Supporting Information

Direct Visualization of Drug-Polymer Phase Separation in Ritonavir-Copovidone Amorphous Solid Dispersions Using *In Situ* Synchrotron X-Ray Fluorescence Imaging of Thin Films

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In-situ X-ray Fluorescence (XRF) experiment:

The *in-situ* XRF measurements are performed at the microprobe hard X-ray 2-ID-E beamline at the Advanced Photon Source, Argonne National Laboratory. The X-ray beam generated by Synchrotron Radiation is selected by Si (111) single crystal monochromator at 10.5 keV. The X-rays are then collimated and focused by a Fresnel zone-plate on the sample to a 0.7 μm×0.7 μm spot size. The energy dispersed four-element silicon drift detector is located perpendicular to the X-ray propagation direction. The sample is raster-scanned by the focused X-ray beam. The recorded spectra regarding individual pixels are then processed by MAPS[1] to individual chemical element maps.

During the XRF experiment, an aliquot (~5 μL) of solution was dispensed onto silicon nitride window (Silson Ltd), and the solvent naturally evaporated in air to form a thin film of ASD. To reduce the data collection time (dwell time) and enhance signal-to-noise ratio, the same procedure was repeated several times to increase the thickness of the film. Control measurements were performed *ex-situ*. Figure S2(a) shows a comparison of integrated spectra of an empty Si_3N_4 window, coated with pure ritonavir and coated with pure copovidone. Figure S2(b)-(c) are sulfur maps of ritonavir and copovidone with a 50 μm×50 μm area. Both figures are plotted with the same color scale. The copovidone doesn't have sulfur signal. Therefore, the phase separation should be monitored with sulfur signals in the ASD thin film.

Scheme 1 shows the humidity chamber for the *in-situ* measurements. One side of the chamber is sealed with Si_3N_4 window and a drop of deionized water is placed inside. The sample window is prepared and dried before sealing the other side of the chamber. When mounted in the X-ray beam, the sample window is located upstream. The humidity chamber is enclosed in the X-ray experimental hutch. It usually takes ~10 minutes between sealing the humidity chamber and alignment of the chamber. A coarse raster scan is performed over a 1000 μm×1000 μm with 10 μm step size. The entire area scan takes ~10 minutes. Figure S3 shows the coarse scans from samples with 20% - 32.5% drug load with 2.5% intervals and 50 % drug load. The 20% drug load does not phase separate. The top part of the image shows uneven patterns because it's reaching the edge of the window. The rest of the image is uniform. Higher drug loads (22.5%-30%) show some degree of phase separation. However, most of the area still have uniform sulfur distribution. The 32.5% drug load shows significant phase separation. At 50% drug load, the ASD thin film is completely phase separated after 10mins. From the coarse scan results, a uniform area of sulfur map is chosen for fine scans. The fine scans cover a region of 500 μm×500 μm with 2.5 μm step size for 30% and 50% drug load and 400 μm×400 μm with 2 μm step size for 20% and 25% drug load. The recorded fine scans have 201×201 pixels with 43minutes of the total data acquisition time. Figure S4 shows fines scans of 20% drug load data. It does not show phase separation for 12 hours. However, with an *ex-situ* measurement of 20% drug load ASD thin film exposed in 100% RH for a week, the sulfur pattern shows clear phase separation (c.f. Figure S5).

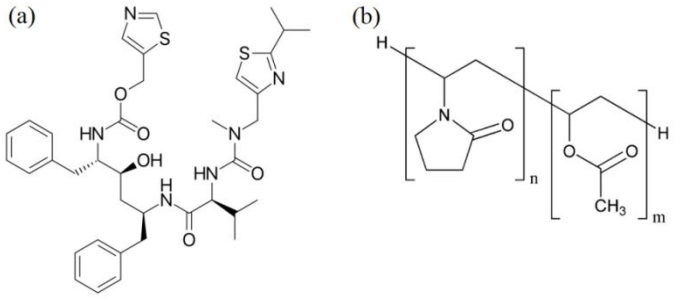

Figure S1. Chemical structures of (a) ritonavir ($C_{37}H_{48}N_6O_5S_2$) and the repeating units of (b) copovidone.

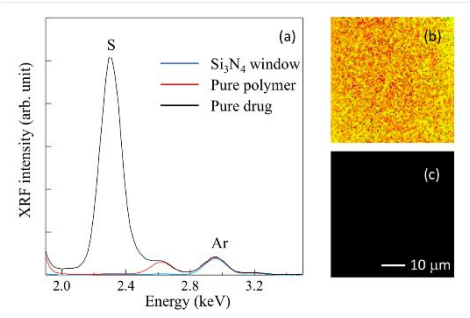

Figure S2. (a) Integrated X-ray fluorescence spectra over an area of 50μm×50μm for pure drug (black), polymer (red) and empty Si_3N_4 window (cyan). (b) The map of sulfur fluorescence signal for pure ritonavir from coarse scan (c) The map of sulfur fluorescence signal for pure polymer from coarse scan. (b) and (c) are plotted in the same color scale.

