqueous solubility. Higher drug release was found in the SDs prepared by hot melt extrusion (HM3) as compared with spray drying technique (SDT2). There is more than 7 fold increases in the solubility of Tolvaptan prepared by HME and SDT compared with pure drug substance. The in vitro drug release profiles in HM3 and SDT2 are found to be comparable to that of drug release profiles of corresponding Innovator product. Physical characterization of hot melt extrudes of HM3 and spray dried mixture of SDT2 by FT-IR, DSC, XRD and SEM revealed that there was a change in crystal structure toward an amorphous form of Tolvaptan. The obtained results suggested that developed Tolvaptan SDs by HME & SDT has potential for oral delivery and might be an efficacious approach for enhancing the therapeutic potential of Tolvaptan.

Key words: Tolvaptan, Hot-melt extrusion, Spray drying technique, Soluplus, Povidone (Kollidon 30)

INTRODUCTION

The oral route of drug administration is the most common and preferred method of delivery due to convenience and ease of ingestion. But a drug with poor aqueous solubility will typically exhibit dissolution rate limited absorption and a drug with poor membrane permeability will typically exhibit permeation rate limited absorption. Hence, two areas of pharmaceutical research that focus on improving the oral bioavailability of active agents include enhancing solubility and dissolution rate of poorly water-soluble drugs and enhancing permeability of poorly permeable drugs [1, 2]. Researchers try to overcome this problem by converting the low energy and low solubility crystal forms to their corresponding highest energy states, i.e, amorphous state [3]. Enhancing the solubility and dissolution rate of drugs can be increased by a well-known process of fabricating solid dispersions [4]. Development of solid dispersions of poorly water soluble drugs is one of the most widely used approaches to enhance the solubility as well as dissolution rate [5]. Solid dispersion (SD) is defined as a dispersion of one or more active ingredients in an inert carrier or matrix in solid state prepared by melting, dissolution in solvent or melting-solvent method [6]. The most relevant technologies for the manufacture of solid dispersions are melting of Excipients or fusion method, embedding of drug by means of spray drying, co- evaporation, co-precipitation, freeze-drying and roll-mixing or co-milling [7]. HME (Hot melt extrusion) has the unique property to maintain the amorphous state of the drug after

the formation of solid dispersion [8]. Spray drying technique (SDT) method consists of dissolving or suspending the drug and polymer in a common solvent or solvent mixture and then drying it into a stream of heated air flow to remove the solvent [9]. Tolvaptan is a selective, competitive vasopressin receptor 2 antagonist used to treat hyponatremia (low blood sodium levles) associated with congestive heart failure, cirrhosis and the syndrome of inappropriate antidiuretic hormone [10]. The aim of the present study was to prepare the solid dispersions of Tolvaptan using different Polymers like Soluplus a novel polymer with amphiphilic properties, Copovidone (Kollidon VA64), Povidone (Kollidon 30) and Hypromellose 2.5cPs. Soluplus has been specially developed for hot melt extrusion [11, 13]. In this study, HME and Spray drying techniques (SDT) are used to obtain Tolvaptan solid dispersions, polymeric carriers are generally chosen depending on criteria such as hydrophilicity, solubility parameter and more over considering the melting point and degradation temperature of carriers / polymers.

MATERIALS AND METHODS

SAMSCA® (Tolvaptan) 30 mg tablets were obtained from Otsuka America Pharmaceutical, Inc., Rockville, MD 20850 (being manufactured by Otsuka Pharmaceutical Co., Ltd., Tokyo, 101-8535 Japan). Tolvaptan drug substance was gifted by Hetero Drugs Ltd, Hyderabad, India. Polyvinyl Caprolactam-Polyvinyl Acetate-Polyethylene Glycol Graft Copolymer (brand name is Soluplus), Povidone (Kollidon 30) and Copovidone (Kollidon VA64) were gifted by BASF,USA. Hypromellose 2.5 cPs was gifted by DOW chemical, USA and hard gelatin capsules were gifted by ACG associated Capsules, Mumbai. All other solvents used were of analytical grade.

Hot Melt Extrusion

Preliminary Solubility Studies of Tolvaptan

Solubility measurements of Tolvaptan were performed according to a published method [12]. Solubility of Tolvaptan was determined by adding an excess amount of Tolvaptan to volumetric flask of 25ml of water and then samples were shaken for the 48 hours at room temperature. Subsequently, the solutions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan by UV/Visible spectrophotometer at λ_{max} 269 nm. In the same Tolvaptan and carrier (Solubilizer), which is suitable for hot melt extrusion of drug substances having melting point above 200°C and hot melt extrusion (HME) at above 150°C and were taken in 1:1 ratios added to volumetric flask of 25ml of water and then samples were shaken for the 48 hours at room temperature. Subsequently, the solutions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan by UV/Visible spectrophotometer at λ_{max} 269 nm.

Preparation of Tolvaptan Solid Dispersions by HME:

Tolvaptan solid dispersions were prepared by using different carriers like Soluplus and Copovidone (Kollidon VA64). Thermo Fischer, HME Parma 24 - Twin Screw Model wasused for the preparation of solid dispersions with the feed rate of 1 to 1.25 Kg/hour, Torque**:**4 Barr and 8 different zones of temperature as from 40˚± 2˚C to 175˚± 2˚C with cooling/chillers zone maintained at 2 - 5˚C (where melt will be converted into the pieces of flakes) shown in **Table 1**.

Table 1: Temperature Ranges To Be Monitored During Processing of Holt Melt Extrusion (HME):

Name of the zone	Temperature
Barrel Conveying Unit (BCU)	40˚C± 2˚C
Zone – I	60˚C± 2˚C
Zone – II	80˚C± 2˚C
Zone – III	100˚± 2˚C
Zone – IV	120˚C˚± 2˚C
Zone – V	140˚C˚± 2˚C
Zone – VI	160˚C˚± 2˚C
Zone – VII	175˚C˚± 2˚C
Die Zone	175˚C˚± 2˚C
Cooling / Chillers zone	Maintained at 2 - 5˚C (where melt will be converted into pieces of flakes)

Formulation Trials with Soluplus:

Table 2: Formulation Plan of Tolvaptan Solid Dispersions

S. No	HME trials→ Ingredients↓	HM1 (mg/unit)	HM2 (mg/unit)	HM3 (mg/unit)
Drug: Carrier ratio (Tolvaptan: Soluplus) →		1:2	1:3	1:4
1	Tolvaptan	30.0	30.0	30.0
2	Soluplus	60.0	90.0	120.0
	Total qty of binary mixture	90.0	120.0	150.0

Tolvaptan and Soluplus were taken in the above mentioned ratios (**Table 2**), sifted together through ASTM #40 mesh and were mixed well in a poly bag for 5 minutes. The above binary mixture was hot melt extruded with keeping above mentioned temperature at different zones. The resulting flakes were opaque in HM1 while transparent in HM2 and HM3 (it indicates the more than 90% crystalline drug substance has been converted into amorphous form, the same has been revealed by DSC / XRD / SEM). The pieces of flakes were crushed into powder using mortar and pestle. The powder was sifted through # 30 mesh, The flow properties of blend were found to be satisfactory and in off-white to light brown coloured granular powder in HM1, where as light brown coloured granular powder in HM2 and HM3.

