Physical, Chemical, and Performance Assessment of Amorphous Solid Dispersions of Ritonavir Prepared by Hot Melt Extrusion

Ritu Kaushik[1], Kevin P. O'Donnell[2]

[1]The Dow Chemical Company, Dow Pharma & Food Solutions, Mumbai, India
[2]The Dow Chemical Company, Dow Pharma & Food Solutions, Midland, MI

Introduction

The use of amorphous solid dispersions (ASDs) to improve the solubility and/or bioavailability of poorly soluble molecules is a highly active area of research in the pharmaceutical industry [1]. Commonly, a hydrophilic polymeric stabilizer is utilized as a major formulation component to provide physical stability against recrystallization of the amorphous drug and to promote dissolution [2]. Hot melt extrusion (HME) is a leading technology in the manufacture of amorphous solid dispersions [3] as the thermal and mechanical energy input during processing can disrupt the crystal lattice of the active ingredient to render it amorphous and promote its incorporation in to the polymeric carrier with sufficient mixing to yield a homogenous dispersion. While hot melt extrusion is a commercially successful technology, the number of pharmaceutically acceptable polymers suitable for use in the process is limited; however, new excipients are being designed with specific utility in extrusion.

Recently, The Dow Chemical Company introduced a new grade of hydroxypropyl methylcellulose (HPMC) designed for hot melt extrusion, AFFINISOL™ HPMC HME (AFFINISOL). This grade of HPMC is characterized by a lower glass transition temperature (T_g; 110 – 115 °C), reduced melt viscosity and minimized color change at elevated temperatures, thereby overcoming the challenges traditionally associated with extruding HPMC [4, 5]. Studies have confirmed AFFINISOL™ to have a broad Hot Melt Extrusion processing window and ability to generate amorphous solid dispersions capable of providing solubility enhancement of poorly soluble compounds [6, 7]. Additionally, AFFINISOL™ has been shown to have a reduced moisture uptake compared to other commonly utilized pharmaceutical extrusion polymers [8] which may improve the physical and potentially chemical stability of manufactured amorphous solid dispersions during storage.

Ritonavir (RTV, Figure 1) is a poorly soluble compound currently commercialized as an amorphous solid dispersions in multiple products [9], each of which employ copovidone as

the carrier polymer. Due to the thermal instability of Ritonavir, which can result in rapid drug degradation if the product temperature exceeds 160 °C [9], hydroxypropyl methylcellulose has not previously been a viable carrier option for the drug in hot melt extrusion. However, in a recent study the impact of extrusion processing parameters on the chemical stability of Ritonavir when prepared as a solid dispersion with AFFINISOL™ confirmed that proper processing conditions can result in the elimination of drug degradation, and that the broad operating window of AFFINISOL™ enables this optimization[10].

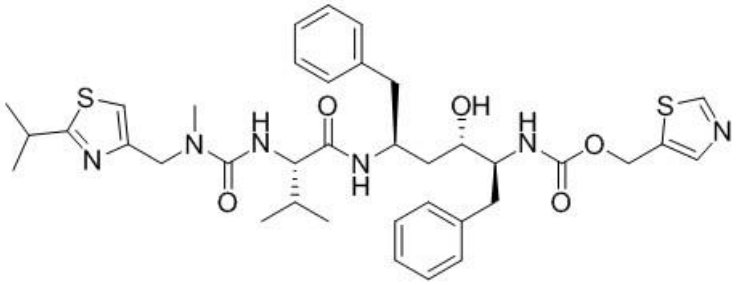


Figure 1 – Chemical structure of ritonavir

Due to the metastable nature of amorphous solid dispersions and potential for recrystallization at temperatures even below the formulation T_g, it is imperative to understand the physical, chemical and performance changes of these specialized formulations over time [11]. While assessing stability at ambient conditions can provide an understanding of potential changes in the formulation at conditions likely to be experienced upon commercialization, these studies take a significant amount of time (> 1 year). The use of accelerated stability testing conditions [12], whereby the formulation is stored at 40 °C, 75% relative humidity for a minimum of six months, can allow for more rapid assessment of these potential changes.

Thus, the purpose of this study was to compare the chemical and physical stability of amorphous solid dispersions of Ritonavir formulated with either AFFINISOL™ or copovidone at 3 drug loads when placed on accelerated stability testing for a period of 6 months. Additionally, dissolution performance of the resulting amorphous solid dispersions before and after stability testing was analyzed to investigate the impact of storage and drug load on performance maintenance with each polymer.

