Crystal Polymorphs Hot Paper

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Using Magnetic Levitation to Separate Mixtures of Crystal Polymorphs**

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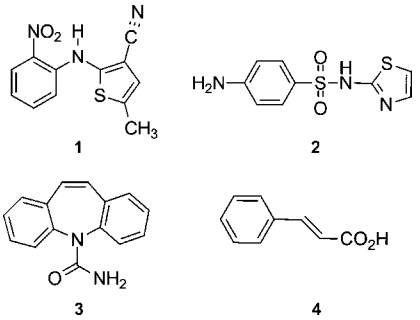
The crystallization of an organic compound can produce a mixture of crystalline solids with different solid-state structures (polymorphs).[1] No convenient method now exists to separate crystals of a single polymorph from a mixture of crystals of different polymorphs. Of the physical properties that might be used to separate polymorphs,[1a] density is attractive for two reasons: 1) Different packings of molecules in a crystal often result in different densities for polymorphs;[1a,2] 2) Separations by density do not destroy the crystals.

Magnetic levitation (MagLev) is a simple system that provides a continuous apparent density gradient in which to measure density and separate objects.[3] MagLev can distinguish small differences in the density ($\Delta\rho = 0.01$–$0.0001\ g\,cm^{-3}$, depending on the type of the experiment) of diamagnetic objects.[4] Although several methods to separate crystals by density exist,[5] MagLev offers four advantages: 1) it separates multiple populations in a single step, 2) it quantifies the density of each population, 3) it is applicable to small crystals (100 μm size), and 4) it provides seed crystals for large-scale crystallization. We used MagLev to separate mixtures of polymorphs of four compounds: 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY) **1**, sulfathiazole **2**, carbamazepine **3**, and *trans*-cinnamic acid **4** (Scheme 1).



Scheme 1. Chemical formulas of compounds analyzed in this study: 1) 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY) **1**, 2) sulfathiazole **2**, 3) carbamazepine **3**, and 4) *trans*-cinnamic acid **4**.

A survey of the Cambridge Structural Database indicates that 3.1–3.5% of the compounds submitted crystallize in different polymorphic forms.[6] A review by Stahly indicated that of 245 small-molecule pharmaceuticals, approximately 90% showed evidence of multiple crystalline and noncrystalline forms, with approximately half of these exhibiting polymorphism.[7] Mixtures of polymorphs are problematic when desired properties depend on a single polymorph.[8] Examples of properties influenced by crystal form include solubility and dissolution rate (which impacts bioavailability in pharmaceuticals),[1b,8] the color of pigments[9] and dyes,[10] and sensitivity towards detonation in explosives.[11]

Methods to obtain single polymorphs from mixtures of polymorphic crystals include selective nucleation,[12] interconversion,[13] isolation based on differences in physical properties,[14] and luck. Success in controlling the kinetics of crystal nucleation and growth in polymorphic systems is limited and largely empirical.[15] Interconversion of polymorphs is restricted to methods that produce thermodynamically stable forms.[8] Separations based on differences in morphology,[16] melting points,[17] or other physical properties can be tedious. Density is, however, a physical property closely linked to molecular packing—the process that forms polymorphs—and provides a means to identify and separate polymorphs.[18] Differences in density between polymorphs may be small ($\Delta\rho \leq 0.01\ g\,cm^{-3}$), and rarely exceed five

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percent.[18] For example, for the compound ROY **1**, the density of form R is 1.438 g cm^{-3}, while that of the form OP is 1.435 g cm^{-3} as calculated from X-ray diffraction (XRD).[19] Methods that separate crystals by density must be able to resolve these small differences.

One of the simplest ways to separate polymorphs by density is the sink–float method.[20] In a fluid with a density between that of two known polymorphs, one type floats while the other sinks. This method can, thus, only separate two crystal forms at once. Steps in density provide multiple bins to separate mixtures,[21] but these systems work best when the density of the objects to be separated is known. A continuous gradient can separate multiple subpopulations with high resolution. The generality of a method based on continuous gradients is helpful when separating polymorphs, because differences in density between crystal forms can be small and precise densities are not known a priori; continuous gradients are, however, often technically demanding to form and to manipulate.