Formulation Trails with Copovidone (Kollidon VA 64)

In the next set of trials, Tolvaptan was hot melt extruded with the most commonly employed polymer for Hot melt extrusion, i.e., Copovidone (Kollidon VA 64) in 1:2, 1:3 and 1:4 ratios.

Table 3: Formulation Plan of Tolvaptan Solid Dispersions

S. No	HME trials→ Ingredients↓	HM4 (mg/unit)	HM5 (mg/unit)	HM6 (mg/unit)
Drug: Carrier ratio (Tolvaptan: Copovidone (Kollidon VA64) →		1:2	1:3	1:4
1	Tolvaptan	30.0	30.0	30.0
2	Copovidone (Kollidon VA64)	60.0	90.0	120.0
	Total qty of binary mixture	90.0	120.0	150.0

Tolvaptan and Copovidone (Kollidon VA64) were taken in the above mentioned ratios (**Table 3**) together through ASTM #40 mesh and were mixed well in a poly bag for 5 minutes. The above binary mixture was hot melt extruded with keeping below mentioned temperature at different zones. The resulting flakes were slightly opaque in HM4 while transparent in HM5 & HM6 (it indicates the drug substance has been converted into more 90% into amorphous form, the same has been revealed by DSC / XRD / SEM). The pieces of flakes were crushed into powder using mortar and pestle. The powder was in granular nature and was sifted through # 30 mesh. The flow properties of blend were found to be satisfactory.

Among the 6 trials by the hot melt extrusion process, the formed flakes at the cooling zone were found to be transparent in HM2, HM3, HM5 and HM6. It indicates that the drug substance has been converted into an amorphous form completely or more than 90%. The resulted powder after crushing the flakes using mortar and pestle was granular in nature and flow properties were found to be good / satisfactory. The granular powder was filled into size "3" hard gelatin capsules and analyzed for the drug release profiles and compared with drug release profiles of pure drug substance and corresponding Innovator product.

Characterization:

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The IR spectrum of the samples was prepared using KBr (spectroscopic grade) disks by means of hydraulic pellet press at pressure of seven to ten tons [13].

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC/min, over a temperature range of 0 to 250ºC [14, 15]**.**

Powder X-ray Diffraction:

A Bruker D8 diffractometer was used to perform powder X-ray diffraction (PXRD) of all samples. A Cu K-α 1 tube was the source, set at 40 KV and 50mA. A scan from 2 to 60^0 2 θ was carried out at a rate of 0.01220^0 2θ/s. The diffractometer was calibrated using powdered α-alumina. Hot-melt extruded samples were ground before analysis [15].

Scanning electron microscopy:

The shape and surface morphology of the Tolvaptan drug substance and Tolvaptan loaded solid dispersions were examined using XL 30 model JEOL 6800 scanning electron microscope (Japan) [15].

Evaluation of Tolvaptan Solid Dispersions:

Solubility Studies of Tolvaptan Solid Dispersion by Hot Melt Extrusion Method:

Hot melt extruded samples of HM1 to HM6 were taken 25 ml of Volumeric flask and make up with purified water. Samples were shaken for the 48 hours at room temperature. Subsequently, the solution was filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan by UV at λ_{max} 269 nm.

Drug Content

Accurately weighed quantity of Tolvaptan solid dispersion which is equivalent to 30mg of Tolvaptan was taken in volumetric flask and the volume is made to 100ml with Methanol. From this 1ml of solution is taken in a 10ml volumetric flask and is made up to 10ml with methanol. This solution is diluted to 10µg/ml and absorbance was measured at λ_{max} 269 nm against blank. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in solid dispersion}}{\text{Theoretical amount of drug in solid dispersion}} \times 100$$

***In Vitro* Release Studies:**

The *in vitro* drug release profiles for each solid dispersion as well as pure drug and corresponding Innovator product, i.e., SAMSCA® (Tolvaptan) 30 mg tablets were performed using USP type - 1 (Basket type) dissolution apparatus. The resulting blend of solid dispersion by HME equivalent to 30mg of Tolvaptan was filled in size 3 hard gelatine capsules were evaluated for dissolution testing. The conditions of dissolution testing are presented in Table 4. The samples are withdrawn at specified time intervals and the obtained samples were analyzed for drug release by using UV/Visible spectrophotometer at 269nm. The different dissolution parameters are summarised in Table 4. The cumulative percentage drug release was calculated.

Table 4: *In Vitro* Dissolution Studies Test Parameters:

Instrument	Electro lab- USP type I dissolution test apparatus.
Dissolution medium	0.22% Sodium Lauryl Sulfate in water
Apparatus	USP type I (Basket type)
Temperature	37±0.5°C
RPM	100
Volume of medium	900 ml.
Sampling intervals	5, 10, 15, 30,45,and 60minutes
Sample volume	10 ml withdrawn and replaced with 10 ml of dissolution medium.

