Experimental report

16/09/2024

Proposal: 6-05-1074 **Council:** 4/2023

Title: Physical stability of co-amorphousmixtures of highly crystallizable drugs probed by fast dynamics

Research area: Physics

This proposal is a new proposal

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Samples: Indomethacin C19H16ClNO4
naproxen C14H14O3

Instrument	Requested days	Allocated days	From	To
IN13	10	10	14/11/2023	24/11/2023

Abstract:

We propose an elastic neutron scattering investigation to study the correlation between the stability of amorphous state of pure and mixed crystallizable drugs and the fast mobility, i.e. the mean square atomic displacement (MSD) originated by the cage dynamics around the glass transition. Recent molecular dynamics simulations indicate a strong correlation between the amplitude of the picosecond rattling motion inside the cage and the much slower structural relaxation, whose dynamics affect the crystallization kinetics.
The elastic intensity of two different drugs, indomethacin and naproxen, respectively good and bad glass-forming, and their mixtures at three different relative weight ratios prepared as co-crystals and amorphized via melt-quenching, will be investigated under temperature scans from very low temperature up to some degrees above Tg. The proposed samples show different fragility and stability versus recrystallization from the amorphous state. The suggested experiment could prove that fast cage vibrational amplitude can be a useful predictor of the stability of the metastable amorphous state.

Experimental report beamtime #6-05-1074 November 2023

Elastic incoherent measurements were performed on four sample as a function of the temperature.
The samples under investigation were Indomethacin (IND) and three coamorphous solution of IND with Naproxen (NAP) at different molar ratio, i.e. NAP:IND 1:1, NAP:IND 1:2, NAP:IND 2:1.
Temperatures investigated span from 20 K up to 450 K. Thanks to the high neutron flux, temperatures from 180 K up to 450 K were investigated by continuous heating at two different rates: 1 K/10 min from 180 K up to 310 K, 1 K/12 min above, while in the range 20-180 K only some selected temperatures were inspected, i.e. 20 K – 60 K - 100 K – 140 K. All samples were hydrogenated, despite the presence of methyl groups. Sample preparation has been done *in loco* by loading the cell with the right amount of specimen, melting in an oven and quenching in liquid nitrogen. We also asked beamtime to perform quasi-elastic scans, and we tried to acquire data but the neutron flux was not enough to obtain reliable data at the temperatures of interest. Differential Scanning Calorimetry (DSC) measurements have been done in house to characterize the thermodynamical behaviour of the four proposed samples and compared with results already available in literature.
It is well known that NAP is almost impossible to amorphize due to its high crystallization tendency[(1)], on the contrary IND crystallizes on very long timescales[2]. As demonstrated in literature[2], by mixing NAP and IND it is possible to decrease NAP crystallization tendency, obtaining a system stable in the amorphous state at room temperature (RT). The most interesting and promising formulations are the three listed above, i.e. NAP:IND 1:1, NAP:IND 1:2, NAP:IND 2:1. In fact, in the NAP:IND 1:1 there is a 1-to-1 interaction of NAP molecules with IND ones, and in the two unbalanced solutions there is a prominent presence of one molecule over the other, with the two have opposite crystallization properties.
The aim of the experiment is to find a connection between thermodynamics and dynamics, namely between the physical stability in the amorphous state and the mean squared displacement of hydrogen atoms. In fact, as demonstrated in literature for proteins[3], there exists a connection between dynamics and thermodynamics, but this connection has rarely been explored in the pharmaceutical field, and never for such kind of samples.
Calorimetric measurements show that all three solutions have a glass transition temperature (T_g) lower than pure IND, highlighting the plasticizing effect that NAP has on IND. In general, the T_g of all three solutions are close to RT (see Fig. 1 for more details).
Elastic measurements present interesting features. First, by looking at the integrated intensity (see Fig. 2), it is possible to note two changes in slope with temperature, qualitatively similar for all the samples. The change at higher temperature can be attributed to the T_g of the sample (being comparable to the calorimetric T_g), whereas the change at low temperature remains difficult to interpret. The most probable explanation is that this change in slope is because of the methyl group. Our conclusion is supported by two evidences: the first is that this change in slope is not found in the mean squared displacement, and the second is that as such low temperature nothing is expected to happen being very far from the T_g.
Moreover, at higher temperatures, only for the sample indomethacin and NAP:IND 2:1 it is possible to note a non-monotonic behaviour first with an uprise of elastic intensity (due to re-crystallization, that induce more rigidity to the system), then to a further decrease in elastic intensity, in occurrence of melting.
When looking at the mean squared displacement (msd), it is possible to note a sudden rise in correspondence of the calorimetric T_g, and another change in slope can be found in correspondence of the temperature when diffusion enters in the time-window of the spectrometer. It must be stressed that for this second feature a deeper analysis is needed to avoid any ambiguities or errors during data analysis.
However, the remarkable result come from the comparison of the msd of all four sample (see Fig. 3). If we consider the msd of pure IND as a reference, it is possible to define two regimes. The first is that of NAP:IND 1:2, showing a lower msd than IND; the second is that of NAP:IND 1:1 and NAP:IND 2:1 presenting a higher msd and hence a faster dynamics. It is reasonable to hypothesize that NAP:IND 1:2 shows a lower msd than IND because of two effects: the anti-plasticizing effect of IND against NAP, and the steric hindrance of NAP on

