Published in final edited form as:

CrystEngComm. ; 14(23): 7891–7894. doi:10.1039/C2CE26285A.

Growth of oriented *p*-aminobenzoic acid crystals by directional freezing

Young Gun Ko^a and Peter X. Ma^{a,b,c,d}

Peter X. Ma: mapx@umich.edu

^aDepartment of Biologic and Materials Sciences, University of Michigan, 1011 North University Ave., Ann Arbor, Michigan 48109-1078, USA. Fax: +1 734 647 2110; Tel: +1 734 647 2209

^bDepartment of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109-1078, USA

^cMacromolecular Science and Engineering Center, University of Michigan, Ann Arbor, Michigan 48109-1078, USA

^dDepartment of Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan 48109-1078, USA

Abstract

Oriented long needle-like *p*-aminobenzoic acid (PABA) crystals are successfully prepared by directional freezing of PABA solution in this work. The width of the oriented crystals is controlled by changing the directional cooling rate, resulting in varying crystal morphologies and thermodynamic properties while maintaining the same chemical structure.

Self-assembled monolayers,¹ Langmuir monolayers,² and well ordered single crystals on a plane³ have been utilized as nucleation templates for organized crystal growth in two dimensions (2D). However, it remains a challenge to design and grow oriented crystals in three dimensions (3D). To control the crystal growth in 3D, specific nucleation-seeds are commonly utilized,⁴ which are often limited in availability. Alternatively, a complex equipment such as chemical vapor deposition (CVD)⁵ or magnetron sputtering⁶ is needed for 3D crystal growth. In this article, we present a novel and facile method to fabricate long and aligned needle-like crystals. This technique has the potential to be broadly utilized to produce nano- and micro-needles and wires for a wide variety of applications, including in optical, electronic, optoelectronic, and biomedical devices.

p-Aminobenzoic acid (PABA) (Fig. 1a) is also called vitamin B_x. However, PABA is not essential to human health, and is therefore not officially classified as a vitamin. However, it is an important biological molecule, acting as a bacterial cofactor involved in the synthesis of folic acid.⁷ PABA is widely distributed in nature. For example, ‘bakers-yeast’ contains 5–6 ppm and ‘brewers-yeast’ 10–100 ppm of PABA.⁸ PABA is a compound displaying polymorphs.⁹ Polymorphs are solid phases which have the same chemical composition but differ in crystal structure.¹⁰ They arise from different ways of crystal packing, different molecular arrangements, and/or different molecular conformations on the lattice. Different solid forms of the same chemical compound have different properties such as different crystal shapes, melting points, densities, stabilities, solubility and bioavailability. Moreover, different polymorphs of a drug can have different rates of uptake by the body, leading to a lower or higher biological activity than desired. For example, the shortage of the AIDS drug

Ritonavir is due to the change of the functional polymorph into new, less soluble polymorphs.¹¹ Therefore, in order to use a specific polymorph of a compound, it is necessary not only to produce the polymorph but also to maintain its crystal structure, solubility, stability and so forth. PABA has two polymorphic forms. One is α form, which looks like a needle¹² and the other is β form, which looks like a prism.¹³ The formation of these polymorphic forms are dependent on the solvent used. Killean et al. summarized how solvents determine the morphological habits (acicular, fibrous, spherulitic, prismatic, and bladed morphologies).¹⁴ The length scale of all crystals is in micrometers or millimeters. They grow without a specific orientation from a solution. To date, there has been no report on either long or oriented needle-like (acicular) crystals of PABA.

In this communication, we report a novel and simple method -directional freezing, which enabled the growth of oriented long needle-like (α polymorph) PABA crystals. The width (the side of the nearly square-shaped cross-section) and the length of the 5 generated needle-like PABA were 2.5–11 μm and 1.3 cm, respectively. However, sea urchin-like PABA crystals were formed by slow evaporation of the solvent (dimethyl carbonate). The commercial PABA crystals, sea urchin-like PABA crystals, and the long needle-like crystals were characterized using field emission gun scanning electron microscopy (FEG SEM) [Philips, XL30FEG], attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) [Perkin Elmer, BX FT-IR system], X-ray diffractometer (XRD) [Rigaku, Rotaflex] and differential scanning calorimetry (DSC) [Perkin Elmer, DSC7].

