M1252

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Dissolution behavior and solubility of marketed amorphous solid dispersions of Tacrolimus

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Abstract

Purpose: To study and evaluate the correlation between experimentally determined liquid-liquid phase separation concentration and maximum concentration obtainable from amorphous solid dispersions
Methods: Differential scanning calorimetry was done for pure tacrolimus monohydrate to determine the melting point and glass transition temperature. Dissolution of 6 marketed tacrolimus products were performed in 40ml of pH 4.5 phosphate buffer and 300 rpm at 37°C using a 0.2cm UV dip probe. After the completion of dissolution the media was filtered through a syringe filter and the filtrate analyzed by HPLC. The LLPS concentration of tacrolimus was determined using light scattering and fluorescence. Tacrolimus solution was made in methanol and then injected at a fixed rate into a stirred phosphate buffer solution of pH 4.5 and the light scattering at 320nm was determined with inflection point of the scattering taken as the LLPS concentration. Fluorescence was also used to confirm the LLPS concentration at 37°C by adding increasing amounts of tacrolimus to a buffer solution containing 1µM of pyrene. LLPS was determined by monitoring the I_3/I_1 peak ratio of the pyrene emission spectrum. Diffusion analysis of tacrolimus was done using a side by side diffusion cell at various concentrations across a cellulose membrane (12-18,000 kD cutoff) with a 2cm dip probe on the receiver side and monitoring done at 205nm. The diffusion of the products was also done after dissolving them in pH 4.5 buffer for an hour and adding it to the donor cell. The stability of tacrolimus supsersaturated solutions was determined by nucleation induction by stirring a 50ml solution of the drug at its LLPS concentration and monitoring the concentration using an ultraviolet dip probe.
Results: Thermal analysis of tacrolimus monohydrate showed a dehydration endotherm followed by a melting event at 121°C resulting in an amorphous solid that had a T_g of 75°C indicating a stable amorphous solid. Dissolution of the 6 tacrolimus products in the non-sink condition resulted in the maximum concentration being reached within 30 minutes for all the product while one particular product had a faster dissolution rate. Light scattering and fluorescence analysis showed that tacrolimus had an LLPS concentration of around 45µg/ml. Diffusion analysis of tacrolimus indicated an LLPS concentration of around 50 µg/ml. The stability of a 50 µg/ml tacrolimus solution based on nucleation induction time was a little over 3 hours. The dissolution samples that were filtered after the experiment also had concentration of around 45-50µg/ml as determined by HPLC and matched the LLPS concentration.
Conclusion: Based on the experimental results it appears that the LLPS concentration is the maximum limit of solubility that an amorphous solid dispersion can reach in solution..

Introduction

Amorphous solid dispersions are a strategy to improve drug solubility and dissolution in cases where the drug crystal solubility in water is very low. The solubility advantage arises from the increase in free energy of the amorphous solid relative to the crystalline form. These formulations generally also contain polymers to prevent their crystallization during processing and storage. This is achieved by decreasing the molecular mobility of the drug by forming interactions between the drug and polymer. The solubility of the amorphous drug depends on the free energy difference between the crystal and the disordered form and can be calculated from thermodynamic and water sorption data. It can also be determined experimentally using various techniques.

It is of interest to see if the experimentally determined amorphous solubility of the pure drug matched that obtained upon dissolution of marketed amorphous solid dispersions which normally contain various other excipients under non-sink dissolution conditions. In this work, 6 different brands of amorphous solid dispersions of tacrolimus were purchased and the dissolution behavior and solubility was compared with the amorphous solubility value obtained for the pure drug.

Methods

Drug used: Tacrolimus Monohydrate
Products studied: Tacrolimus 5mg capsules from the following companies were used Astellas Pharma, Mylan, Panacea Biotech, Dr. Reddys, Intas Pharma and Sandoz.
Spectroscopic analysis: X-ray powder diffraction (XRPD) was done using a Rikagu Smartlab at angles between 5-40° 2θ at a scan speed of 20°/min. Fourier transform infra-red (FTIR) data was obtained using a Bruker Vertex 70 where 128 scans were averaged for each sample over a wavenumber region of 400-4000 cm^{-1} using a Golden Gate bridge ATR accessory.
Thermal analysis: The melting point, melting enthalpy and the glass transition temperature was determined using a differential scanning calorimeter (DSC) (TA Instruments) at 10°C/min heating rate.
Amorphous solubility: This was determined using different techniques ranging from UV scattering, fluororescence spectroscopy (using pyrene), diffusion and ultracentrifugation.
Dissolution: This was done in 40 and 900ml in a dissolution media consisting of phosphate solution pH 4.5 at 37°C. The drug concentration was determined using a UV dip probe from SI photonics.
HPLC: Chromatographic analysis was done using an Agilent Infinity system using a Zorbax XDB C-18 analytical column with a mobile phase of Acetonitrile:Water (70:30) and a detection wavelength of 205nm.
Particle analysis: The capsule contents were dissolved in 10ml, filtered through a 0.7µm glass filter and the filtrate was analyzed for particles using the Nanoparticle tracking analyzer (NTA, Malvern) using scattering of light by the particles.

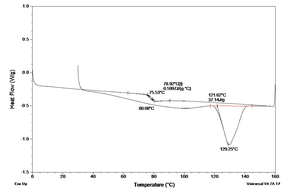
Results and Discussion

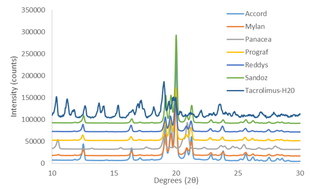
Characterization of dispersions:

Capsules manufactured by 6 companies were evaluated for capsule content weight, the batch number and the contents as mentioned in the product insert.
Most of the contents match the innovator product.
Panacea had beta-lactose anhydrate instead of the monohydrate form of lactose. Mylan had a small amount of the anhydrous form. Dr. Reddy's insert was missing HPMC even though it contained this polymer.