Materials

Ritonavir was purchased from Indo Overseas Trading Agencies-India. AFFINISOL™ HPMC HME 15LV was obtained from The Dow Chemical Company (Midland, MI). Copovidone was purchased from Spectrum Chemical (Tucson, AZ). Acetonitrile, potassium dihydrogen orthophosphate, methanol and hydrochloric acid used in the study were Analytical grades procured from Orion Scientific Suppliers Pvt. Ltd. (Mumbai, India).

Methods

Hot Melt Extrusion (HME):

The formulations were prepared by mixing ritonavir and AFFINISOL™ or copovidone in a blender at different drug:polymer ratios as described in Table 1 below. Melt extrusions were carried out in a Thermo Fisher Pharma 11 (Thermo Fisher -Germany) twin screw extruder and blends were manually fed into the extruder. The screw kneading elements used have 30°, 60° & 90° configurations. The process conditions utilized in Hot Melt Extrusion for each formulation are shown in Table 1.

Table 1 – Hot Melt extrusion formulations and processing conditions

Batch	Formulation	Ritonavir (wt%)	Polymer (wt%)	Temperature Profile (°C)	Screw Speed (RPM)
1	Ritonavir:AFFINISOL™	25	75	25-70-120-140-140-140-140-140	100
2	Ritonavir:Copovidone	25	75	25-70-120-120-120-120-120-120	150
3	Ritonavir:AFFINISOL™	33.3	66.6	25-70-120-140-140-140-140-140	100
4	Ritonavir:Copovidone	33.3	66.6	25-70-120-120-120-120-120-120	150
5	Ritonavir:AFFINISOL™	50	50	25-70-120-140-140-140-140-140	100
6	Ritonavir:Copovidone	50	50	25-70-120-120-120-120-120-120	150

Differential Scanning Calorimetry (DSC):

The thermal behavior of ritonavir and the extrudates were recorded using differential scanning calorimetry (TQ2000, TA Instruments). The differential scanning calorimetry experiments were run under a dry nitrogen atmosphere at purge gas flow rate of 50 mL/min. The samples were weighed into an aluminum pan, crimped and heated at a ramp rate of 10 °C/min from 25 °C to 225 °C. Data analysis was performed in TA Instruments Universal Analysis software.

Powder X-ray Diffraction (XRD)

X-ray diffraction patterns were recorded using a PANalytical - Empyrean (Netherlands) X-Ray diffractometer. The samples were placed in a zero background sample holder and incorporated on a spinner stage. The X-ray was applied from a Cu Kα source at a voltage of 40 kV and a current intensity of 20 mA. The samples were analyzed over a 2θ range of 5°–50° with a step size of 0.002^0.

High Performance Liquid Chromatography (HPLC)

The ritonavir drug content of the extrudates and dissolution samples was evaluated using HPLC (Agilent 1260). The mobile phase was composed of acetonitrile and phosphate buffer (adjusted to pH of 4.0) in ratio of 55:45 at flow rate of 1 mL/min. The column used was an Eclipse plus C8 4.6 x 150 mm, 5 µm and detection of ritonavir was determined at 246 nm.

Dissolution Studies

In vitro dissolution studies were conducted in a USP II dissolution apparatus (Electrolab, India) under sink conditions. A quantity equivalent to 100 mg of ritonavir from extruded samples was weighed and filled into hard gelatin capsules. Dissolution of ritonavir from the capsules was performed in triplicate in 0.1 N HCl with the temperature maintained 37 °C at a paddle speed of 75 RPM. The samples were collected at time points of 10, 20, 30, 45, 60 and 120 minutes and analyzed by high performance liquid chromatography after filtering the samples through a 0.45 µm PVDF filter.

Stability:

Extrudates of ritonavir were charged for an accelerated stability study in a chamber (Newtronic equipment company private Ltd) maintained at a temperature of 40 ºC and relative humidity of 75%. Extrudates were packed in triple laminated aluminum pouches and

loaded into the stability chamber. The samples were collected at time points of 1, 2, 3 and 6 months and analyzed for chemical stability by high performance liquid chromatography as well as dissolution performance. Ritonavir physical state and thermal stability were characterized by X-ray diffraction and differential scanning calorimetry, respectively.

Results and Discussion

Hot Melt Extrusion

Formulations of ritonavir with both AFFINISOL™ HPMC HME 15LV and copovidone were successfully extruded at all conditions outlined in Table 1. No processing challenges were observed during the trials. AFFINISOL™ extrudates were light yellow in color and transparent (Figure 2) and copovidone extrudates were colorless and transparent (Figure 3); these observations indicated homogenous mixing and amorphization of the drug both with AFFINISOL™ and copovidone. However, in the case of copovidone moisture droplets were observed on the extrudates at all drug:polymer ratios which could be attributed to the hygroscopic nature of copovidone. Depending on polymer storage conditions, prior to use it may be necessary to dry copovidone to remove moisture from the material; this does not appear necessary with AFFINISOL™.