To demonstrate the use of MagLev to separate crystal polymorphs, we used the four compounds in Scheme 1. We selected them for four reasons: 1) Each compound exhibits polymorphism. 2) The density and structure of each crystal form has been characterized by single-crystal XRD, and the densities measured by MagLev can be compared to values calculated from crystal structures. 3) The polymorphs have different shapes, which makes their visual identification straightforward.[22] 4) These crystals have densities in the most convenient working range of MagLev (0.8–2.0 g cm^{-3}).[3a]

We have described the MagLev device and procedures in detail elsewhere.[3a] Briefly, two permanent magnets (NdFeB; 5 cm × 5 cm × 2.5 cm) with like poles facing at a distance of 4.5 cm, generates a linear gradient in the magnetic field with a minimum in the field located at the vertical midpoint between the magnets (d/2; Figure 1). When suspended in a solution containing paramagnetic ions and placed between the magnets, a diamagnetic object will levitate at a height, h (m), where the gravitational (F_g) and magnetic forces (F_m) acting on it are balanced [Eq. (1)].[3a]

$$h = \frac{(\rho_s - \rho_m) g \mu_0 d^2}{(\chi_s - \chi_m) 4 B_0^2} + \frac{d}{2} \quad (1)$$

In this equation, ρ_m (kg m^{-3}) is the density of the paramagnetic medium, ρ_s (kg m^{-3}) is the density of the levitating sample, g is the acceleration due to gravity (m s^{-2}), χ_m and χ_s (both unitless) are the magnetic susceptibilities of the paramagnetic medium and the suspended sample, respectively, μ_0 (T m A^{-1}) is the magnetic permeability of free space, and B_0 (T) is the magnetic field strength at the surface of the magnet.

We crystallized each compound as a mixture of polymorphs (see Supporting Information for details). Approximately 0.5 mg of the mixture of crystals was placed into a cuvette filled with an aqueous solution of manganese(II) chloride ($MnCl_2$), and the container was placed between the magnets. The densities and magnetic susceptibilities of the paramagnetic solutions were adjusted empirically (concentrations were in the range 0.7–3.0 M) to achieve levitation, and to allow separation of the crystals by density (see Supporting Information for details). To minimize the adhesion of air bubbles to the surface of the crystals, we added 1 % Tween 20 to the paramagnetic solution, introduced the crystals, and degassed and sonicated the mixture. Polymorphs required seconds to minutes to reach their equilibrium levitation heights, h, once placed in the MagLev device (Figure 2).

The average density of the crystals calculated using MagLev (n = 7) correlate well with that obtained using

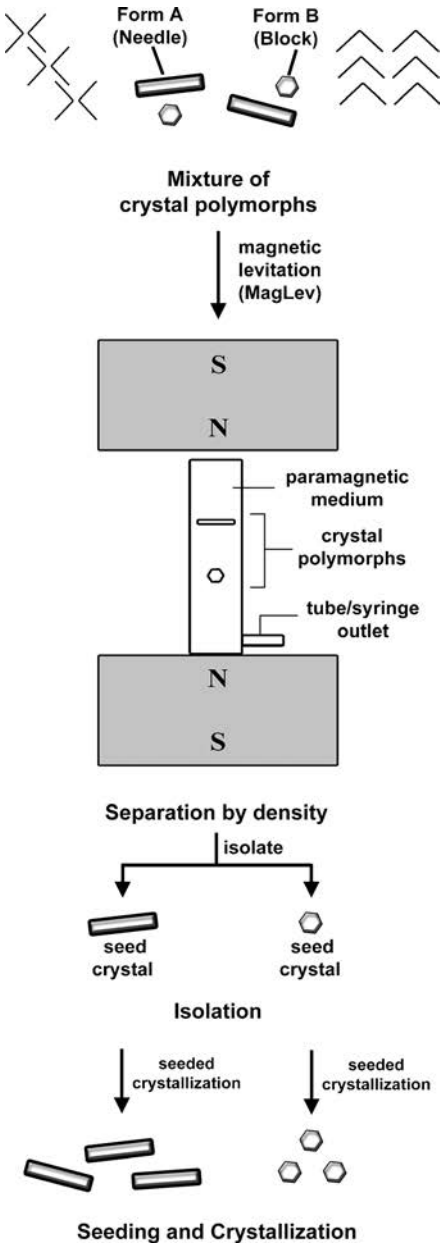

Figure 1. Separation, isolation, and seeding of crystal forms using MagLev, for a polymorphic system that crystallizes as Form A (needles) and Form B (blocks). The compound adopts different structural orientations in Form A and B. MagLev enables separation and isolation of these two crystal forms by their difference in density and enables the subsequent use of these forms to seed crystallizations of single polymorphs.