Fig. 1b and c show FEG SEM images of commercial PABA crystals and sea urchin-like PABA crystals. Commercial PABA was purchased from Sigma Chemical Co. The commercial PABA material is in the form of short bundled needles. The average length of the bundle is ca. 150 μm , and the width of the short needle is ca. 5 μm . To fabricate the sea urchin-like PABA crystal, the PABA raw material was dissolved in dimethyl carbonate (DMC, purchased from Aldrich Chemical Co.). The PABA was crystallized by the slow evaporation of the DMC (2.5 wt/v% PABA/DMC solution was used in this study.) for 1 day at 60°C. Thus formed PABA crystals were random in direction. The obtained aggregates of PABA crystals looked like sea urchins. Therefore, they are called sea urchin-like PABA in this study. Its average size was 350 μm , and the width of its needle was ca. 15 μm . The oriented and aligned needle-like PABA crystals were fabricated by pouring a 2.5 wt/v% PABA/DMC solution into a PTFE mold whose top and bottom caps were made of stainless steel (Fig. 1d). The mold was surrounded by insulating styrofoam and placed between two stainless steel rods. The two rods were immersed in a bath of liquid nitrogen to freeze the mold. The bath of liquid nitrogen was placed in a bath of ethanol to reduce the volatilization of the liquid nitrogen. A computer, two proportional–integral–derivative (PID) temperature controllers, and two heating tapes were used to control the temperatures of the top and bottom stainless steel rods. The cooling rate of each rod was controlled individually using the connected heating tape and its PID temperature controller. Various cooling rates (0.25, 0.50 and 1.00°C/min) were used to fabricate different needle-like PABA crystals. The two rods were cooled from 15°C to –20°C with 5°C temperature difference between the top and bottom rods. The temperature difference was utilized to control the thermal transfer (freezing from the bottom to top) of the PABA/DMC solution in the mold. After freezing, the mold was transferred into a freeze-drying vessel to be freeze-dried (in the temperature range of –5°C to –10°C in an ice/salt bath) and under vacuum (pressure lower than 0.5 mmHg) for 1 week. Fig. 1h shows the fabrication process of the oriented and aligned needle-like PABA crystals. The α form PABA crystals were formed from the PABA/DMC solution with their c axis in the direction of the freezing direction. DMC was also frozen (below the freezing point of ca. 2°C). Frozen DMC was removed by sublimation. At the end, oriented needle-like PABA crystals were obtained in the mold. Fig. 1i shows a FEG SEM image of oriented needle-like PABA crystals, which were fabricated at the cooling rate of –0.5°C/

min. The length of the fabricated crystals was ca. 1.3 cm, which is approximately the same as the depth of the PABA/DMC solution in the mold (was ca. 1.5 cm). This data indicates that the needle-like crystal grew from the bottom to the top of the solution. Therefore, a deeper PABA/DMC solution would allow for longer PABA needle formation. All needle-like crystals were oriented along the freezing direction (from the bottom to the top), i.e., the longitudinal direction of the needles is along the c axis of the crystals. The cross-section of the needle-like PABA was a rhombus (slightly off a square shape), which is the shape of the α form PABA crystal structure. This is the first report that the long and oriented PABA crystals are grown by directional freezing.

The width of the needle-like PABA crystals was controlled by varying the cooling rate. Fig. 2 shows that reducing the cooling rate increased the width of the needle-like PABA crystals. The needle-like PABA crystals that were fabricated at the cooling rate of -0.25 °C/min exhibited a width of about 3 times larger than that of the PABA crystals that were fabricated at -1 °C/min. In this set-up, the temperature decrease (the heat transfer)¹⁵ was the driving force for PABA crystal formation. The mold filled with PABA/DMC solution was cooled from its bottom to its top. Therefore, the crystallization of PABA was driven along the length direction. After the formation of PABA nuclei on the bottom, the crystals growth was driven by the temperature difference.