RESULTS AND DISCUSSION

Results and Discussion for Tolvaptan Solid Dispersions by Hot Melt Extrusion Method

Preliminary solubility studies of Tolvaptan

In case of solid dispersions initially preliminary solubility analysis were carried out to select the appropriate qty of polymer, which are suitable for hot melt extrusion of drug substances having melting point above 200°C and hot melt extrusion above 150°C for the preparation of solid dispersion by HME process. Trials are made with the suitable polymer available for the hot melt extrusion, which should be stable at the temperature in the range of 150°C to 200°C. From the physical mixtures of drug and Soluplus in the ratio of 1:4 shown highest drug solubility i.e. 0.33mg/ml, when compared with all other ratios, presented in **Table 5.**

Table 5: Preliminary Solubility Studies of Tolvaptan

S.No.	Physical mixture	Solubility (mg/ml)
1.	Pure drug	0.04±0.02
2.	Tolvaptan: Soluplus (1:1)	0.27±0.11
3.	Tolvaptan: Soluplus (1:2)	0.28±0.12
4.	Tolvaptan: Soluplus (1:3)	0.30±0.09
5.	Tolvaptan: Soluplus (1:4)	0.33±0.03
6.	Tolvaptan: Soluplus (1:5)	0.29±0.06
7.	Tolvaptan: Kollidon VA 64 (1:2)	0.27±0.07
8.	Tolvaptan: Kollidon VA 64 (1:3)	0.26±0.09
9.	Tolvaptan: Kollidon VA 64 (1:4)	0.31±0.12
10.	Tolvaptan: Kollidon VA 64 (1:5)	0.30±0.04

Preparation of Tolvaptan Solid Dispersions

Solid dispersions of Tolvaptan were prepared with Soluplus in different ratios like 1:2, 1:3 and 1:4 (HM1 – HM3) and in another set of formulation trials were carried out with Copovidone (Kollidon VA 64) in the ratios of 1:2, 1:3 and 1:4 (HM4 – HM6) by HME method. In the present investigation, 6 formulations were prepared and their complete composition is shown in **Table 2 & 3.** All the solid dispersions prepared were found to be free flowing granular powder. The resulted powder of Solid dispersions was shown in **Figure 1.**

Figure 1: Tolvaptan Solid Dispersions by HME (with Soluplus in 1:4 ratios)

Characterization:

The prominent peaks of Tolvaptan was observed **(Figure 2)** the region of 3420.26 cm^{-1} due to the (Polymeric OH stretching), a peak at 3263.66 cm^{-1} due to (aromatic H(-C=C-)H stretching) and a peak at 3086.21 cm^{-1} due to (aromatic C-H stretching). At the lower frequencies 741 cm^{-1} (C-Cl), 1195.91 cm^{-1} (C-N stretching), 1357.93 cm^{-1} (N-H stretching), 1681.98 cm^{-1} (C=O stretching) observed. Soluplus **(Figure 3)** shows the prominent peak at 3410.26cm^{-1} due to polymeric OH stretching a peak at 2978.19 cm^{-1} due to the (aliphatic CH_3 stretching).

Physical mixture **(Figure: 4)** of the drug and Soluplus shows summation of the spectra of the drug and Soluplus equivalent to the addition of the spectrum of polymer and drug. This indicates that interaction has occurred with simple physical mixture of drug and polymer. In case of formulation HM3 **(Figure 5)** peaks related to C-H stretching remains unchanged. This indicates that overall symmetry of the molecule might not be significantly changed.

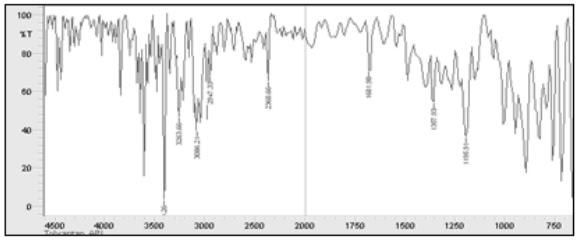


Figure 2: FTIR spectra of pure drug

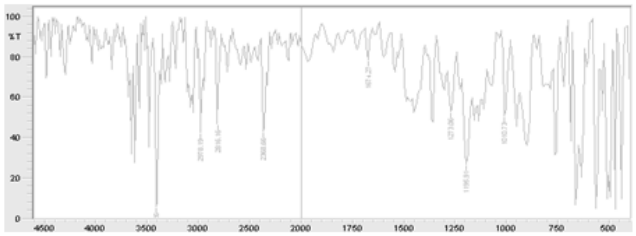


Figure 3: FTIR spectra of Soluplus

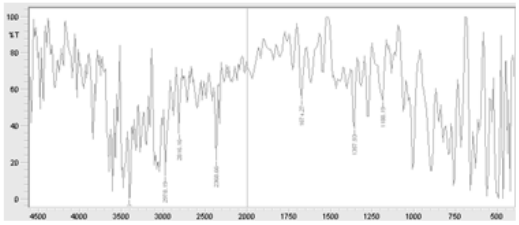


Figure 4: FTIR spectra of physical

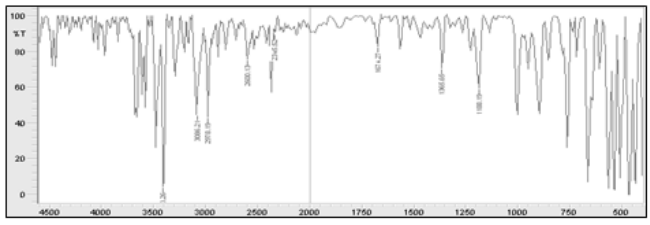


Figure 5: FTIR spectra of formulation HM3
Mixture of Tolvaptan and Soluplus (1:4)

Differential Scanning Calorimetry:

The DSC thermo grams of Pure Tolvaptan showed in F**igure 6,** sharp endothermic peak at melting point (225^0C), indicating that the drug is highly crystalline. The absence of drug peak in the solid dispersion formulation (HM3 (Tolvaptan & Soluplus (1:4)) indicating the drug was in amorphous form.

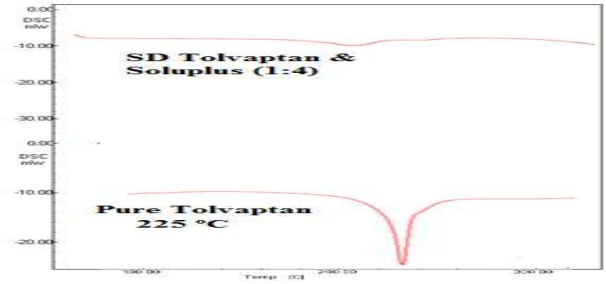
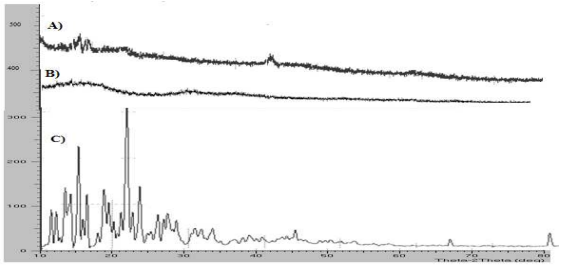


Figure 6: DSC Thermograms of Pure Drug and Formulation HM3

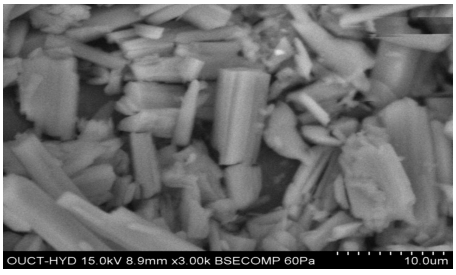
XRD Analysis

The XRD of Tolvaptan consist of sharp multiple peaks, indicating the crystalline nature of the drug. In case of SD (Tolvapatn with Soluplus (1:4)) when exposed to X-ray beam, disappearance of all crystalline endothermic peaks and characteristic intensities of Tolvaptan. This indicates complete transformation of crystalline Tolvaptan into amorphous form during HME process. From the XRD studies, it is confirmed that the drug substance in hot melt extruded granules (HM3) has been converted into amorphous form (**Figure 7**).