Figure 2 – Images of Ritonavir:AFFINISOL™ extrudates; left to right: 1:1, 1:2, and 1:3

Figure 3 – Images of Ritonavir:copovidone extrudates; left to right: 1:1, 1:2, 1:3

Differential Scanning Calorimetry (DSC):

The differential scanning calorimetry thermogram of pure ritonavir showed a sharp endothermic peak at 122.12 °C attributed to melting of the crystalline drug. In contrast, the extrudates of ritonavir with AFFINISOL™ and copovidone showed no endothermic melting events nor exothermic recrystallization events indicating the extrudates immediately following manufacture were rendered amorphous. The stability samples with AFFINISOL™ stored for at 40 °C/75% RH displayed identical thermal profiles to those of the initial samples indicating no change in drug morphology (i.e. recrystallization) occurred during storage (Figure 4). Additionally, AFFINISOL™ extrudates exhibited a single T_g indicating no phase separation occurred upon storage (Figure 5). This demonstrates the potential of AFFINISOL™ HPMC HME to inhibit ritonavir recrystallization during storage for at least 6 months at accelerated conditions.

Similar to the AFFINISOL™ formulations, copovidone formulations following storage showed no recrystallization exotherms or melting endotherms indicating the drug remained amorphous. However, in the case of the copovidone 3 and 6 month stability samples an endothermic event in the range of 54 to 70 °C was observed at all drug:polymer ratios; this corresponds to glass transition of pure amorphous ritonavir indicating that phase separation of ritonavir from the amorphous dispersion occurred upon storage. This thermal analysis did not indicate recrystallization occurred over the tested time period suggesting ritonavir separated into amorphous drug rich domains; such physical instability is undesired in an amorphous dispersion. The 6 month differential scanning calorimetry thermograms of the 1:2 and 1:3 Ritonavir:Polymer formulations containing either copovidone or AFFINISOL™ are shown in Figure 5.