The crystal structure of the α form is based on dimers formed by the association of the carboxylic acid groups.¹⁶ In this study, the sea urchin-like PABA crystals and the needle-like PABA crystals were fabricated with the commercial PABA crystals. The change of hydrogen bonding between carboxylic acid groups and amine groups in PABA could occur due to the potential twinning or disorder¹⁷ in the crystals during the change of PABA morphology. FT-IR is able to detect the changes in hydrogen bonding or dimer structure.¹⁸ In this study, ATR FT-IR (ATR accessory with a KRS-5 crystal) was used, and the spectra of the samples were recorded with 1 cm^{-1} resolution and 16 scanning times. Fig. 3a shows that there is no new peak or peak shift. Accordingly, there should be no change in chemical structure during the change of PABA crystal morphology. To examine the change in crystal morphology, the XRD patterns of PABA crystals were recorded at room temperature using the nickel-filtered Cu K α radiation (wavelength $\lambda = 0.154\text{ nm}$, 40 kV and 100 mA) in the 2θ range from 2 to 50° at a scanning rate of $2^\circ/\text{min}$. As shown in Fig. 3b, whereas there were no changes in the number or the positions of peaks for all samples, there were meaningful differences in the peak intensity of the different PABA samples. To compare the peak intensities between the crystal planes (002) and (202) of all samples (PDF no. 49-2187), the peak intensities of the (202) plane of all samples were normalized to the same value. The (002) peak intensities of the samples were found in the following order: needle-like PABA fabricated at -1 °C/min > needle-like PABA fabricated at -0.5 °C/min > needle-like PABA fabricated at -0.25 °C/min > sea urchin-like PABA > PABA raw material. Interestingly, this order matched the order of the length-ratio value (length of needle / width of needle in the sample). Since direction [002] is along c axis, this result indicates that PABA crystal grows faster in the direction of c axis than in other directions, and the ratios of the growth speeds (c axis over other directions) increase with increasing cooling rates.

To examine the possible changes in thermal behavior associated with the changes in crystal morphology, DSC measurements were performed from 30 to 300 °C for all samples. All measurements were run with a sample mass of ca. $5.4 \sim 6.1\text{ mg}$ in a sealed Al pan. The standard heating rate of 10 °C/min was employed under nitrogen atmosphere. There were small but significant differences in DSC thermograms. All samples showed the typical DSC curves of α form crystals; there was one endothermal peak between 190.5 °C and 192.4 °C as shown in Fig. 4. In the case of the β form, there are other endothermal peak due to the transformation from $\beta \rightarrow \alpha$ form.¹⁹ The melting points and the enthalpies of all PABA