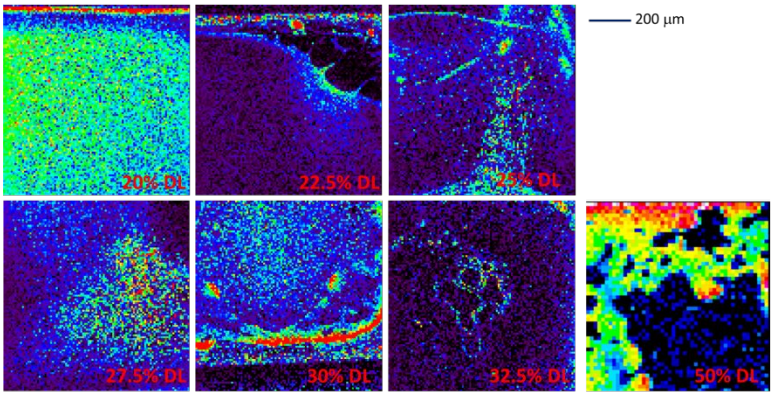

Figure S3. Coarse mapping of sulfur fluorescence signals for ritonavir-PVPVA ASDs at various drug loadings. From top left to bottom right, the drug load increases from 20 wt% to 32.5 wt%, with an increment of 2.5 wt%. The last figure is from 50 wt% DL. Prior to actual measurements, there was ~10 minutes time for experimental set-up and beam alignment.

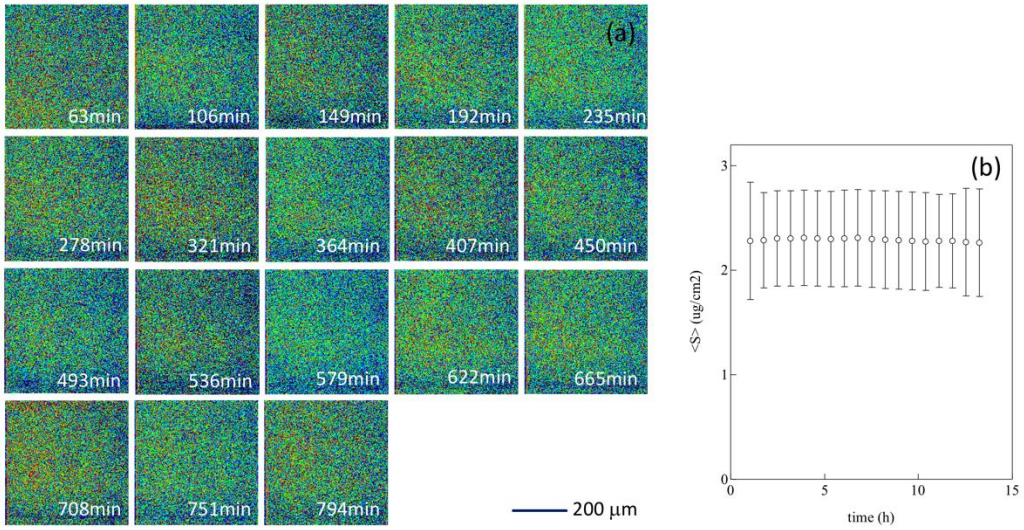

Figure S4. (a) Fine scans of ritonavir-PVPVA ASD film at 20 wt% DL in 100% RH over a period of 12 hours no phase separation detected. (b) the average sulfur concentration in fines scans as a function of time stamp.

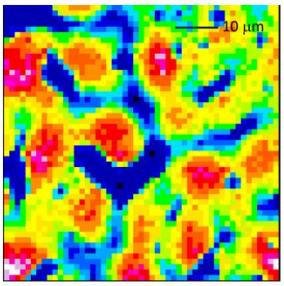

Figure S5. An *ex-situ* measurement of a 50 µm × 50 µm area fine scan of a ritonavir-PVPVA ASD film at 20 wt% DL in 100% RH over a week. AAPS happened and has reached the equilibrium. No crystallization was observed under the same condition using polarized light microscopy

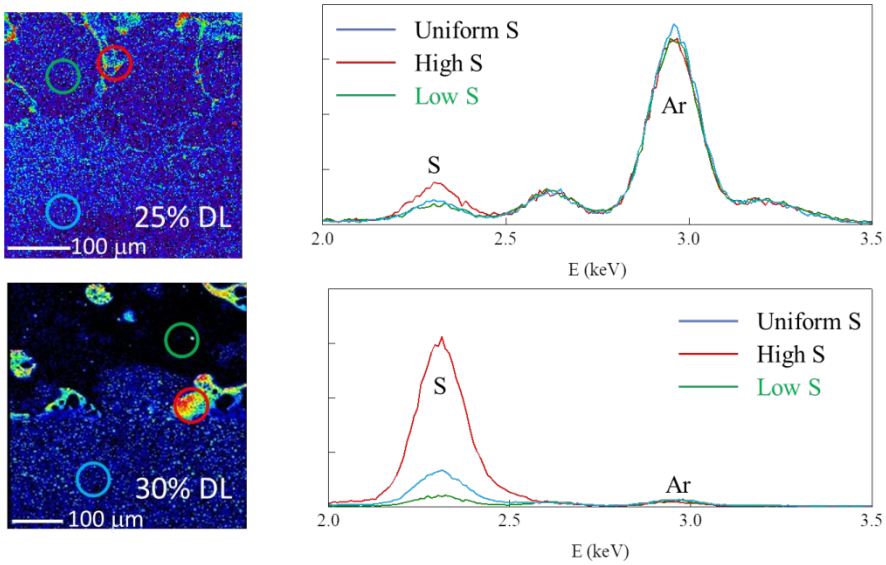

Figure S6. The fine scans with the AAPS onset for 25% and 30% DL. The integrated spectra are extracted from the marked area to present the RTV rich (red), RTV deficient (green) and RTV uniform (blue) areas. The curves are normalized by the Argon intensity, which is contributed from the trace amount of Argon gas in the sample environment. For 25% DL, the contrast of the RTV rich and deficient regions is 34%, and for 30% DL, the contrast is 93%.

Reference:

(1) Vogt, S.; Maser, J.; Jacobsen, C. Data analysis for X-ray fluorescence imaging. *J. Phys. IV France* **2003**, 104, 617–622.