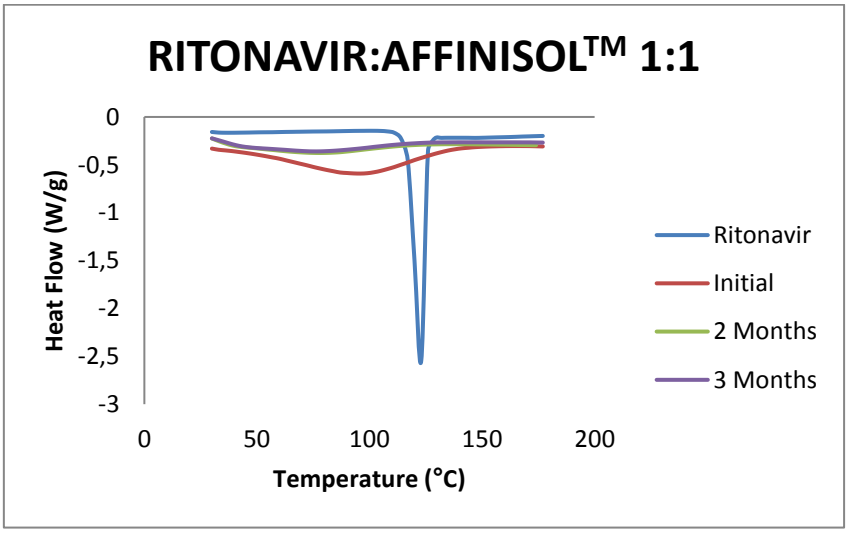
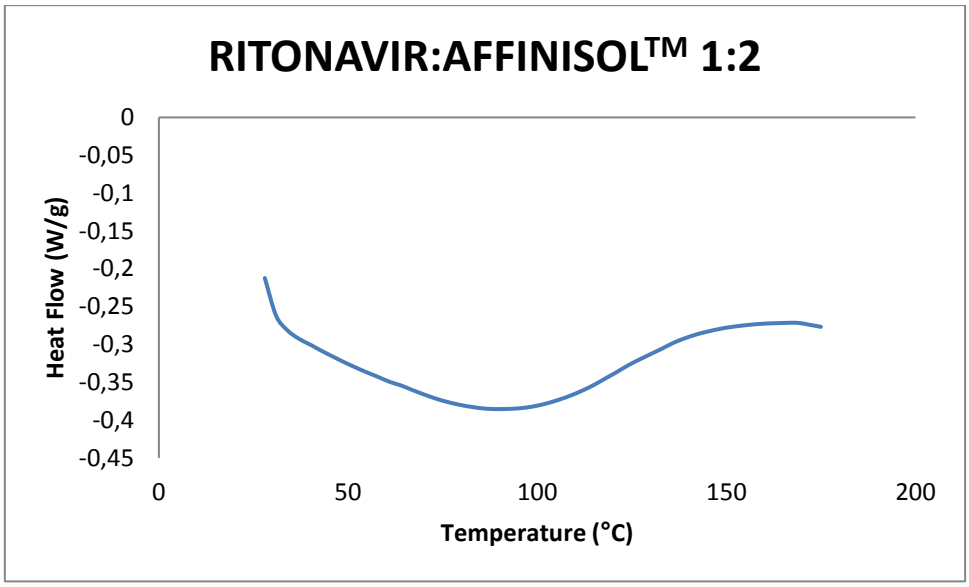
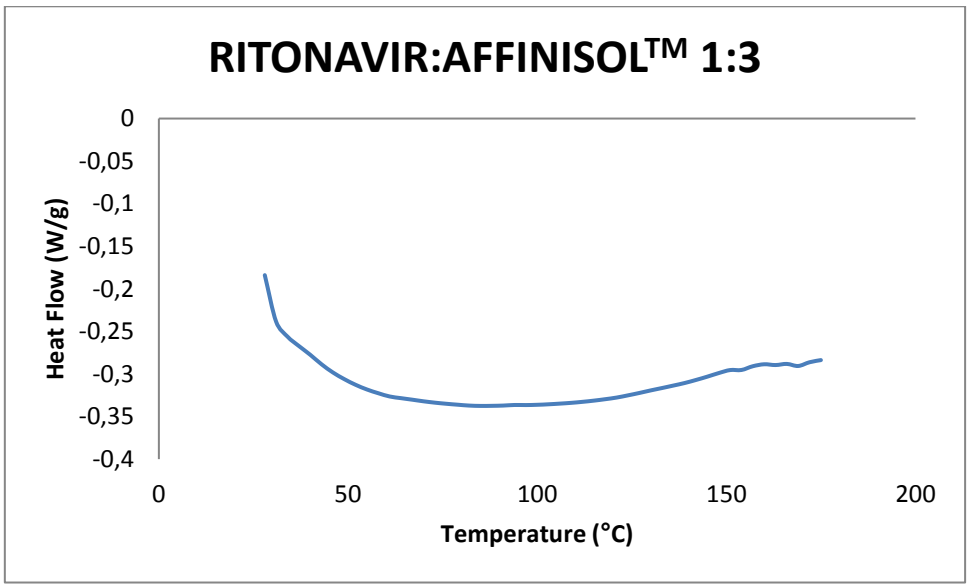


Figure 4: Example differential scanning calorimetry thermogram of stability samples of Ritonavir:AFFINISOL™ amorphous solid dispersions: Initial, 2 and 3 months.