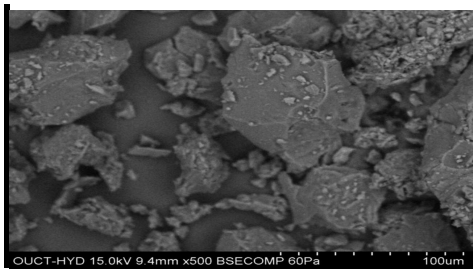


A) Formulation HM3 B) Placebo C) Tolvaptan pure drug

Figure 7: powder x-ray diffraction patterns of Tolvaptan Pure Drug, Placebo and HM3



A



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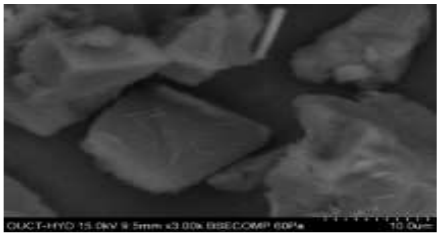

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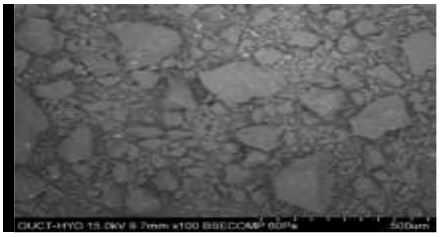


D

Figure 8: SEM images of Tolvaptan pure drug (A), SD formulation HM3 (B, C & D)

Scanning Electron Microscopy:
Surface micrographs of SD by hot melt extruded powder (HM3) and pure Tolvaptan were determined using SEM technique. The SEM micrograph of pure Tolvaptan (**Figure 8A**) was observed with large crystalline forms of drug agglomerates with ordered shape and size. The surface characteristics of SD of formulation HM3 (**Figure 8B, C & D**) showed rough disordered and intact structures, which subsequently help to dissolve drug when comes in contact with aqueous fluid.

Evaluation of Parameters:
Solubility Studies of Tolvaptan Solid Dispersions:
Six formulations of solid dispersions by hot melt extrusion method were prepared, 3 formulations each with Soluplus and with Copovidone (Kollidone VA64). After preparation of solid dispersion solubility analysis was carried out, this is compared with physical mixtures of pure drug substance itself. The formulation with Soluplus in the ratio of 1:4 (drug to carrier) which had shown increased solubility by more than 8 fold as compared to that of the pure drug (Pure drug solubility is 0.04). The results are tabulated in **Table 6**.

Table 6: Solubility Studies of Solid Dispersions Prepared By Hot Melt Extrusion Method

S.No.	Formulation	Solubility (mg/ml)
1.	Pure drug	0.04±0.02
With Soluplus		
2.	HM1 (1:2)	0.31±0.12
3.	HM2 (1:3)	0.33±0.08
4.	HM3 (1:4)	0.35±0.06
With Copovidone (Kollidon VA64)		
5.	HM4 (1:2)	0.32±0.09
6.	HM5 (1:3)	0.33±0.05
7.	HM6 (1:4)	0.34±0.07

Drug content:
Actual drug content of all 6 formulations are shown in **Table 7**. The drug content of the prepared solid dispersions was found to be in the range of 87.3- 98.1%. Maximum % drug content i.e. 97 % was found in the formulation HM6.

Table 7: % Drug Content for different formulations of Tolvaptan Solid dispersions

S.No.	Formulation code	(%) Drug content
1.	HM1	91±3.7
2.	HM2	92±2.9
3.	HM3	93±2.4
4.	HM4	96±3.3
5.	HM5	95±2.6
6.	HM6	97±1.1

***In Vitro* Dissolution Studies**

Table 8: *In Vitro* Dissolution Profiles of Pure Drug, Different Formulations of Tolvaptan Solid Dispersions (HM2, HM3, HM5 and HM6) and Innovator product

Time in Min	Cumulative % drug release					
	Pure drug	SAMSCA® 30 mg Tablets	With Soluplus		With Copovidone	
			HM2 (1:3)	HM3 (1:4)	HM5 (1:3)	HM6 (1:4)
0	0	0	0	0	0	0
5	12.2±4.9	32.4±2.6	35.6±4.2	45.7±3.8	34.5±4.4	30.9±3.7
10	18.6±4.1	49.4±2.2	52.2±3.6	55.7±4.1	48.6±3.2	45.7±3.5
20	22.1±3.6	72.8±1.6	63.6±3.3	67.8±3.5	58.6±2.7	57.5±3.1
30	28.9±3.3	79.2±1.9	66.3±2.3	78.5±2.6	65.2±2.2	64.2±2.8
45	32.5±2.6	89.8±1.2	75.3±1.6	86.1±1.5	73.8±1.7	73.6±1.9
60	39.6±2.7	94.2±0.9	89.1±1.3	93.5±1.2	83.6±0.8	79.2±0.6

