**Combined Synchrotron XRD/Raman Measurements: In Situ Identification of Polymorphic Transitions during Crystallization Processes**

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A combination of two analytical methods, time-resolved X-ray diffraction (XRD) and Raman spectroscopy, is presented as a novel tool for crystallization studies. An acoustic levitator was employed as sample environment. This setup enables the acquisition of XRD and Raman data in situ simultaneously within a 20 s period and hence permits investigation of polymorphic phase transitions during the crystallization process in different solvents (methanol, ethanol, acetone, dichloromethane, acetonitrile). These real time measurements allow the determination of the phase content from the onset of the first crystalline molecular assemblies to the stable system. To evaluate the capability of this approach, the setup was applied to elucidate the crystallization process of the polymorphic compound nifedipine. The results indicate the existence of solvent-dependent transient phases during the crystallization process. The quality of the data allowed the assignment of the lattice constants of the hitherto unknown crystal structure of the β-polymorph.

**Introduction**

The polymorphism of organic compounds is a subject of great interest for both basic research and industrial applications, especially for pharmaceutical and chemical industry. Polymorphism is defined as the ability of a chemical substance to crystallize in two or more different crystal structures. Each polymorphic form has the same chemical structure but differs in the arrangement of molecules in the unit cell. The variations in the arrangement of the atoms, ions, or molecules result in different physical properties of the polymorphs, such as color, solubility, bioavailability, or reactivity. Consequently, the development of methods permitting in situ real time diagnostics of polymorphic forms is urgently needed. Crystallization processes involve both nucleation and growth of a crystalline phase. In the context of polymorphism, nucleation is pivotal in the selective crystallization of polymorphs and in the stabilization of intermediate stages. Therefore, studies of the initial steps are essential when characterizing intermolecular interactions and identifying solvents that may promote or preclude the crystallization of a specific polymorph.

X-ray diffraction (XRD) is in most cases the primary method utilized to study crystallization processes. Developments in the area of synchrotron XRD, i.e., fast CCD detectors for crystallographic data collection, led to an increasing use of in situ powder diffraction to investigate the formation of crystalline solids in real time. A prerequisite for XRD, on the other hand, is the existence of a crystalline solid consisting of several unit cells, typically more than 10 per edge. Since organic polymorphs are characterized by small variations in their enthalpy of formation, formation, and dissolution of crystalline seeds, below the detection limit of XRD, can be expected. Thus, in order to elucidate the structure of transient crystalline or amorphous intermediates, additional techniques are required to monitor their existence. Raman spectroscopy provides in situ monitoring of the crystal formation and of the transformation from one polymorph to another. This holds especially for those cases where amorphous stages can be observed. Hence, vibrational spectroscopy has found broad application in the characterization of pharmaceuticals and their matrices.

To avoid any surface contributions to the crystallization process, we have chosen acoustically levitated droplets as sample environment rather than any conventional reaction vessel, a technique proven to be promising in previous experiments. Extensive studies have been performed on the evaporation and drying processes occurring in these droplets. Santesson et al. provide a review on the wide use of acoustic levitation in analytical and bioanalytical chemistry applications. Several groups have used that method either in combination with Raman spectroscopy or X-ray diffraction. To our knowledge, this is the first study...
in levitated droplets using a combination of synchrotron XRD and Raman spectroscopy for simultaneous investigations.

The compound nifedipine (4-(2-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine), which is well-known for its polymorphism, was selected as a model system for the investigations. In medical treatments, nifedipine acts as dihydropyridine calcium antagonist. Despite this pharmaceutical importance, the wide use of the substance, as well as knowledge of its polymorphism, the crystal structures of the nifedipine polymorphs still remain unknown. Only the crystal structure of α-nifedipine (form I) was reported in 1980 by Triggle et al. Subsequently, three monotropic related modifications and four dioxane solvates were described in the literature.

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The authors describe three different crystalline forms (α, β, γ) and a glassy form (g). Additionally, the crystallization and the polymorphic changes of amorphous nifedipine have been investigated by using FT-IR imaging under a controlled humidity and temperature environment by Chan and Kazarian.

In order to cover a broad range of transformation, different solvents (methanol, ethanol, acetone, dichloromethane, acetonitrile) were chosen in our investigation of the nifedipine crystallization process. The α-form of nifedipine is the thermodynamically most stable form and is expected to be favored in a very slow evaporation process, where the molecules can self-assemble over an extended period of time into their lowest energy configuration. The intermediate phases β- and amorphous g-nifedipine are kinetically favored and formed preferentially under fast evaporation conditions.