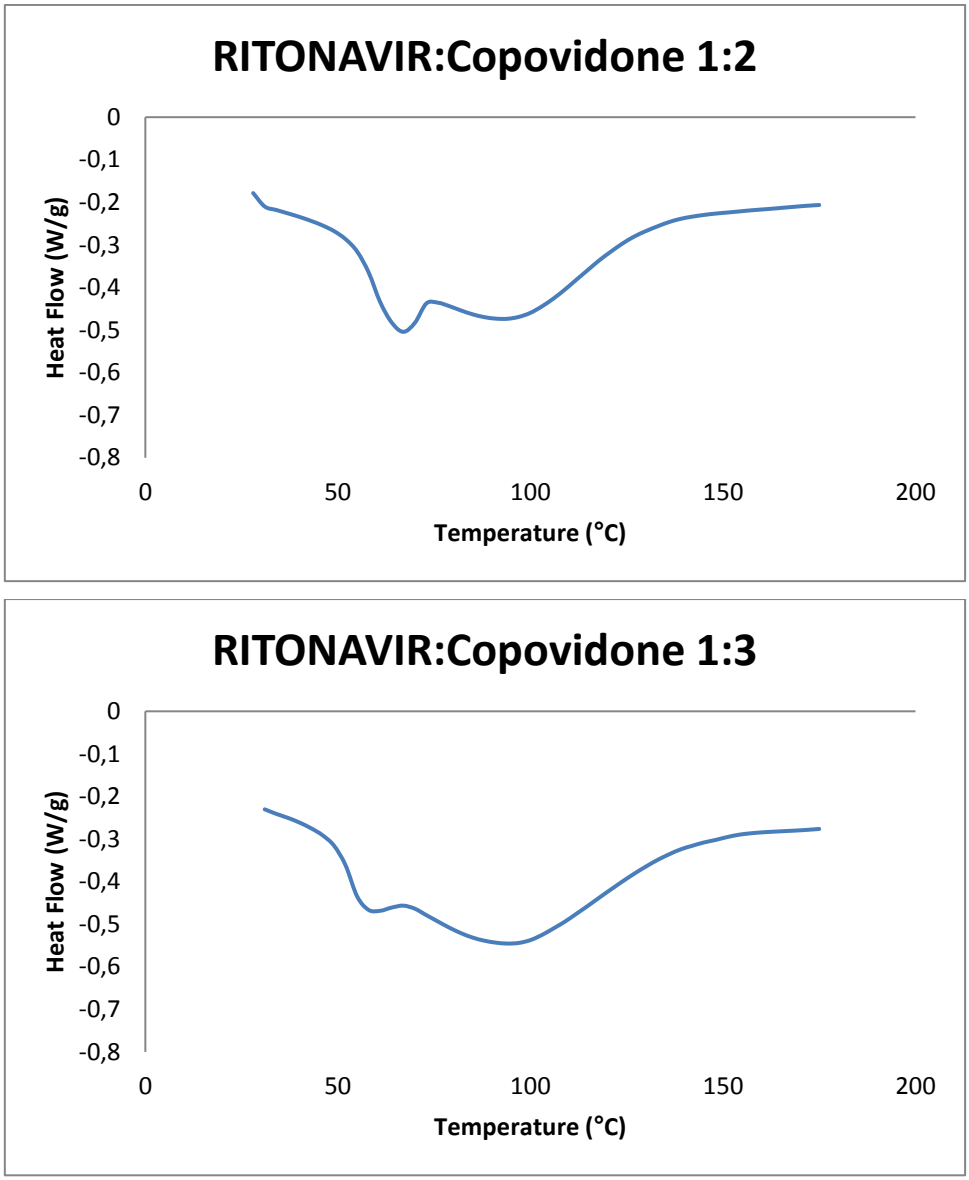


Figure 5: Differential scanning calorimetry thermograms of 6 month stability samples of Ritonavir:AFFINISOL™ and Ritonavir:copovidone. The respective 1:1 drug loads provided identical results to the examples shown here.

Powder X-ray Diffraction

The X-ray diffraction patterns of pure Ritonavir showed characteristic crystalline peaks between 2θ of 5°- 45°. However, the extruded samples of the Ritonavir:AFFINISOL™ formulations did not show crystalline peaks in this range and displayed only an amorphous halo. This was true for the initial and stability samples (Figure 6). The results of AFFINISOL™ X-ray diffraction were in agreement with differential scanning calorimetry observations; the drug was rendered and remained amorphous over the course of the study. The minor peaks in the AFFINISOL™ extrudates at approximately 32° and 45 °are due to

minor residual sodium chloride present in hydroxypropyl methylcellulose following manufacture (typically < 1% present).

Similarly, for copovidone there were no differences in the X-ray diffraction patterns of the initial samples compared to the stability samples confirming the drug remained amorphous upon storage. Therefore, the observations in the thermal analysis are attributed to ritonavir separating as amorphous domains within the amorphous solid dispersions.

