Experimental report

05/07/2017

Proposal: 9-12-476 **Council:** 4/2016

Title: Nucleation Kinetics and Growth of Poorly Water-Soluble Fenofibrate Drug Nanoparticles

Research area: Physics

This proposal is a new proposal

Main proposer: Dennis NOLL

Experimental team: Jano HEPPT
Torben SCHINDLER
Isabel SCHULDES
Dennis NOLL

Local contacts: Lionel PORCAR

Samples: hydroxypropyl methylcellulose (HPMC)
Poloxamer 188 (also known as Pluronic F-68)
Fenofibrate - C20H21ClO4
hydroxypropylcellulose (HPC)

Instrument	Requested days	Allocated days	From	To
D22	3	2	05/07/2016	07/07/2016

Abstract:

Precipitation is an established and nowadays widely used bottom-up approach for the production of nanosuspensions of poorly water-soluble active pharmaceutical ingredients. To enhance their bioavailability, suitable stabilizers have to be selected to inhibit agglomeration in order to produce smaller particles. The purpose of the proposed experiment is to study the influence of three structurally different polymeric stabilizers (HPC, HPMC, poloxamer 188) on the nucleation kinetics and growth of organic drug nanoparticles prepared by precipitation. We will use fenofibrate as a model system, since reliable stabilizers are well known as a result of preliminary precipitation as well as media milling experiments and its formation kinetics take place on a time range well suitable for the stopped-flow method. The SANS measurements will be complemented by own SAXS measurements.
Assuming a time of over 17 h only for the high amount of mixing processes needed for one of three proposed sample systems, adding the time needed for the high amount of manual refillings and regarding calibration, background and transmission measurements, we apply for three days of beamtime at the D22 instrument.

Experimental Report – Instrument: D22 – Proposal No. 9-12-476 – Schedule: 05/07/16 – 07/07/16

Nucleation Kinetics and Growth of Poorly Water-Soluble Fenofibrate Drug Nanoparticles

Antisolvent precipitation is an established and nowadays widely used bottom-up approach for the production of nanosuspensions of poorly water-soluble active pharmaceutical ingredients (APIs). Since nanosuspensions are thermodynamically unstable colloidal systems, suitable steric, electrosteric, or electrostatic stabilizers have to be selected to inhibit agglomeration. It is intended to enhance the bioavailability of these drugs by producing smaller particles. Therefore, the precipitation process has to be optimized and the influence of stabilizers on the nucleation kinetics, which is almost unknown in the field of organic nanoparticles, has to be studied.
For example, experiments on the precipitation of perylene were performed using a combination of the stopped-flow method and UV/Vis-spectroscopy.[1,2] The formation of the particles was observed by measuring the decrease of the perylene monomer's absorption peak. It has been concluded that the formation of perylene nanoparticles takes place in the first tens of milliseconds and is governed by the classical nucleation theory. Recent studies address the polymer adsorption onto drug nanoparticles using SANS and it has been demonstrated, that a combination of SAXS and SANS measurements is highly suitable to characterize the stabilizer layer adsorbed onto organic triglyceride nanoparticles.[3-6]

However, there is a lack of experiments studying the structure of organic nanoparticles and their stabilizer layer during the precipitation process, so the purpose of the proposed experiment was to study the influence of three structurally different polymeric stabilizers (HPC, HPMC, poloxamer 188) on the nucleation and growth of the model API fenofibrate, prepared by precipitation. To achieve good statistics with a high temporal resolution on a time range of milliseconds to few minutes, the stopped-flow method in combination with SANS is highly efficient, since it allows a large amount of reproducible mixing processes with any desired maximum measurement time and small waste.

Since these experiments were the first combined stopped-flow SANS experiments for the sample system of the model API fenofibrate, there was no experience in how the neutron scattering intensity would be and which temporal resolution is achievable. Every sample system had the same composition: 35 mg fenofibrate / 1 ml ethanol had been mixed together with 25 wt% stabilizer (relative to fenofibrate) / 9 ml D_2O, so that the mixing ratio always has been 1 : 9 = solvent : antisolvent. The concentration of the final API suspension was 35 mg / 10 ml = 0.35 wt%. Due to the low concentration we intended to repeat the mixing processes of each sample system (one stabilizer) very often, up to 3900 times, but the scattered intensity was pretty good, which allowed to perform less mixing processes per sample system with still a good temporal resolution of up to 50 ms. Therefore, we could study more different stabilizer systems, and also perform temperature dependent experiments and vary the contrast for one sample system (SDS as stabilizer). 10 mixing processes per sample system were enough to achieve a temporal resolution of 50 ms and additional 10 mixing processes were performed to measure with a temporal resolution of 1 s on a time range up to 300 s. A preliminary analysis for four different sample systems is shown in fig 2. For this analysis spherical particles are assumed as observed by