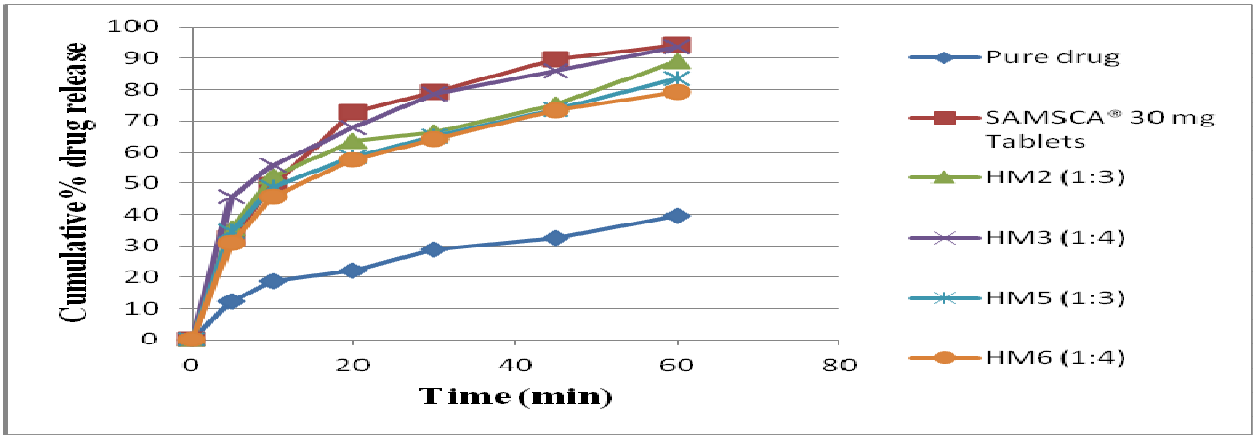


Figure 9: *In vitro* dissolution profiles of pure drug, Innovator product and solid dispersion of Tolvaptan by HME (HM2, HM3, HM5 and HM6)

Among the 6 solid dispersion formulations by the hot melt extrusion process, the formed flakes at the cooling zone were found to be transparent in HM2, HM3, HM5 and HM6. It indicates that the drug substance has been converted into an amorphous (more than 90%). The resulted powder after crushing the flakes using mortar and pestle was free flowing granular in nature. The granular powder was filled into size "3" hard gelatin capsules and evaluated for the drug release profiles in FDA recommended dissolution medium and compared against the drug release profiles of pure drug substance and corresponding innovator product in the same media.

The drug release data obtained for formulations HM2, HM3, HM5 & HM6 are tabulated in **Table 8**. The Table shows the cumulative percent drug released as a function of time for all formulations. Cumulative percent drug released after 90 min was 89.1%, 93.5%, 83.6% and 79.2% for HM2, HM3, HM5 and HM6 respectively while for pure drug 39.6 % in 60 min and for Innovator product 94.2% in 60 minutes.

In vitro studies revealed that there is marked increase in the dissolution rate of Tolvaptan from all the solid dispersions when compared to pure Tolvaptan itself. From the *in vitro* drug release profiles, it is clearly evident that the rate of drug release and its profiles in HM3 are comparable to that of rate and drug release profiles of Innovator product and more over on higher side as compared with rate and drug release profiles in rest of the formulations. This may be attributed to the increase in drug wettability, conversion to amorphous form and solubilization of the drug due to hydrophilic carrier. Comparative drug release profiles for solid dispersion by HME process, drug substance and corresponding Innovator product was depicted in **Figure 9**.

SPRAY DRYING TECHNIQUE

Preliminary solubility studies of Tolvaptan:

Solubility of Tolvaptan was determined by adding an excess amount of Tolvaptan to volumetric flask of 25ml of water and then samples were shaken for the 48 hours at room temperature. Subsequently, the solutions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan by UV/Visible spectrophotometer at λ_{max} 269 nm. In the same Tolvaptan and water soluble carriers like Povidone (Kollidon 30), hydrophilic carrier Soluplus, mixture of Soluplus and water soluble and hydrophilic Hypromellose 2.5 cPs were taken in 1:1 ratios, added to volumetric flask of 25ml of water and then samples were shaken for the 48 hours at room temperature. Subsequently, the solutions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Tolvaptan by UV/Visible spectrophotometer at λ_{max} 269 nm.

Preparation of Tolvaptan solid dispersions by spray drying:

Step – 1: Tolvaptan was slowly added to 2:1 solvent mixtures of dichloromethane and methanol (Drug to solvent ratio is 1:7.5) under continuous stirring and stirred well till to get a clear solution.

Step – 2: In one set of formulations**,** taken with Povidone (Kollidon 30) – SDT1(drug to carrier ratio, 1:2) and SDT2 (drug to carrier ratio, 3:1) / in another set of formulations taken with Soluplus - SDT3 (drug to carrier ratio, 1:2) and SDT5 (drug to carrier ratio, 3:1) and also with mixture of Soluplus and Hypromellose 2.5 cPs – SDT4 (drug to carrier ratio, 3:1:1) added to the step 1 of drug solution and stirred well till to get a clear solution (with Povidone), whereas with Soluplus uniform drug dispersion. The above solution / dispersion was subjected to spray drying using BUCHI spray dryer (Inlet air temperature 60 – 70°C, Aspiration 90 - 100%; Nozzle tip: 0.2 mm; Nitrogen gas cylinder). The majority of the spray dried powder was collected in the drying chamber cylinder with aspiration below 90% and it was found to be coarser powder as compared to spray dried powder, which was collected in

Extraction cyclone cylinder where aspiration above 90% to 100%. Spray dried powder was found to be coarser with nozzle size more than 0.4 mm, coarser grade powder was collected in drying chamber cylinder. The parameters to maintain during the process are summarized in **Table 9** and the composition is shown in **Table 10.**

Table 9: Parameters considered during spray drying

Spray drying parameters:	
Inlet temperature	60 - 70°C
Pump rate for spraying Solution	25 – 35%
Nitrogen gas pressure	30mm Hg
Nozzle tip:	0.2 mm
Nozzle diameter:	0.4 mm
Cap diameter:	1.4 mm

Table 10: Composition of Tolvaptan solid dispersions by Spray drying technique (SDT)

S. No	Ingredients (Units)	SDT1	SDT2	SDT3	SDT4	SDT5
		Qty (mg)	Qty (mg)	Qty (mg)	Qty (mg)	Qty (mg)
1.	Tolvaptan	30.0	30.0	30.0	30.0	30.0
2.	Povidone (Kollidon 30)	60.0	10.0	-	-	-
3.	Soluplus	-	-	60.0	10.0	10.0
4	Hypromellose 2.5cPs	-	-	-	10.0	-
5.	Dichloromethane (2 parts)	q.s	q.s	q.s	q.s	q.s
6.	Methanol (1 parts)	q.s	q.s	q.s	q.s	q.s

Solubility studies of Tolvaptan solid dispersions by Spray drying technique

The resulting Spray dried mixture, SDT1 to SDT4 were added 25 ml of volumetric flask of purified water. The samples were shaken for the 48 hours at room temperature. Subsequently, the solutions / dispersion were filtered through a Whatman filter paper no 1. Filtered solution was analyzed for the tolvaptan by UV/Visible spectrophotometer at λ_{max} 269 nm.

