Limitations in the Pharmacopeial Dissolution Testing of Marketed Tacrolimus Amorphous Solid Dispersions

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Purpose
With the increase in the number of poorly soluble compounds in the drug development pipeline, amorphous solid dispersions (ASDs) are gaining increasing importance as a solubility enhancement strategy. Since the amorphous drug in an ASD has higher free energy, it can undergo crystallization in the solid formulation during manufacture or during storage over the shelf life. From a performance standpoint, crystallization leads to decrease in the free energy and a corresponding decrease in the solubility advantage of the amorphous form. Compendial dissolution testing serves as a quality control tool for the changes in the formulation that can possibly lead to a negative impact on the performance of a formulation. With respect to an ASD, ideally, a dissolution method should be able to discriminate formulations with varying levels of crystallinity so that any amount of crystallization of the drug (manufacturing or storage induced) in an ASD can be detected during dissolution testing. Thus it becomes important to assess the discriminatory power of the conventional dissolution testing methods to determine how sensitive they are with respect to crystallinity detection and discrimination. The purpose of this study was to employ United States Pharmacopeia (USP) dissolution tests for two marketed formulations of tacrolimus, the innovator product and a generic version. The sensitivity towards crystallinity detection of non-conventional low-sink and non-sink dissolution methods was also tested for comparison with the USP methods.

Methods
Amorphous solubility determination: A methanolic stock solution of tacrolimus (5 mg/mL) was prepared and infused in the dissolution medium, stirred at 300 rpm at 37°C. The tacrolimus stock was added using a syringe pump at a controlled rate and the extinction was measured at a non-absorbing wavelength of 380nm using a dip probe ultraviolet (UV) spectrophotometer. The inflection in the extinction versus concentration graph indicates the amorphous solubility.

Dissolution testing: It was observed that the tacrolimus in the generic formulation crystallizes when exposed to 40°C/75%RH. The amorphous tacrolimus was allowed to crystallize to the maximum extent (confirmed using X-ray powder diffraction) and different amounts of fully crystallized generic capsule contents were added to the fresh generic capsule contents using geometric mixing to obtain the required levels of crystallinity. The crystalline formulations thus obtained were filled in size 3 clear gelatin capsules. USP dissolution tests were performed on the capsules according to the method outlined in the USP monograph of tacrolimus capsules. Briefly, for dissolution test I, III and IV the dissolution medium is pH 4.5 water containing hydroxypropylcellulose (50 μg/mL) and dissolution was performed at 37°C with stirring speeds of 50 rpm. The method is single point testing and the sample withdrawal time is 90 min for test I and III and it is 120 min for test IV. The test II uses SLS at the concentration of 1g/L in a solution containing 1.38 g/L of monobasic sodium phosphate. The time for testing according to method II is 60 min at 37°C with 50rpm stirring. Dissolution of generic capsules with different levels of crystallinity was also performed in lower volumes viz. 450mL, 100mL and 40mL.

Results
The amorphous solubility of tacrolimus was found out to be 50μg/mL. The amorphous tacrolimus present in the generic was found to be fully crystallized in ~ 4 weeks using the X-ray calibration (range: 15%-100% crystallinity). For the USP test I, III and IV, it was found that formulations containing 30% crystallinity passed the tolerance criteria of 80%. For USP test II, all crystalline formulations passed except the 100% crystalline generic. The volume of 100mL seemed to be most discriminatory. It was observed that even fresh generic shows signs of crystallization in 100mL volume. The 40 mL volume that serves as non-sink showed maximum concentrations up to the amorphous solubility of tacrolimus and eventually showed a decrease in concentrations.

Conclusion
A marketed generic formulation crystallized when exposed to stressful environmental conditions whereas the innovator product did not. It was observed that USP dissolution tests I, III and IV are non-discriminatory up to 30% crystallinity in the formulation. USP dissolution test II was found to be least sensitive to crystallinity as even 90% crystalline formulations passed the tolerance criteria. It was observed that a 100mL dissolution volume, equivalent to the 1X sink to amorphous solubility, was the most discriminatory and could even detect the crystal seeds thought to be present in fresh generic, that were beyond the detection limits of X-ray. The results of this study also suggest that the medium components should also be carefully selected since surfactants that are present in many dissolution media can lead to solubilization of the crystalline drug and can therefore lead to a decrease in the power of dissolution in detecting crystallinity.