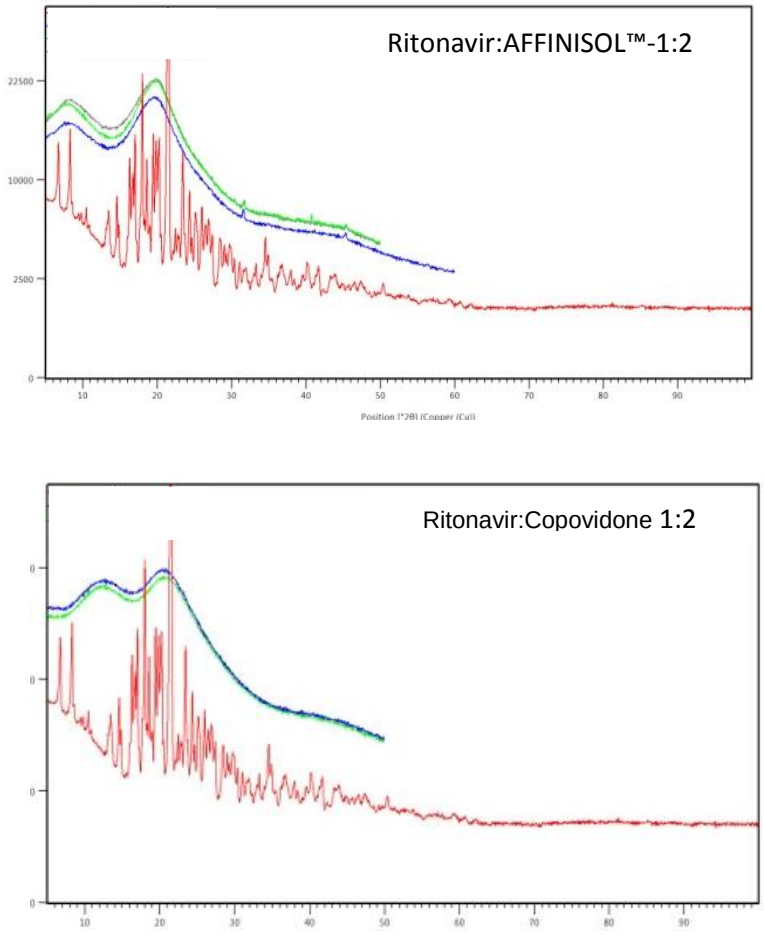


Figure 6: Example X-ray diffraction patterns of Ritonavir:AFFINISOL™ and Ritonavir:Copovidone amorphous solid dispersions. Red – Crystalline Ritonavir, Blue – Immediately following production, Green – 3 Month stability sample, Grey – 6 Month stability sample.

Drug Content:

The ritonavir content was determined by high performance liquid chromatography and the drug recovery values of the extruded batches are shown in Tables 2 and 3; primary impurities were not quantified. There was no degradation beyond that initially observed following storage at accelerated stability conditions for a period of 6 months for either AFFINISOL™ or copovidone amorphous solid dispersions. The observed variation in drug recovery was due to extraction efficiency and test variability.

Table 2 – Ritonavir recovery from AFFINISOL™ extrudates

Time	RITONAVIR: AFFINISOL™ (1:1)	RITONAVIR: AFFINISOL™ (1:2)	RITONAVIR: AFFINISOL™ (1:3)
Initial	99 %	96%	95%
6M	99%	97%	98%

Table 3 – Ritonavir recovery from copovidone extrudates

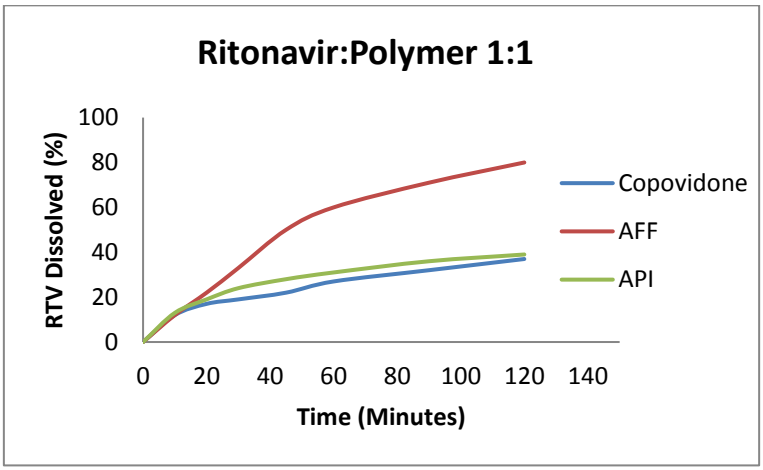
Time	RITONAVIR: :Copovidone (1:1)	RITONAVIR: :Copovidone (1:2)	RITONAVIR: :Copovidone (1:3)
Initial	96%	94%	95%
6M	97%	95%	97%

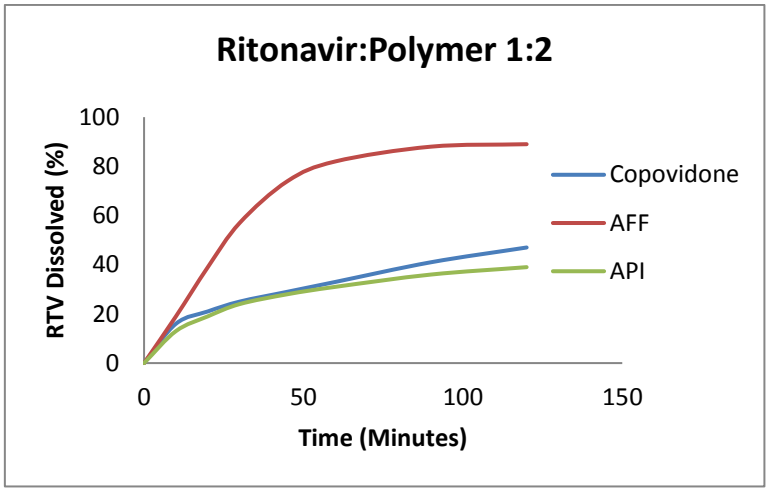
Dissolution studies

Solid dispersions of Ritonavir formulated with AFFINISOL™ at a high drug load of 50% w/w showed significant improvements in the rate and extent of dissolution in 0.1N HCl over the time period studied compared to the crystalline drug as well as those formulated with copovidone as shown in Figure 7. At this 1:1 drug:polymer ratio, the dissolution profile of the copovidone formulation matched that of the pure crystalline API, releasing approximately 40% of the Ritonavir over the course of the study, while the AFFINISOL™ formulation provided faster and more complete dissolution. Similar results were observed with the 33% w/w drug loaded formulations; in this case approximately 80% of drug release in 60 minutes