An approach to understanding the formation processes and polymorphic transitions has to involve information on both the structural characterization of final crystalline stages and also of intermediate stages. A setup combining XRD and Raman spectroscopy enables one to observe these stages simultaneously. The X-ray diffraction pattern is possible. In fortunate cases, structure solutions can be obtained using different solvents: ethanol (19 mM), methanol (26 mM), acetone (182 mM), dichloromethane (231 mM), and acetonitrile (72 mM). The solutions were stirred vigorously. In all cases, the concentrations were chosen significantly below the saturation point to avoid the presence of any crystalline material in the starting solutions.

Acoustic Levitation. An acoustic levitator (tec5 AG, Oberursel, Germany) was used as sample holder. Acoustic levitation is a versatile technique for sample handling. A detailed description of the experimental setup can be found in refs 6, 7, and 24. Applying this contact-free method, solid and liquid samples can be positioned in a gaseous environment by means of a stationary ultrasonic field. In a typical experiment, a droplet with a volume of about 4 μL was manually injected into the acoustic levitator with a common Eppendorf pipet (size 0.5–1 μL, Eppendorf, Germany). The ambient air temperature at the sample position was 296 ± 1 K. The relative humidity was RH = 60 ± 5%. A series of tests indicated that the temperature and pressure conditions in the direct environment of the droplet show only minimal fluctuations as a benefit of the controllable conditions prevailing in the experimental hutch. Moreover, the evaporation rate can be controlled via regulating the temperature and the chemical potential of the respective solvent. In this experiment the conditions were chosen to meet the needs and intrinsic time scales of the XRD and Raman data acquisition procedures. The sample remains in a fixed position during the measurement even after evaporation of the solvent. The position stability of the droplet, measured as a displacement smaller than 20 μm, allowed more than 30 min of data acquisition time. A sketch of the setup is depicted in Figure 2.

Methods. Ex situ Raman measurements of the crystallized final products were performed with a LabRam single stage spectrophotograph (Horiba Jobin-Yvon, Bensheim, Germany) at an excitation wavelength of 785 nm using a notch filter and a liquid nitrogen cooled CCD detector (256 × 1024 pixels). At a laser power of 1.8 mW (corresponding to an intensity of 3 × 10^4 W/cm² when using a 50 × microscope objective), an acquisition time of 10 × 10 s was sufficient to obtain high-quality data from most of the samples. Single crystals obtained from the evaporated solutions mentioned above were placed on conventional microscope slides (1 mm, Menzel, Braunschweig, Germany) for measurements.

The in situ Raman spectra were recorded directly from the levitated droplet (spot diameter 1 mm) and detected via a fiber-optical RXN spectrometer (Kaiser Optical Systems, Ecully, France), equipped with a 785 nm diode laser (irradiance ~10 W/cm² in the experiment) and a thermoelectrically cooled CCD.

Materials. Nifedipine (≥98%) was purchased from Sigma-Aldrich (CAS 21829-25-4) and used without further purification. The X-ray diffraction pattern (Bruker AXS, D5000, Cu Kα radiation) revealed that the sample consisted of the α-modification (P21/c, a = 10.923(5) Å, b = 10.326(6) Å, c = 14.814(7) Å, β = 92.70(6)°, V = 1669.03(494) Å³).

Sample Preparation. Solutions of nifedipine were prepared using different solvents: ethanol (19 mM), methanol (26 mM), acetone (182 mM), dichloromethane (231 mM), and acetonitrile (72 mM). The solutions were stirred vigorously. In all cases, the concentrations were chosen significantly below the saturation point to avoid the presence of any crystalline material in the starting solutions.

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Figure 1. Structure of nifedipine (4-(2-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine).
detector. The Raman scattered light was collected in a 180° backscattering geometry.