Evaluation of tolvaptan solid dispersions by spray drying technique:

Solid dispersions obtained from the spray drying technique (SDT) were tested for drug substance solubility. The solid dispersion showing good solubility, and were further studied for drug content and *in-vitro* drug release studies.

Drug content

Accurately weighed quantity of Tolvaptan solid dispersion which is equivalent to 30mg of Tolvaptan was taken in volumetric flask and the volume is made to 100ml with Methanol. From this 1ml of solution is taken in a 10ml volumetric flask and is made up to 10ml with methanol. This solution is diluted to 10µg/ml and absorbance was measured at λ_{max} 269 nm against blank. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in solid dispersion}}{\text{Theoretical amount of drug in solid dispersion}} \times 100$$

***In vitro* release studies:**

The *in vitro* drug release profiles for each solid dispersion as well as pure drug and its corresponding Innovator product was performed using USP type 1 (Basket) dissolution apparatus. The resulting blend of solid dispersion by SDT equivalent to 30 mg of Tolvaptan was filled in size "3" hard gelatin capsules and was evaluated for dissolution profiles. The conditions and parameters of dissolution testing are presented in **Table 11.**

The samples are withdrawn at specified time intervals and the obtained samples were analyzed for drug release by using UV/Visible spectrophotometer at λ_{max} 269nm. The cumulative percentage release was calculated.

Table 11: *In Vitro* dissolution studies test parameters

Instrument	Electro lab - USP type I Dissolution test apparatus.
Dissolution medium	0.22% Sodium Lauryl Sulfate in water
Apparatus	USP apparatus-I (Basket type)
Temperature	37±0.5^{0}C
RPM	100
Volume of medium	900 ml.
Sampling intervals	5, 10, 15, 30,45 & 60 minutes
Sample volume	10 ml withdrawn and replaced with 10 ml of dissolution medium.

Results and Discussion for Solid Dispersion of Tolvaptan By Spray Drying Technique

Preliminary Solubility Studies of Tolvaptan

In case of solid dispersions initially preliminary solubility analysis were carried out to select the appropriate water soluble or / hydrophilic carriers for the preparation of solid dispersion. Trials are made with the suitable polymer available for the spray drying method. Finally solubility studies were made with the water soluble carrier in different drug: carrier ratios.

From these physical mixtures **(Table 12**) of drug and Povidone (Kollidon 30) in the ratio of 3:1 shown highest drug solubility i.e. 0.29 mg/ml, more than 7 fold increased as compared to that of pure drug.

Table 12: Preliminary Solubility Studies of Tolvaptan

S.No.	Physical mixture	Solubility (mg/ml)
1.	Pure drug	0.04±0.02
2.	Drug: Kollidon 30 (1:2)	0.21±0.08
3.	Drug: Kollidon 30 (3:1)	0.29±0.05
4.	Drug : Soluplus (1:2)	0.28±0.03
5.	Drug : Soluplus: HPMC 2.5cPs (3:1:1)	0.22±0.07
6.	Drug : Soluplus (3:1)	0.24±0.06

Preparation of Tolvaptan Solid Dispersions by SDT

Solid dispersions of Tolvaptan were prepared by using Soluplus, Povidone (Kollidon 30) and mixture of Soluplus and HPMC 2.5cPs (1:1 ratio). In the present investigation 5 formulations were prepared and their complete composition is shown in **Table 10**. The resulting spray dried powder was found to be fine and fluffy in nature was filled in size "3" hard gelatin capsules and evaluated for dissolution profiles.

Figure 10: Tolvaptan Solid Dispersions by SDT (SDT2)

Characterization:

FTIR Studies of Tolvaptan Solid Dispersion By Spray Drying Method:

The prominent peaks of Tolvaptan was observed **(Figure 11)** the region of 3410.26 cm^{-1} due to the (Polymeric OH stretching), a peak at 3263.66 cm^{-1} due to (aromatic H(-C=C-)H stretching) and a peak at 3086.21 cm^{-1} due to (aromatic C-H stretching). At the lower frequencies 741 cm^{-1} (C-Cl), 1195.91 cm^{-1} (C-N stretching), 1357.93 cm^{-1} (N-H stretching), 1681.98 cm^{-1} (C=O stretching) observed**.** Povidone (Kollidon 30) **(Figure 12)** shows the prominent peak at 3410.26 cm^{-1} due to polymeric OH stretching, a peak at 2978.19 cm^{-1} due to the (aliphatic CH_3 stretching).

Physical mixture **(Figure 13)** of the drug and Povidone (Kollidon 30) shows summation of the spectra of the drug and Povidone (Kollidon 30) equivalent to the addition of the spectrum of polymer and drug. This indicates that interaction has occurred with simple physical mixture of drug and polymer. In case of solid dispersion **(Figure 14)** of the drug and Kollidon 30 shows overlapping of O-H and N-H group and broadening of peak was observed. However other peaks related to C-H stretching remains unchanged. This indicates that overall symmetry of the molecule might not be significantly changed.

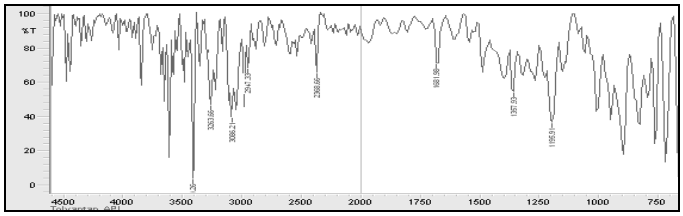


Figure 11: FTIR spectra of pure drug

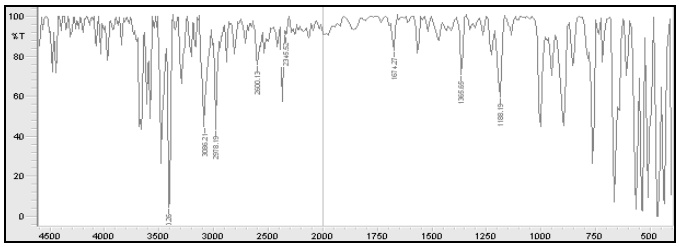


Figure 12: FTIR spectra of Povidone (Kollidon 30)