IND molecules, creating a further slowdown of the overall dynamics. On the contrary, the acceleration of NAP:IND 1:1 and NAP:IND 2:1 dynamics can be attributed to the presence of NAP, with NAP:IND 2:1 being slightly faster than NAP:IND 1:1.In the relevant temperature range for this discussion (higher than 270 K), the contribution of methyl group rotation in elastic scattering should not play any role, since it is faster than the time window of the spectrometer.

The obtained results are remarkable since NAP:IND 1:1 is the most stable coamorphous solution upon storage at RT, but it must be taken into account that both molecules where hydrogenated, hence the measured msd is the average of NAP and IND contributions. Nevertheless, the obtained results show the non-trivial connection between dynamics and thermodynamics, highlighting the importance of such studies in the understanding of such phenomena.

References:

[1] A. Minecka, E. Kaminska, M. Tarnacka, I. Grudzka-Flak, M. Bartoszek, K. Wolnica, M. Dulski, K. Kaminski, M. Paluch, *Mol. Pharmaceutics* 15, 4764−4776 (**2018**).

[2] K. Lobmann, R. Laitinen, H. Grohganz, K. C. Gordon, C. Strachan, T. Rades, *Mol. Pharmaceutics* 8, 1919–1928 (**2011**).

[3] M. Katava, G. Stirnemann, M. Zanatta, S. Capaccioli, M. Pachetti, K. L. Ngai, F. Sterponea, A. Paciaroni, *Proc. Natl. Acad. Sci. USA* 114(35), 9361-9366 (**2017**).

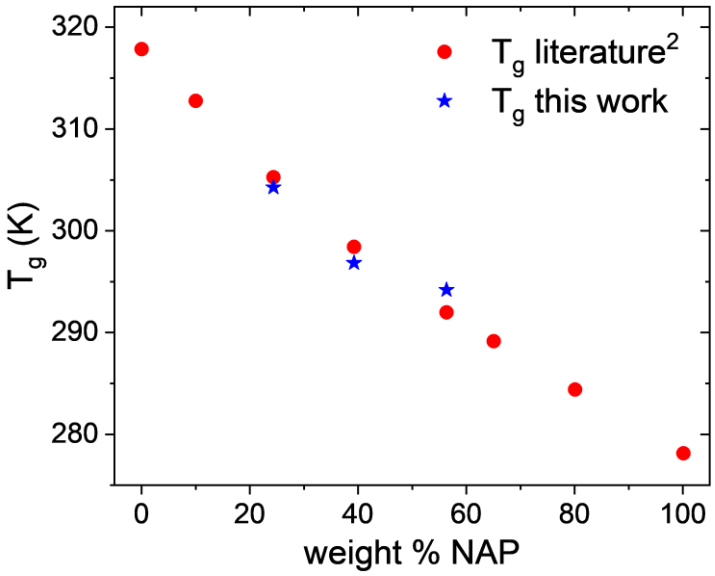

Figure 1: Glass transition temperature of coamorphous solutions as a function of the weight percentage of NAP. Red dots are taken from Ref. 2, blue stars are our data.