The in situ X-ray diffraction experiments were performed at the synchrotron micro focus beamline μSpot (BESSY II of the Helmholtz Centre Berlin for Materials and Energy). Providing a divergence of less than 1 mrad (horizontally and vertically), the focusing scheme of the beamline is designed to provide a beam diameter of 20–100 μm at a photon flux of 1 × 10^9 s⁻¹ at a ring current of 100 mA. The experiments were carried out employing a wavelength of 1.033 58 Å using a double crystal monochromator (Si 111). Scattered intensities were collected 200 mm behind the sample position with a two-dimensional X-ray detector (MarMosaic, CCD 3072 × 3072 pixel with a point spread function width of about 100 μm). A more detailed description of the beamline can be found in ref 25. The obtained scattering images were processed and converted into diagrams of scattered intensities versus scattering vector q (q is defined in terms of the scattering angle θ and the wavelength λ of the radiation; thus q = 4πλ sin θ) employing an algorithm from the computer program FIT2D.26

Results and Discussion

Prior to the in situ measurements, ex situ Raman and single crystal X-ray experiments were carried out with crystals obtained from solutions with different solvents in order to prove the accordance with previously published Raman spectra and XRD patterns, respectively.20,21 In all cases, the XRD pattern obtained from the crystals could be precisely assigned to the theoretical diffraction pattern of α-nifedipine, calculated from the crystal structure (CSD entry: BICCIZ). As was shown by Chan et al.,20 the prominent C−O and C=C stretching modes of the ester carbonyls and the dihydropyridine rings, respectively, can be used as sensitive indicators of intermolecular H-bond formation which differs in the different nifedipine polymorphs. The Raman microspectra obtained ex situ from the crystals at 785 nm excitation are in good agreement with the FT-Raman spectra of α-nifedipine excited at 1064 nm that were published previously20 (compare band positions listed in Table 1 in the Supporting Information). In α-nifedipine, the frequency of the C=O stretching doublet at ~1680 cm⁻¹ results from strong intermolecular hydrogen bonds between ester carbonyls of one molecule with the NH group of another molecule.

Figure 2. Setup of the XRD/Raman experiment used to follow the crystallization of nifedipine in levitated droplets.

Figure 3. Ex situ micro-Raman spectra of α-nifedipine obtained by crystallization from acetonitrile solution.

In a typical in situ experiment a droplet of a half-saturated solution of nifedipine with a volume of about 4 μL was placed into and levitated in the middle wave node of an acoustic levitator. The ambient air temperature was 296 ± 1 K, and the relative humidity RH = 60 ± 5%. The sample remained in a fixed position during the measurement, even after complete evaporation of the solvent. During the experiment, the position of the droplet was determined, as a displacement of the center of gravity, to be smaller than 20 μm. This allowed for XRD and Raman spectroscopy data acquisition in a time span of more than 30 min until no further change in sample volume was measured, indicating the completed evaporation of the solvent.

Figure 4 displays the scattering curves obtained during the evaporation process of nifedipine in methanol, ethanol, acetone, dichloromethane, and acetonitrile. At the beginning of each experiment, the scattering curves exhibit the typical form of amorphous scattering with broad maxima of solutions. While the solvent is constantly evaporating, stable crystallites form as indicated by the presence of Debye rings.

Acetonitrile. Investigating a solution of nifedipine in acetonitrile, the first Bragg reflexes in the diffraction pattern appear after a period of 11 min. The 2D-CDD frames show spotted Debye–Scherrer rings, which are assigned to the α-polymorph, and become nearly closed rings by the end of the measurement. A closure of the rings is interpreted as diffraction contribution of randomly orientated crystallites. Interestingly, the simultaneously recorded Raman spectra indicate clearly that, prior to the crystallization of the α-polymorph, an intermediate stage is formed in the midsection of the experiment (see, e.g., the 11 min spectrum in Figure 5). The spectra at the beginning of the measurement can be assigned to the solvent acetonitrile. These characteristic modes diminish, and the formation of the amorphous form, so-called γ-nifedipine,20 can be observed. After 13 min, a transformation to the thermodynamically stable α-nifedipine proceeds, as evidenced by the appearance of the characteristic red-shifted C=O stretching frequency around 1680 cm⁻¹. Over time, the Raman signals become more pronounced owing to the fact that the widths of the bands decrease due to the proceeding crystallization process. An assignment of some important Raman bands to the different polymorphs can be found in Table 1 (see Supporting Information). In this case the initial transformation can be followed only from Raman spectroscopy, since the lack of pronounced reflections in the XRD pattern can be attributed to the presence of solvent, an amorphous phase, and a mixture of both.

Dichloromethane. In the case of nifedipine solved in dichloromethane, the first Bragg reflexes appear after 70 s. The diffraction


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pattern indicates the formation of the α-polymorph. The absence of preferred orientation in the growth of the nifedipine crystals is attested by the clean and spot-free Debye–Scherrer rings in the 2D-CCD frames. Compared to the findings reported for acetonitrile, the simultaneously recorded Raman spectra (not shown) also reveal the existence of an intermediate stage which can be assigned to the spectra of g-nifedipine (see dashed line in Figure 7). The final product is again identified as R-nifedipine.