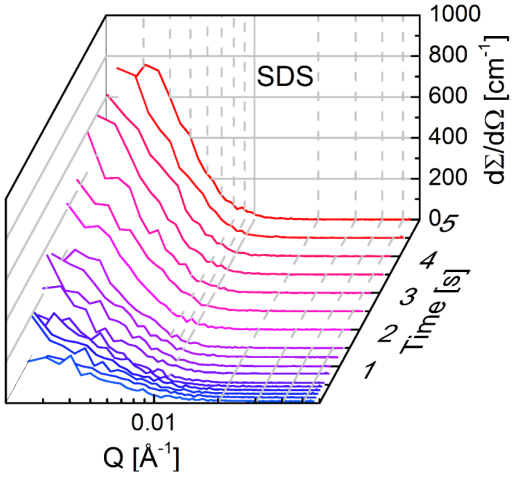

Figure 1: First in situ SANS data measured at the D22 instrument that show the possibility of combined SANS and stopped-flow experiments to study the growth of fenofibrate particles produced by antisolvent precipitation.

electron microscopy for organic nanoparticles produced by antisolvent precipitation. Further, the analyses show that the smallest average diameter is already over 20 nm during the first 50 ms which leads to the need of a higher temporal resolution to also study the nucleation during and directly after the mixing process.

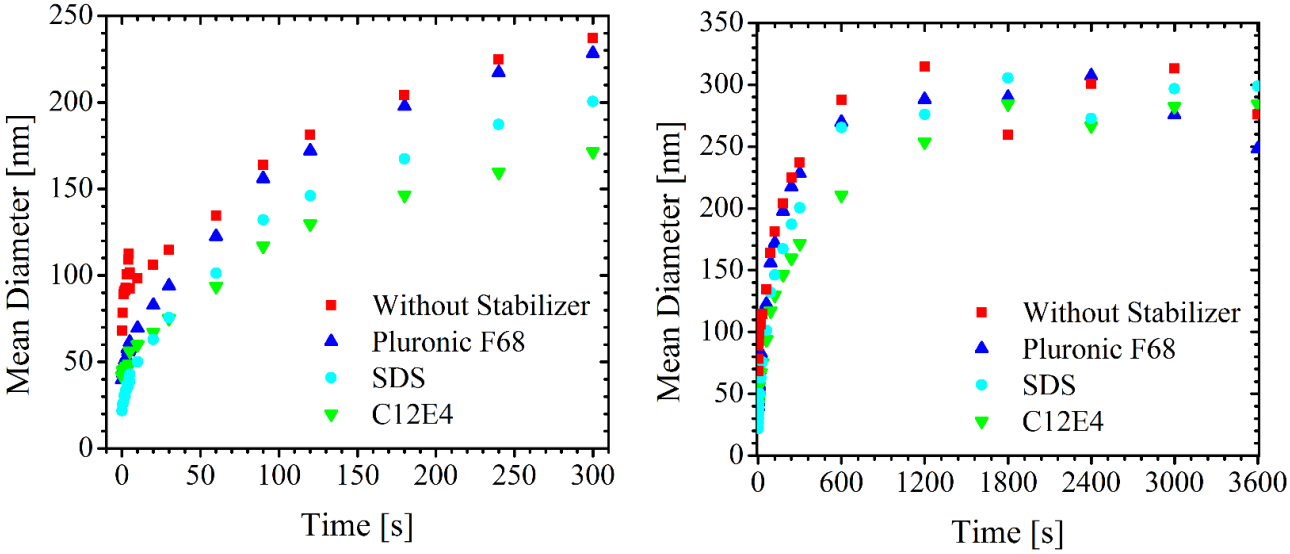

Figure 2: For each mixing process fenofibrate solved in ethanol was mixed together with the stabilizer solved in D_2O with a ratio of 1 : 9. The temporal resolution of 50 ms during the first 15 s was achieved by 10 mixing processes and a wavelength of 6 Å. The range of 15 – 300 s was measured with a temporal resolution of 1 s and with a longer wavelength of 12 Å.
Assuming spherical particles preliminary analyses show the growth of fenifibrate nanoparticles over a range of 300 s and that the smallest particles already have average diameters of over 20 nm during the first 50 ms.

The analyses in fig. 2 also show that the assumption of spherical particles does not fit anymore after 600 s and that the nanoparticles agglomerate. Further analyses and especially the combination with SAXS should enable to study the role of different stabilizers during the precipitation process of organic nanoparticles. However, recent observations show that it is not easily possible to compare stopped-flow SAXS and SANS data, since SAXS is measured in a capillary and SANS in a cuvette and the mixed sample solutions show different behaviors in both geometries.
Additionally, a higher temporal resolution up to 1 ms should be easily achievable by performing up to 500 mixing processes for each sample system at the D22 instrument. This experiment is already proposed at the ILL and yields the opportunity to study the structure and the stabilization mechanism of poorly water-soluble organic nanoparticles during their nucleation for the very first time. This proposed experiment should be complemented by SAXS measurements with a similar high temporal resolution proposed at the ESRF.

[1] J. Mori et al., J. Cryst. Growth **311** (2009) 553
[2] D. Oliveira et al., Jpn. J. Appl. Phys. **48** (2009) 105003
[3] D. J. Goodwin et al., Mol. Pharm **10** (2013) 4146
[4] M. Schmiele et al., Phys. Rev. E **87** (2013) 062316
[5] M. Schmiele et al., J. Phys. Chem. B **118** (2014) 8808
[6] M. Schmiele et al., PCCP **17** (2015) 17939