crystals are summarized in Table 1. The raw PABA material, sea urchin-like PABA, needle-like PABA fabricated at the cooling rate of $-0.25\text{ }^{\circ}\text{C}/\text{min}$ showed the same melting point of $192.4\text{ }^{\circ}\text{C}$. For the needle-like PABA crystals fabricated at the cooling rates of -0.5 and $-1\text{ }^{\circ}\text{C}/\text{min}$, a slightly lower melting point of $190.5\text{ }^{\circ}\text{C}$ was obtained. The enthalpies of fusion, ΔH_{fus} , of the PABA crystals were all different. The slow crystallization (sea-urchin-like PABA) resulted in the highest enthalpy of fusion. For the needle-like PABA crystals fabricated at different cooling rates, increasing cooling rate resulted in decreasing ΔH_{fus} (from $-0.25\text{ }^{\circ}\text{C}/\text{min}$ to $-1\text{ }^{\circ}\text{C}/\text{min}$). It appears that the melting point and the enthalpy of fusion of the PABA crystals are related to the width of each PABA crystals. The overall trend is that both the melting point and ΔH_{fus} decreases with decreasing width of the crystals (Table 1). The ΔH_{fus} is more responsive than melting point to the width change. The raw PABA material has a width of $\sim 5\text{ }\mu\text{m}$, falling between those of the needle-like PABA crystals fabricated at cooling rates of $-0.5\text{ }^{\circ}\text{C}/\text{min}$ and $-1.0\text{ }^{\circ}\text{C}/\text{min}$. Correspondingly, the ΔH_{fus} of the raw PABA material falls also between those of the PABA crystals fabricated at cooling rates of $-0.5\text{ }^{\circ}\text{C}/\text{min}$ and $-1.0\text{ }^{\circ}\text{C}/\text{min}$. However, the melting point of raw PABA is not between those of the PABA crystals fabricated at cooling rates of $-0.5\text{ }^{\circ}\text{C}/\text{min}$ and $-1.0\text{ }^{\circ}\text{C}/\text{min}$. This exception could be explained by the fact that the raw PABA crystals were bundled small needle-like PABA, or alternatively due to the lower accuracy or less responsiveness of the melting point to the width in this system. Corroborating with our observation, others reported that the values of the transformation temperature and heat of transformation are very dependent on the crystal size.¹⁹ Similarly, it was reported that the melting point and enthalpy generally increased with increasing cross-sectional area of the nano- and micro-sized GaN wires.²⁰

In summary, a sea urchin-like PABA crystal was fabricated by the slow evaporation of the solvent (DMC). Long and aligned needle-like PABA crystals were successfully fabricated by the directional freezing, resulting in the crystal orientation parallel to the c axis. The length of the fabricated needle-like PABA was 1.3 cm (the depth of the solution) and the widths were in the range of 2.5 to $11\text{ }\mu\text{m}$. The slower cooling rates produced the needle-like PABA crystals with the larger widths. There was no detectable change in hydrogen bonding during the morphology change from the PABA raw crystal to the sea urchin-like PABA crystal or the needle-like PABA crystals. However, the directional freezing produced long and oriented PABA crystals with c axis parallel to the longitudinal direction of the needle-like crystals. Both the melting point and enthalpy of fusion increased with the increasing width (decreasing cooling rate) of the resulted needle-like PABA crystals. The new directional crystallization method provides a simple and convenient way to grow long and aligned crystals with controllable width and therefore adjustable enthalpy of fusion and melting point, which may be potentially used as micro-/nano-wires in electric/optical applications and biomimetic fibers in biomedical applications.

Acknowledgments

The authors gratefully acknowledge the financial support from the NIH (NIDCR DE022327, DE015384 & DE017689) and DOD (W81XWH-12-2-0008).

Notes and references

1. Kim K, Centrone A, Hatton TA, Myerson AS. *CrystEngComm*. 2011; 13:1127–1131. Stephens CJ, Kim YY, Evans SD, Meldrum FC, Christenson HK. *J Am Chem Soc*. 2011; 133:5210–5213. [PubMed: 21425847]
2. Pichon BP, Bomans PHH, Frederik PM, Sommerdijk NAJM. *J Am Chem Soc*. 2008; 130:4034–4040. [PubMed: 18303894] Uysal A, Stripe B, Kim K, Dutta P. *CrystEngComm*. 2010; 12:2025–2028. Popescu DC, Smulders MMJ, Pichon BP, Chebotareva N, Kwak SY, van Asselen OJL,