for the AFFINISOL™ formulation (Figure 7). It should be noted that the 33% w/w drug loaded AFFINISOL™ formulation provided faster release compared to the 50% drug loaded AFFINISOL™ formulation. This indicates potential for AFFINISOL™ HPMC HME to provide significantly improved dissolution performance in aqueous media at high drug loading. At 25% w/w drug loading the copovidone formulation displayed faster dissolution compared to the AFFINISOL™ equivalent. The release profile of 1:3 Ritonavir:AFFINISOL™ formulation being slower than that of the 1:2 formulation warrants additional study as previous work with other APIs has demonstrated a low drug loading to provide very immediate release with this polymer[6].

The stability samples analyzed at 1, 2, 3 & 6 months showed no significant changes in dissolution profiles compared to the initial profiles for both copovidone and AFFINISOL™ formulations as shown in Figure 8 (Note: only initial, 3 and 6 month data is presented for clarity. As all drug loads maintained stable performance, the 1:2 Ritonavir:Polymer formulations are presented). Though phase separation was observed in the thermal data of the copovidone formulations at 3 and 6 months, it was not reflected in the drug release profiles. This supports the prior conclusion derived from the differential scanning calorimetry and X-ray diffraction analysis that Ritonavir phase separated as amorphous domains in the copovidone formulations.





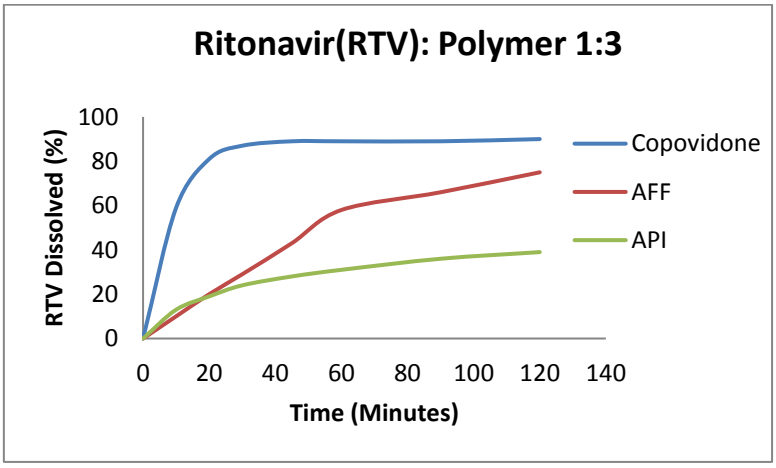
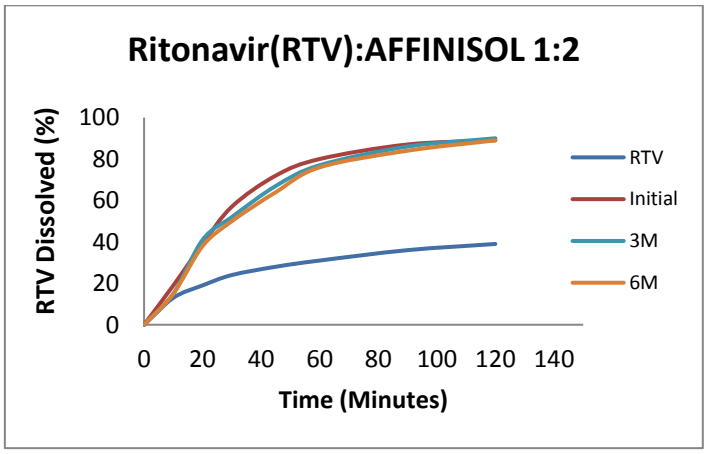


Figure 7: Dissolution profile of Ritonavir extrudes immediately following manufacture demonstrating the impact of drug load on performance.