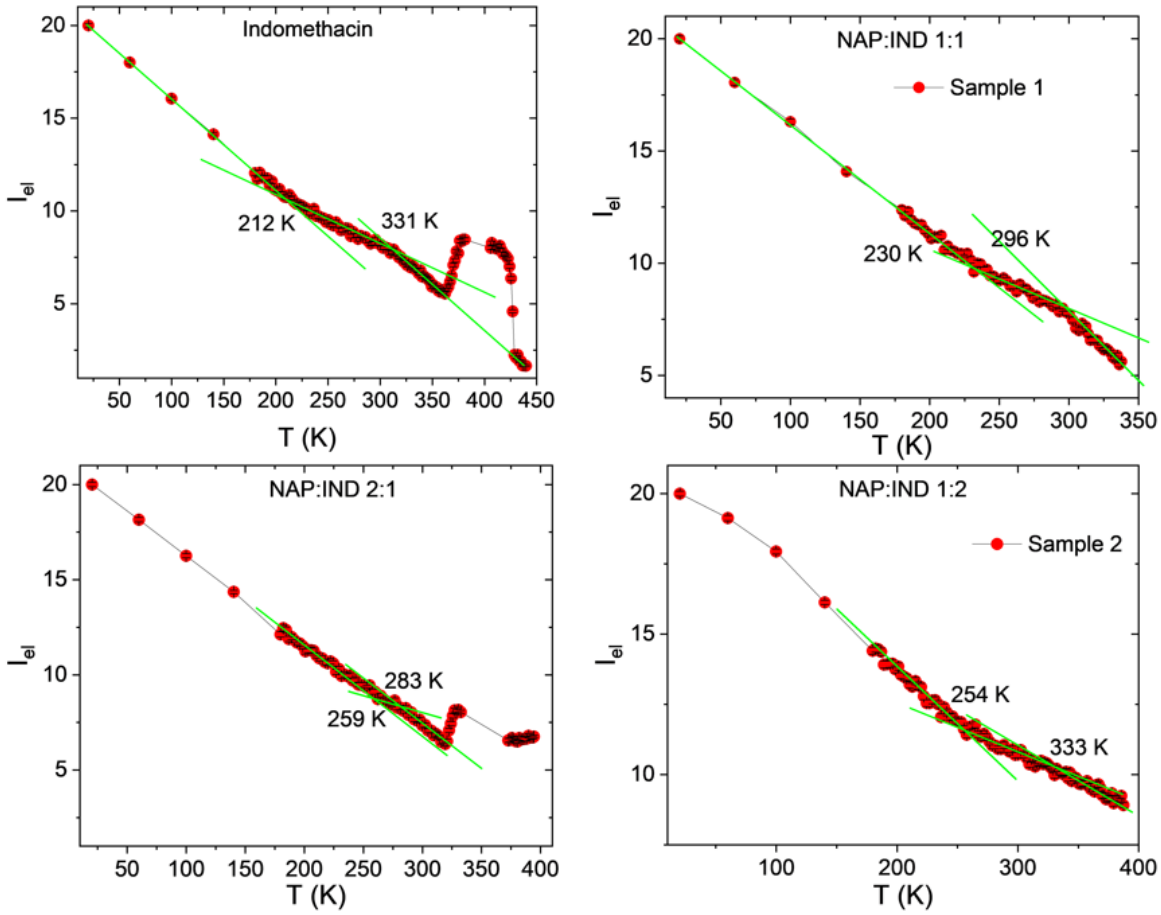

Figure 2: integrated intensity as a function of the temperature for all investigated samples. Green lines help in the visualization of the change in slope. The sudden rise in intensity in the case of IND and NAP:IND 2:1 can be attributed to crystallization.

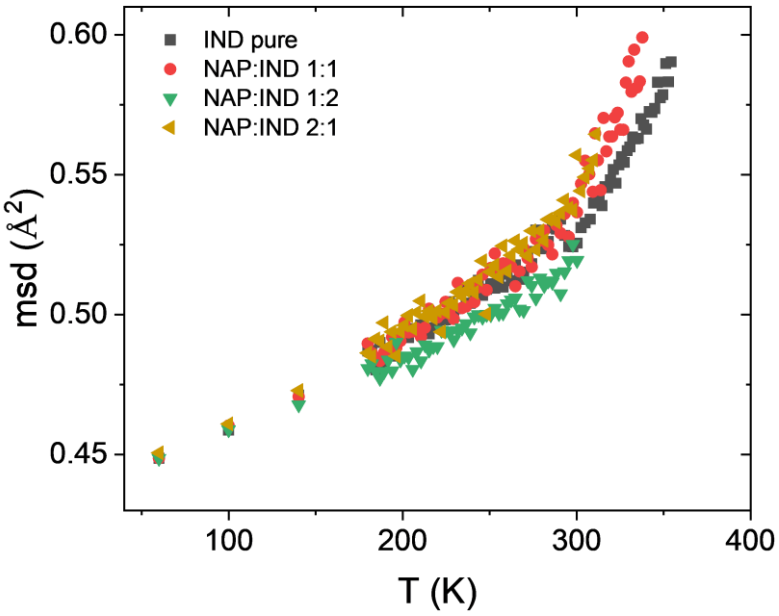

Figure 3: msd as a function of the temperature for all investigated samples.