Terahertz Dynamics of Amorphous (Bio)Pharmaceutical Mixtures

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***Abstract*—The molecular dynamics of amorphous materials and its relation to the physico-chemical changes in the organic glasses is still a topic of a dispute in the scientific community. Terahertz spectroscopy however provides very important experimental evidence on the fast dynamics in the overlapping region between the molecular relaxations and structural excitations, which is extremely sensitive to both dynamical and structural changes in the amorphous systems. We show how this information can be utilised to enhance the long-term stability of amorphous pharmaceutical systems and cryo-preserved proteins.**

I. INTRODUCTION

Organic amorphous materials are involved in many aspects of life, from a sugar candy to their pivotal role in suspension of desert insect life during drought [1]. Two applications of amorphous materials are particularly industrially important: First, amorphous solids can be used to significantly enhance the water solubility in pharmaceutical formulation. Second, vitrified solids play a central role for the preservation of food and proteins in the absence of water.

The molecular dynamics in amorphous materials are traditionally assessed by broadband dielectric spectroscopy. Such measurements allow measuring the primary and secondary relaxations that occur in disordered solids. The primary relaxation is related to the type of motion associated with diffusion observed in liquids. Upon cooling it vanishes below the glass transition temperature, T_g, where a liquid loses its ability to flow and forms an amorphous solid, or a glass. The secondary relaxations prevail at temperatures below T_g and can originate from inter- or intra-molecular motions. The former type of secondary relaxation is of a higher practical significance as it involves localised movements of whole molecules that are significant in the crystallisation of the glass [2]. The frequency region between the fastest relaxations and lowest structural excitations (i.e. usually at MHz/GHz to THz frequencies) merely exhibits weakly frequency-dependant dielectric losses and is commonly referred to as the region of nearly constant losses (NCL) in the glassy state [3]. This behaviour is usually explained to originate from the caged molecular dynamics [3]. However, dielectric spectroscopy cannot easily access the terahertz frequency range where molecular relaxations overlap with structural excitations. This is in particular true for temperatures below T_g.

Terahertz spectroscopy (THz-TDS) offers a straightforward way to acquire measurements in the 100 GHz – 5 THz range. Previous terahertz studies of amorphous systems focused on understanding the fundamental origin of terahertz losses over a wide temperature range [4]. It has been shown that the losses in the terahertz region originate from both the relaxational and vibrational response of the molecules. A significant outcome of the previous studies was the discovery of the onset of molecular mobility at around 0.67 T_g, which seems to be linked to the secondary relaxational dynamics as described above [4].

II. RESULTS

Amorphous naproxen has an extraordinarily strong tendency to crystallise even at temperatures below its T_g = 279 K. The terahertz spectra in Fig. 1(a) reveal that the baseline absorption in the spectra of naproxen decreases upon heating and a broad vibrational resonance peak emerges at a frequency of around 1.5 THz. Such behaviour is characteristic of continuous crystallisation of naproxen upon heating and we found that the sample was fully crystalline at 270 K. The absorption changes with temperature in a linear fashion, as shown in Fig. 1(b). The linear analysis reveals that the decrease in absorption becomes about 4 times faster at temperatures above 190 K ~ 0.67 T_g. We previously reported that this temperature is associated with the onset of local mobility in amorphous systems due to the molecular mobility

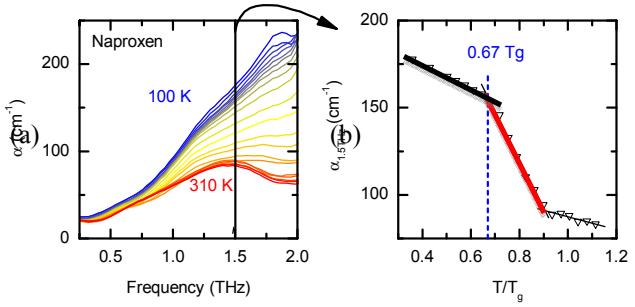

Fig. 1. (a) Terahertz absorption spectra of quench-cooled naproxen in the thermal range 100−310 K. (b) Terahertz absorption coefficient at 1.2 THz, 1.5 THz, and 1.8 THz as a function of rescaled temperature T/T_g.

that originates from the secondary relaxation [4]. It can be therefore concluded that the secondary relaxation is directly facilitating the crystallisation above 0.67 T_g in amorphous naproxen.

In the second case we studied four amorphous drug compounds: paracetamol, indomethacin, flufenamic acid and simvastatin. Similar to the case of amorphous polyalcoholos [4], the absorption changes around 0.67 T_g and 1.0 T_g, as can be seen in Fig. 2. The rate of increase in the absorption above 0.67 T_g varies across the samples: It is largest for amorphous paracetamol and flufenamic acid and lower in the other two drugs. Monitoring of the time to the onset of crystallisation by means of X-ray powder diffraction confirmed that these two samples have the poorest stability and tend to crystallise in a matter of a few minutes at ambient conditions. In contrast, amorphous simvastatin exhibits only weak temperature dependence of absorption below T_g and remains amorphous under the same conditions for more than 80 days. Indomethacin, which exhibits moderate change in absorption with temperature, remains amorphous for about 7 days. The results show a strong link between the stability of amorphous drugs and the onset of local mobility due to secondary relaxation at 0.67 T_g. Terahertz spectroscopy offers an

analytical method to quantify this mobility and can be potentially used to predict the stability of amorphous drugs [5].