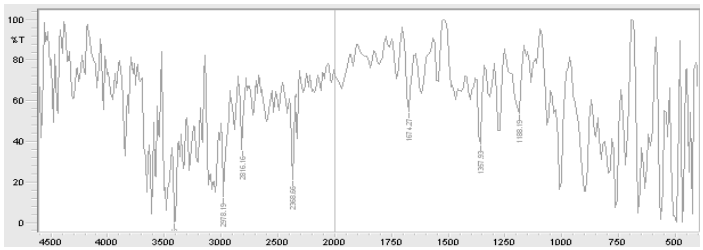


Figure 13: FTIR spectra of physical mixture of Tolvaptan: Povidone (Kollidon 30)

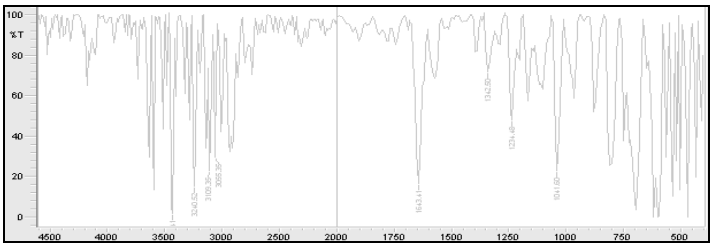


Figure 14: FTIR spectra of formulation SDT2 solid dispersion prepared by spray drying method

Differential scanning calorimetry:

The DSC thermo grams of Pure Tolvaptan showed in F**igure 15,** sharp endothermic peak at melting point (225 ^{0}C), indicating that the drug is highly crystalline. The absence of drug peak in the solid dispersion formulation SDT2 (Tolvaptan & Kollidon 30 (3:1)) indicating the drug was in amorphous form.

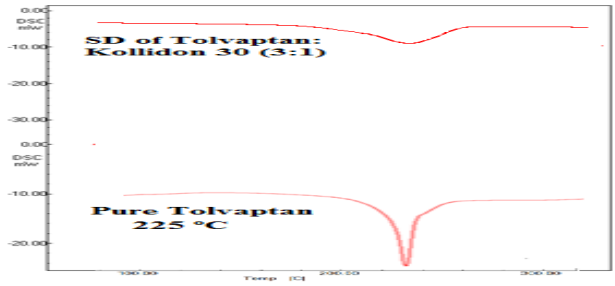
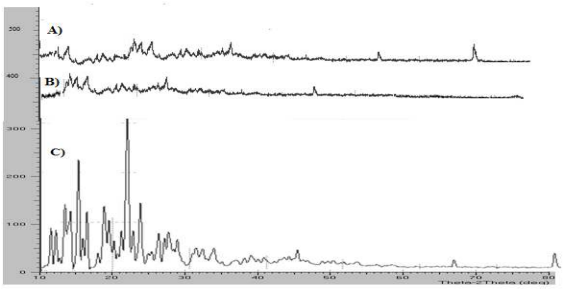


Figure 15: DSC thermograms of pure drug and formulation SDT2

XRD Analysis

The XRD of Tolvaptan consist of sharp multiple peaks, indicating the crystalline nature of the drug. In case of SDT2 (Tolvapatn with Kollidon (3:1)) when exposed to X-ray beam, disappearance of all crystalline endothermic peaks and characteristic intensities of Tolvaptan. This indicates complete transformation of crystalline Tolvaptan into

amorphous form during SDT process. From the XRD studies, it is confirmed that the drug substance by SDT of the formulation (SDT2) has been converted into amorphous form (**Figure 16**).



A) Formulation SDT2 B) Placebo C) Tolvaptan pure drug
Figure 16: Powder X-ray diffraction patterns of Tolvaptan pure drug, placebo and formulation

Scanning electron microscopy:
Surface micrographs of prepared spray dried powder (SDT2) and pure Tolvaptan were determined using SEM technique. The SEM micrograph of pure Tolvaptan (**Figure 17A**) was observed with large crystalline forms of drug agglomerates with ordered shape and size. The surface characteristics of SD of formulation SDT2 (**Figure 17B, C & D**) shown rough disordered and intact structures, which subsequently help to dissolve drug when comes in contact with aqueous fluid.

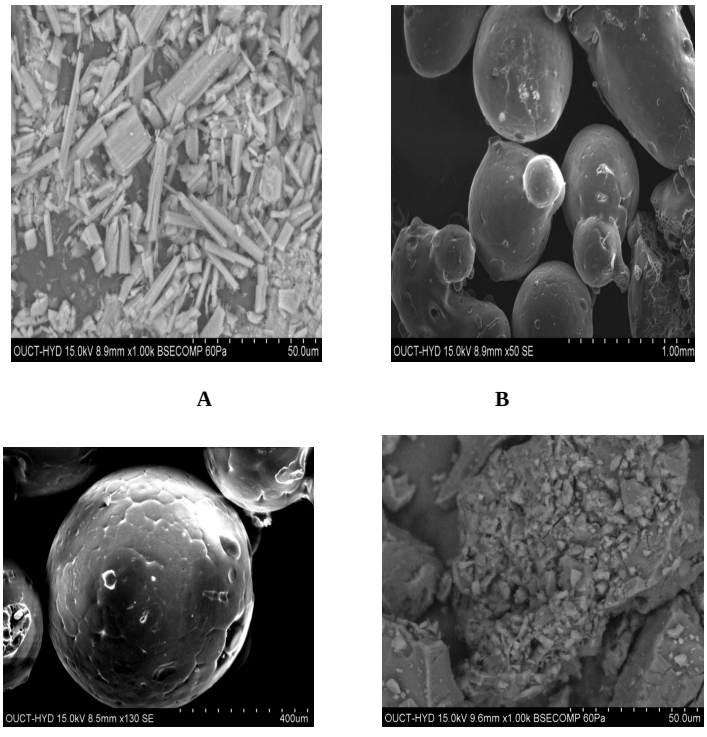


Figure 17: SEM images of Tolvaptan pure drug (A), SD formulation SDT2 (B, C & D)

Evaluation of Parameters:
Solubility Studies of Tolvaptan Solid Dispersions:
Five formulations of solid dispersions were prepared by spray drying method with their respective carriers. After preparation of solid dispersion solubility analysis was carried out, this is compared with pure drug substance itself. The formulation (SDT2) with Povidone (Kollidon 30) in the ratio of 3:1 (drug to carrier) which had shown increased solubility, the solubility is more than 8 fold increased as compared to that of the pure drug (Pure drug solubility is 0.04 mg/mL). The results are tabulated in **Table 13.**

Table 13: Solubility studies of solid dispersions prepared by spray drying method

S.No.	Formulation	Solubility (mg/ml)
1.	Pure drug	0.04±0.02
2.	SDT1	0.27±0.07
3.	SDT2	0.32±0.10
4.	SDT3	0.31±0.08
5.	SDT4	0.29±0.14
6.	SDT5	0.30±0.11

Drug content:

Actual drug content of all 5 formulations are shown in **Table 14**. The drug content of the prepared solid dispersions was found to be in the range of **90.2% – 95.3%**. Maximum % drug content i.e. 95.3 % was found in the formulation SD2.