- Sijbesma RP, DiMasi E, Sommerdijk NAJM. *J Am Chem Soc.* 2007; 129:14058–14067. [PubMed: 17944471]
3. Wang J, Kaur I, Diaconescu B, Tang JM, Miller GP, Pohl K. *ACS Nano.* 2011; 5:1792–1797. [PubMed: 21319850]
4. Zheng Y, Wong ML, Alberts B, Mitchison T. *Nature.* 1995; 378:578–583. [PubMed: 8524390]
5. Shi J, Sun C, Starr MB, Wang X. *Nano Lett.* 2011; 11:624–631. [PubMed: 21261290]
6. Kang SW, Mohanta SK, Kim YY, Cho HK. *Cryst Growth Des.* 2008; 8:1458–1460.
7. Robinson, FA. *The Vitamin Co-factors of Enzyme Systems.* Pergamon; London: 1966.
8. Vasilieva S. *Mutat Res.* 2001; 496:89–95. [PubMed: 11551484]
9. Gracin S, Uusi-Penttilä M, Rasmuson ÅC. *Cryst Growth Des.* 2005; 5:1787–1794.
10. Lian Y. *CrystEngComm.* 2007; 9:847–851.
11. Chemburkar SR, Bauer J, Deming K, Spiwek H, Patel K, Morris J, Henry R, Spanton S, Dziki W, Porter W, Quick J, Bauer P, Donaubauer J, Narayanan BA, Soldani M, Riley D, McFarland K. *Org Process Res Dev.* 2000; 4:413–417.
12. Athimoolam S, Natarajan S. *Acta Cryst.* 2007; C63:o514–o517.
13. Gracin S, Andreas F. *Acta Cryst.* 2005; E61:o1242–o1244.
14. Killeen RCG, Tollin P, Watson DG, Young DW. *Acta Cryst.* 1965; 19:482–483. [PubMed: 5896918]
15. González-Cinca R, Ramírez-Piscina L, Casademunt J, Hernández-Machado A, Tóth-Katona T, Börzsönyi T, Buka Á. *J Cryst Growth.* 1998; 193:712–719.
16. Lai TF, Marsh RE. *Acta Cryst.* 1967; 22:885–893.
17. Dornberger-Schiff K, Dunitz JD. *Acta Cryst.* 1965; 19:471–472.
18. O'Sullivan B, Glennon B. *Org Process Res Dev.* 2005; 9:884–889.
19. Gracin S, Rasmuson ÅC. *Cryst Growth Des.* 2004; 4:1013–1023.
20. Wang Z, Zu X, Gao F, Weber WJ. *J Appl Phys.* 2007; 101:043511.

Fig. 1.

(a) *p*-Aminobenzoic acid, (b) A FEG SEM image of PABA raw materials, and (c) sea urchin-like PABA, (d) Schematic of the apparatus to directionally freeze the PABA solution. The freezing temperature-time profiles (cooling rate) of two cooling rods contacting the top and bottom of the PABA solution chamber; (e) $-0.25\text{ }^{\circ}\text{C/min}$, (f) $-0.5\text{ }^{\circ}\text{C/min}$, and (g) $-1\text{ }^{\circ}\text{C/min}$. (h) Fabrication process of aligned needle-like PABA crystals. (i) FEG SEM image of the oriented needle-like PABA crystals generated by the directional freezing technique.

Fig. 2. FEG SEM images of needle-like PABA crystals. PABA crystals were fabricated at the cooling rate of (a) $-1\text{ }^{\circ}\text{C/min}$, (b) $-0.5\text{ }^{\circ}\text{C/min}$, and (c) $-0.25\text{ }^{\circ}\text{C/min}$. (d) Width of needle-like PABA crystals decreases with increasing cooling rates.

Fig. 3. (a) ATR FT-IR spectra, (b) XRD patterns, and (c) stacked XRD patterns of various PABA crystals. Note: (c) focuses on a 2θ range of 8–14.2° to highlight the peaks of interest.

Fig. 4. DSC thermograms of various PABA crystals (inset: temperature range from 180 °C to 200 °C).

Table 1

Melting temperature, and enthalpy change of PABA crystals

PABA crystals	T _m [°C]	ΔH_{fus} [kJ/mol]	Width [μm]
raw material	192.4	24.49	~ 5
sea urchin	192.4	28.51	~ 15
needle (−0.25 °C/min)	192.4	26.15	~ 9
needle (−0.5 °C/min)	190.5	25.67	~ 6
needle (−1 °C/min)	190.5	23.14	~ 3