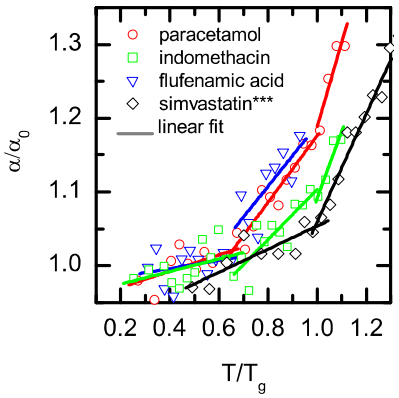

Fig. 2. Terahertz absorption as a function of temperature at 1 THz for amorphous drugs: paracetamol, indomethacin, flufenamic acid and simvastatin. The x-axis was rescaled by T_g in order to compare the samples at isochronal condition with respect to the primary dielectric relaxation. The y-axis was rescaled by the low-temperature terahertz absorption in order to reflect on the different level of absorption originating from a different density of hydrogen bonding and molecular dipolar moment in the samples. The higher increase in terahertz absorption above 0.67 T_g is strongly linked to the stability of amorphous drugs.

Lastly, we examined the feasibility of terahertz spectroscopy to study freeze-dried proteins embedded in glassy sugar matrices. Recently it was shown that smaller and molecularly more flexible sugars stabilised proteins better compared to larger and more rigid sugars [6]. This is most likely because they are less affected by steric hindrance and therewith better capable of forming hydrogen bonds with the protein. In order to investigate this using THz-TDS we have studied bovine

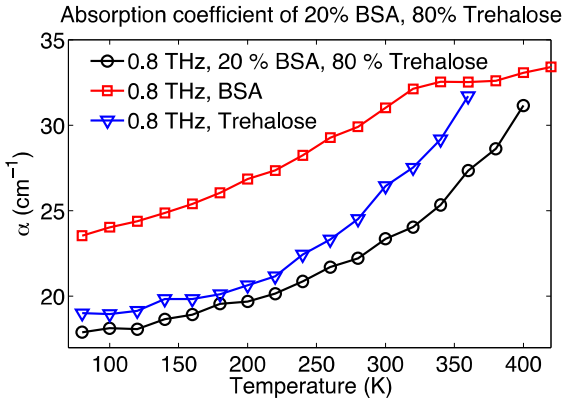

Fig. 3. Terahertz absorption spectra of freeze-dried BSA, trehalose and BSA/trehalose mixture at 0.8 THz as a function of temperature.

serum albumin (BSA) freeze-dried with three different sugars: trehalose, inulin 4 kDa and dextran 70 kDa.

The comparison between the terahertz spectra of the protein/sugar mixture, pure protein and pure sugar can be used to estimate the relative hydrogen bonding between the components. If the protein and sugar do not interact strongly the mixed system forms effectively a phase-separated system, and hence the resulting absorption of the mixture is merely a linear combination of the absorption of the constituent components. On the other hand, if the mixing results in strong bonding between the protein and sugar the resulting absorption deviates from the sum of absorption of the pure compounds. Terahertz absorption of the freeze dried BSA/trehalose formulation is clearly lower than that of pure BSA or pure trehalose (Fig. 3). In contrast, the absorption of BSA/dextran is close to the linear combination of absorption of pure BSA and pure dextran. The absorption of BSA/inulin suggests some bonding between BSA and inulin being present though not as strong as for the case of BSA and trehalose. A previous stability study showed that trehalose has the best stabilisation effect on BSA, while dextran performs the poorest [6]. The terahertz spectroscopy study confirms that the bonding between protein and sugar molecules is a significant factor in protein stabilisation.

The results also suggest that terahertz spectroscopy can be used to assess the mobility of the protein itself. Fig. 3 shows that the mobility of protein increases with temperature (red data points) until it denatures at around 360 K [7]. There is no clear onset of local mobility around 0.67 T_g in such systems. Instead, the increase in the terahertz absorption is continuous much like the observations made by neutron scattering [8] and previous terahertz spectroscopy studies [9].

III. Summary

Terahertz spectroscopy can be used to characterise amorphous systems of high relevance to pharmaceutical and biopharmaceutical industry. While the crystallisation of amorphous drugs and degradation of proteins in glassy matrices are completely different phenomena we show that they are ruled by the same molecular dynamics. In particular, we show that

1. The molecular dynamics, which evolves above 0.67 T_g, can play an important role in the crystallisation of amorphous small organic molecules below T_g.
2. There is a strong correlation between the molecular mobility above 0.67 T_g and the long-term stability of amorphous drugs, which allows utilising THz-TDS as an analytical technique for stability prediction.
3. THz-TDS can be used to assess the long-term stability of cryo-preserved proteins in glassy sugar matrices.

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