Manufacturer	Avg. powder weight (mg)	Lot no.	Expiry date	Inactive ingredients
Dr. Reddys	139.4	C402194	01/2016	Croscarmellose Na, Lactose MH and mag stearate, HPMC
Panacea Biotech	141.3	4233501	01/2016	Anhydrous lactose, Hypromellose 2910, CCSNa, mag stearate
For: Accord healthcare By: Intas Pharma. Ltd.	132	R09717	07/2016	Lactose MH, hypromellose E5, CCS-Na, mag stearate
Astellas Pharma Tech. Co. (Prograf)	137.5	047354	09/2016	Lactose MH NF, hypromellose USP, CCS-Na NF, mag stearate NF
Mylan	99.8	3057525	01/2016	Anhyd lactose, CCS-Na, hypromellose, lactose MH, Mag. Stearate, SLS
Sandoz	242	047354	02/2016	Croscarmellose Na, HPMC, lactose MH and Mag stearate

Compound property	
Molecular weight	804 g/mol
Melting temperature	120°C
Melting enthalpy	40 J/g
Glass transition temperature	76°C
Crystalline solubility	1.4 µg/mL

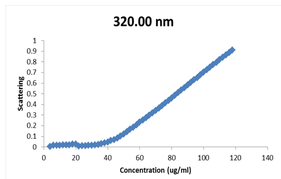




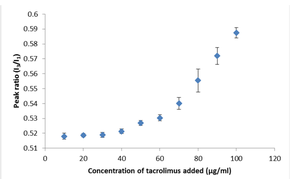
The XRPD patterns exhibited mostly the overwhelming presence of lactose. The difference in the pattern of Panacea capsules was due to the beta-anhydrous lactose form being present. The amorphous form of tacrolimus had a high T_g of 76°C.

Amorphous solubility:

UV scattering



Fluorescence

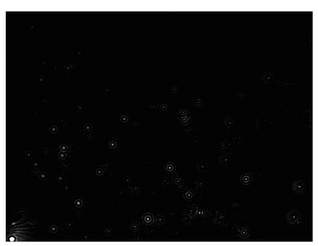


The amorphous solubility of tacrolimus in pH 4.5 buffer was found to be between 45-50µg/mL using different measurement techniques.

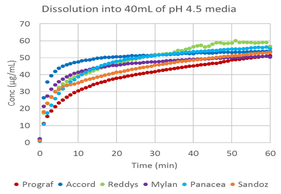
Technique	Amorphous solubility
UV scattering	45µg/mL
Fluorescence	40-50µg/mL
Diffusion analysis	50-60µg/mL
Ultracentrifuge	49 (1.5) µg/mL

Particle analysis:

Particle size analysis of the products after dissolution in extreme non-sink condition and filtration, using the NTA showed that there were particles present in the media with a particle size range of between 250-300nm.



Dissolution of the products in non-sink condition:

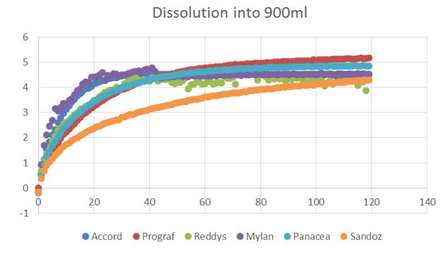


Products	Tacro conc. (ug/ml)
Prograf	50.1(3)
Accord	50.3(2)
Mylan	48.5(3)
Reddys	49.6(3)
Panacea	49.5(0)
Sandoz	47.3(2)
LLPS 37°C	49(1)
Crystal solubility	1.4(0.1)

The dissolution rate of Prograf was the slowest among the brands studied while Accord dissolved the fastest. Drug concentration higher than amorphous solubility was seen due to particle scattering in the turbid media. Filtration and HPLC analysis showed that the drug concentration after dissolution matched the amorphous solubility.

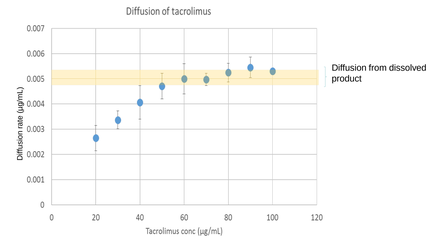
Dissolution into sink condition:

Accord and Mylan appeared to dissolve the fastest with Sandoz dissolving the slowest. Most of the drug dissolved into the media within 60 minutes and had similar dissolution profiles except for the Sandoz brand.



Diffusion of pure drug and dispersions

Flux data of the pure tacrolimus showed a plateau formation at around 50-60µg/mL. The diffusion rate of the drug after complete dissolution of the capsule contents was in the same range as the rate of the drug at the amorphous solubility.



Conclusions

Tacrolimus was found to form an amorphous solid with a high T_g. The crystalline solubility of the hydrate was very low at 1.4µg/mL while the amorphous solubility was 35 times higher at around 50µg/mL. The formulation ingredients of the different brands were similar to the innovator product except for Panacea. Prograf and Sandoz had the slowest dissolution rate when dissolved in a non sink and sink condition respectively. In non-sink conditions, the dissolution rate of Accord was the fastest among the brands and all the brands dissolved to free drug concentrations that did not exceed the amorphous solubility of 50µg/mL. Diffusion analysis also showed that the free drug concentration in the media was the same as that at which LLPS is formed.

Acknowledgments

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