Methanol. Starting from a methanolic solution, the first spotted Debye–Scherrer rings were detected after 10 min. Closed rings related to the R-polymorph are monitored at the end of the measurement. The appropriate set of Raman spectra consist of methanol at the beginning of the measurement leading directly to the R-polymorph without the appearance of any intermediate stages.

Ethanol. During the evaporation process of an ethanolic solution, the first Bragg reflexes are detectable after 6 min. The 2D CCD frames show significantly spotted Debye–Scherrer rings. These rings can be attributed not only to R-polymorph but also to a further modification of nifedipine. That second phase diminishes at the end of the experiment so that only the R-polymorph is detectable. The concomitantly recorded Raman spectra reveal the appearance of an intermediate phase during the crystallization process, which can be assigned to the spectra of β-nifedipine. 20 This intermediate phase diminishes as soon as the characteristic modes of the α-polymorph begin to develop.
Acetone. Immediately (10 s) after the start of the experiment, the first Debye–Scherrer rings are detectable in the 2D-CCD frames, appearing fully closed but displaying spots. This phenomenon is in agreement with the existence of randomly distributed crystals coexisting with larger orientated entities. The diffraction patterns show reflexes of the α-polymorph and concomitant reflexes of a second phase. The latter is identical to the one detected already in ethanolic solution and diminishes at the end of the experiment. The less spotted Debye–Scherrer rings indicate the growth of uniformly distributed crystallites. The simultaneously acquired Raman spectra show the appearance of the same second phase (β-NIF) as in ethanolic solution. The final spectra indicate the formation of the stable α-polymorph.

A closer inspection of the diffraction patterns derived from the crystallization from ethanolic solution, which indicate the formation of a second crystalline phase, was carried out. After omitting the peaks of the α-form, attempts were made to index the remaining peaks using the TREOR autoindexing program. They could be assigned to a monoclinic cell with the lattice constants \( a = 9.793 \text{ Å}, b = 13.897 \text{ Å}, c = 14.276 \text{ Å}, \alpha = 119.70^\circ, \beta = 87.89^\circ, \gamma = 81.90^\circ, \) and \( V = 1657 \text{ Å}^3 \). The derived volume of the β-form is slightly smaller than the volume of the unit cell of α-nifedipine \( (V' = 1669 \text{ Å}^3) \). In crystalline solids, molecules typically pack together to maximize intermolecular interactions. Accordingly, the most stable polymorph of a compound lacking strongly directional intermolecular interactions is commonly the one with the highest density. This “density rule” usually holds for crystals the structures of which are dominated by van der Waals interactions, whereas crystals with strong hydrogen bonding usually do not obey this rule. An example for the latter case is the compound Ritonavir. Here, the more stable crystalline structure (form II) has a lower density (1.25 g/cm\(^3\)) than form I (1.28 g/cm\(^3\)). In our case we obtain values of 1.37 g/cm\(^3\) for α-nifedipine and 1.39 g/cm\(^3\) for β-nifedipine.

Figure 6 shows the XRD patterns of the final stages of crystallized nifedipine from solutions of the selected solvents.

Summary and Conclusions

In this study, a novel combined XRD/Raman setup including an acoustic levitator was established and applied to investigate the crystallization process of nifedipine using various solvents. This experimental setup is capable of providing comprehensive and simultaneous information from orthogonal analytical methods in a single experiment, making it a valuable tool for the screening of crystallization processes in situ while avoiding the influences of container walls. The combination proved to be a powerful technique for detecting different stages in the crystallization process. Thereby, it allows deeper insight into subtle events during the growth process of the crystallites in the time course of further solvent evaporation.

The main aspects of the solvent screening can be described as follows: The crystallization process of nifedipine exhibits a strong dependence upon the solvent. As for acetone and ethanol, the β-modification is formed as intermediate. In case of acetonitrile and dichloromethane the glassy form of nifedipine is formed prior to the transformation to the α-modification. These observations obtained through in situ monitoring with XRD and Raman spectroscopy are consistent with Ostwald’s rule of stages. From the XRD data obtained it was possible to assign a unit cell to the β-modification, which possesses a slightly smaller volume compared to the α-modification.

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Supporting Information Available: A table containing the respective wavenumbers and suggested assignments derived from the Raman spectra. This material is available free of charge via the Internet at http://pubs.acs.org.