Table 14: % Drug Content for different formulations of Tolvaptan Solid dispersions

S.No.	Formulation	%Drug content
1.	SDT1	90.2 ±3.4
2.	SDT2	95.3 ±2.7
3.	SDT3	94.3±3.1
4.	SDT4	92.4±1,8
5.	SDT5	93.6±2.6

***In Vitro* Dissolution Studies**

Table 15: *In Vitro* Dissolution Profiles of Pure Drug, Innovator Product and Different Formulations of Tolvaptan Solid Dispersions (SDT1-SDT5)

Time in Min	Cumulative % drug release						
	Pure drug	SAMSCA® (tolvaptan) 30 mg tablets	SDT1	SDT2	SDT3	SDT4	SDT5
0	0	0	0	0	0	0	0
5	12.2±4.9	32.4±2.6	29.4±4.8	36.2±3.2	35.6±3.8	31.8±3.9	33.5±3.6
10	18.6±4.1	49.4±2.2	43.6±4.1	54.2±2.5	47.5±3.1	44.5±4.2	44.8±2.6
20	22.1±3.6	72.8±1.6	53.2±2.8	66.3±3.1	57.5±2.7	55.4±2.4	57.5±2.5
30	28.9±3.3	79.2±1.9	62.2±3.2	79.2±1.6	68.2±1.7	65.6±1.8	66.5±1.4
45	32.5±2.6	89.8±1.2	72.6±2.3	85.8±1.3	74.5±2.3	73.4±1.3	74.2±0.8
60	39.6±2.7	94.2±0.9	79.1±1.7	91.4±0.8	89.3±1.1	81.3±0.6	84.6±1.4

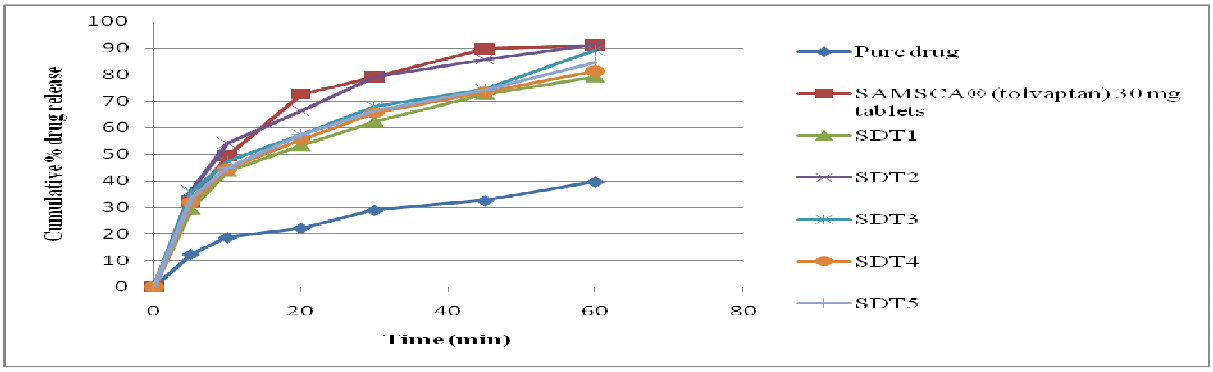


Figure 18: *In Vitro* Dissolution Profile of Pure Drug, Innovator Product and Tolvaptan Solid Dispersion by Spray Drying Technique (SDT1-SDT5)

The drug release data obtained for formulations SDT1-SDT5 are tabulated in Table 15. The Table shows the cumulative percent drug released as a function of time for all formulations. Cumulative percent drug released after 60 min was found to be 79.1%, 91..4%, 89.3%, 81.3% and 84.6% for SDT1-SDT5 respectively, 39.6 % in 60 min for pure drug and 94.2% in 60 min for Innovator product. *In vitro* studies revealed that there is marked increase in the dissolution rate of tolvaptan from all the solid dispersions when compared to pure tolvaptan itself. From the *in vitro* drug release profiles, it can be seen that formulation SDT2 containing Povidone (Kollidon 30) (3:1 ratio of drug: Kollidon 30) shown higher drug release i.e. 91.4 % compared with other formulations. From the *in vitro* drug release profiles, it is clearly evident that the rate of drug release and its profiles in SDT2 are comparable to that of rate and drug release profiles of Innovator product and more over on higher side as compared with rate and drug release profiles in rest of the formulations. This may be due to conversion from crystalline to amorphous form and

solubilization of the drug due to hydrophilic carrier. The graphical representation drug release profiles of solid dispersions of SDT1 to SDT5 were depicted in **Figure 18**.

CONCLUSION

In the present investigation the solid dispersions of the water insoluble drug substance Tolvaptan was successfully prepared by Hot-melt extrustion and Spray drying technique. The *in-vitro* dissolution test showed a significant increase in the rate and % of cumulative drug release of solid dispersions prepared by HME (93.5%) & SDT(91.4%) as compared with pure Tolvaptan (39.6%) in 60 minutes and found to be comparable with the drug release from the innovator product (94.2%) [SAMSCA® (tolvaptan) 30 mg tablets]. The rate of drug release was slightly higher from Tolvaptan solid dispersion by HME when compared with SDT. The increase in the rate of drug release of Tolvaptan is in the order of solid dispersions of HME>SDT> Pure drug substance itself. The mechanism involved are solubilization and improved wetting of the drug substance within hydrophilic carriers rich microenvironment formed at the surface of the drug substance. The crystalline form of the drug substance has been converted into amorphous form with solid dispersion of hydrophilc polymer by HME & SDT. Results from FT-IR concluded that there was no well defined interaction between Tolvaptan and carriers. DSC, XRD and SEM showed a change in crystal structure toward an amorphous form of Tolvaptan. Finally it could be concluded that solid dispersion of Tolvaptan using hydrophilic polymers by HME & SDT would improved the aqueous solubility, dissolution rate, permeability and thereby enhancing its systemic availability.

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