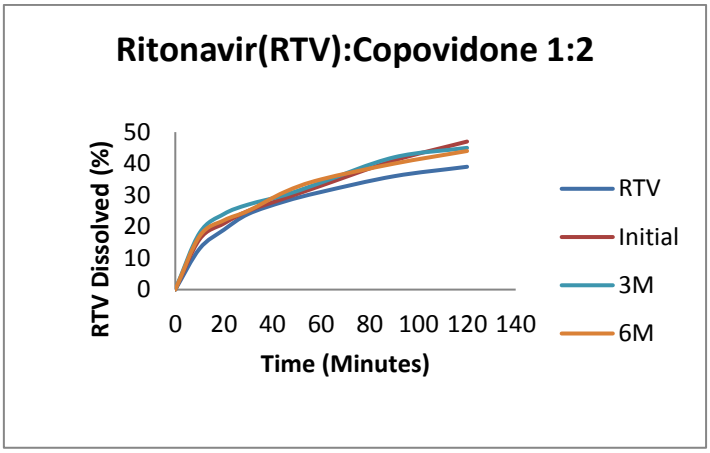


Figure 8: Example dissolution profiles of stability samples of Ritonavir:AFFINISOL™ and Ritonavir:copovidone extrudates. All formulations maintained performance relative to the dissolution profile observed immediately following manufacture.

Conclusions

AFFINISOL™ HPMC HME and copovidone are capable of forming amorphous solid dispersions with the antiretroviral compound ritonavir by hot melt extrusion. AFFINISOL™ was able to provide greater physical stability and improved dissolution at higher drug loads than copovidone. While thermal analysis indicated physical changes upon storage at accelerated conditions occurred for the copovidone formulations, these did not manifest as performance changes in dissolution testing.

1. Kawabata, Y., et al., *Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications.* International Journal of Pharmaceutics, 2011. **420**(1): p. 1-10.
2. Miller, J.M., et al., *A Win–Win Solution in Oral Delivery of Lipophilic Drugs: Supersaturation via Amorphous Solid Dispersions Increases Apparent Solubility without Sacrifice of Intestinal Membrane Permeability.* Molecular Pharmaceutics, 2012. **9**(7): p. 2009-2016.
3. LaFountaine, J.S., J.W. McGinity, and R.O. Williams, *Challenges and Strategies in Thermal Processing of Amorphous Solid Dispersions: A Review.* AAPS PharmSciTech, 2016. **17**(1): p. 43-55.
4. Coppens, K.A., et al., *Hypromellose, ethylcellulose, and polyethylene oxide use in hot melt extrusion.* 2006.
5. O'Donnell, K.P. and W.H.H. Woodward, *Dielectric spectroscopy for the determination of the glass transition temperature of pharmaceutical solid dispersions.* Drug Development and Industrial Pharmacy, 2015. **41**(6): p. 959-968.

6. Huang, S., et al., *A New Extrudable Form of Hypromellose: AFFINISOL™ HPMC HME.* AAPS PharmSciTech, 2016. **17**(1): p. 106-119.
7. O'DOnnell, K.P., Shrestha, U., Rickard, M., Keene, E., Vanchura, B., Mayfield, D. *The Influence of Apparent Viscosity of AFFINISOL™ HPMC HME on Hot Melt Extrusion Processability and Drug Release from Amorphous Solid Dispersions*. 2016 [cited 2016 September 14, 2016]; Technical White Paper].
8. O'Donnell, K.P., Zhao, J., Dan, F., Shrestha, U., Porter III, W. *The Effect of HME Processing, Polymer Type, and MW on Polymer-Water Interactions of Pharmaceutical Excipients for Extrusion*. in *AAPS Annual Meeting and Exposition*. 2015. Orlando, FL.
9. LaFountaine, J.S., et al., *Enabling thermal processing of ritonavir–polyvinyl alcohol amorphous solid dispersions by KinetiSol® Dispersing.* European Journal of Pharmaceutics and Biopharmaceutics, 2016. **101**: p. 72-81.
10. Kaushik, R., O'Donnell, K.P., Singh, G. *Impact of Extrusion Process Parameters on Drug Recovery and Dissolution Performance of Solid Dispersions of Ritonavir and AFFINISOL™ HPMC HME*. 2016 [cited 2016 September 14, 2016]; Available from: http://www.pharma-excipients.ch/2016/07/05/impact-of-extrusion-process-parameters-on-drug-recovery-and-dissolution-performance-of-solid-dispersions-of-ritonavir-and-affinisoltm-hpmc-hme/.
11. Zhu, D., et al., *Modeling Physical Stability of Amorphous Solids Based on Temperature and Moisture Stresses.* Journal of Pharmaceutical Sciences, 2016. **105**(9): p. 2932-2939.
12. *Stability Testing of New Drug Substances and Products Q1A(R2)*, I.C.o. Harmonisation, Editor. 2003.