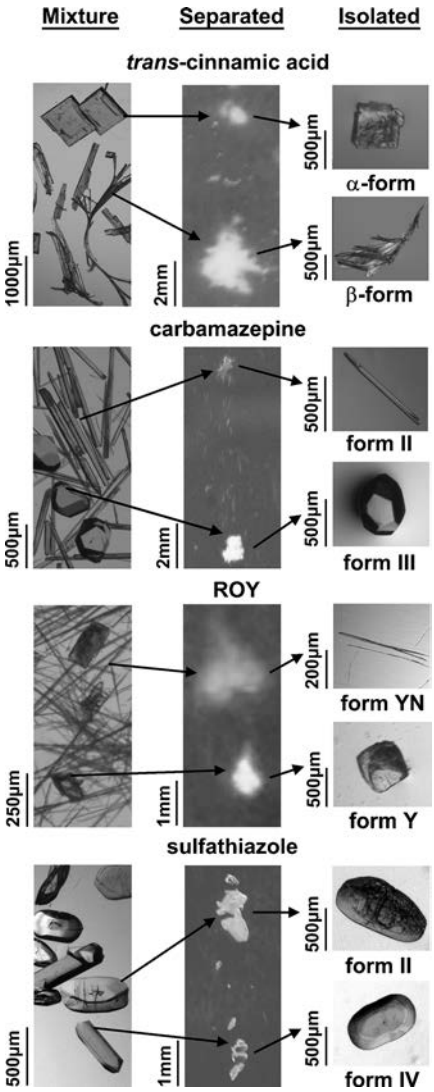

Figure 2. Photographs showing mixtures of crystal polymorphs (left), their separation using MagLev levitating at their respective equilibrium heights (middle), and their isolated forms (right). A mixture of α (top; $\rho = 1.251 \pm 0.006\ g\,cm^{-3}$) and β (bottom; $\rho = 1.268 \pm 0.005\ g\,cm^{-3}$) polymorphs of *trans*-cinnamic acid **4** are separated in MagLev using an aqueous solution of 0.7 M $MnCl_2$, 0.9 M $ZnCl_2$, 0.8 mL glycerine, 1% Tween 20. A mixture of form YN (top; $\rho = 1.419 \pm 0.010\ g\,cm^{-3}$), form Y (bottom; $\rho = 1.450 \pm 0.010\ g\,cm^{-3}$) of ROY **1** is separated in MagLev using an aqueous solution of 3.0 M $MnCl_2$, 0.5 M $ZnCl_2$, 1% Tween 20. A mixture of form II (top; $\rho = 1.541 \pm 0.001\ g\,cm^{-3}$) and form IV (bottom; $\rho = 1.580 \pm 0.001\ g\,cm^{-3}$) of sulfathiazole **2** is separated in MagLev using an aqueous solution of 0.965 M $MnCl_2$, 4.19 M $ZnCl_2$, 1% Tween 20. A mixture of form II (top; $\rho = 1.271 \pm 0.004\ g\,cm^{-3}$) and form III (bottom; $\rho = 1.320 \pm 0.004\ g\,cm^{-3}$) of carbamazepine **3** is separated in MagLev using an aqueous solution of 2 M $MnCl_2$, 1 M $ZnCl_2$, 1% Tween 20.

XRD (Figure 3) with an R^2 value of 0.993. For example, sulfathiazole **2** gives these densities ($g\,cm^{-3}$): form II, 1.541 ± 0.001 (MagLev) and 1.546[23] (XRD); form IV, 1.580 ± 0.001 (MagLev) and 1.600[23] (XRD). The difference in density between the crystal forms determined by MagLev and XRD are $\Delta\rho = 0.005\ g\,cm^{-3}$ for form II and $\Delta\rho = 0.02\ g\,cm^{-3}$ for form IV.

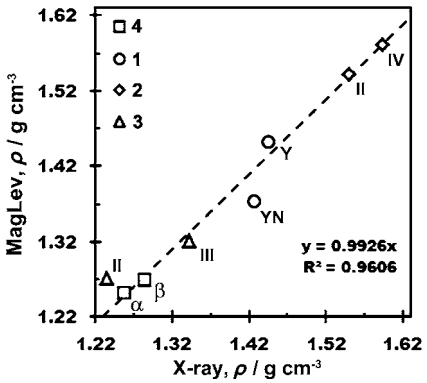

Figure 3. Comparison of the densities of crystal polymorphs measured using MagLev ($n = 7$) and estimated from XRD. The error bars are smaller than the data points and are not shown for clarity.

After separation of the polymorphs by density using MagLev, we isolated samples of each form to use as seeds for selective nucleation. We used two syringes—each coupled to the container by tubing—to withdraw crystals levitating at specific locations (see Supporting Information for details). The crystal structure of each isolate was confirmed using XRD. The unit cell parameters were collected for seven crystals from each isolation container and compared with unit cell parameters from seven random crystals collected before being placed in the paramagnetic media. No difference in crystal form was observed among parameters of the unit cells of the crystals before and after separation. Given that the isolated crystals maintained their form, we demonstrated their utility as seeds for further crystallization using form Y of ROY **1** and form II of sulfathiazole **2** (see Supporting Information for details).

MagLev is the first convenient technique capable of separating crystal polymorphs. This capability is useful in isolating seed crystals for crystallization,[24] identifying the presence of multiple crystalline forms,[25] and separating mixtures of crystal forms when crystal morphologies and/or shapes are visually indistinguishable. This technique is also applicable for separations of other crystal types that include pseudopolymorphs (e.g. solvates) and chiral systems (see Supporting Information). We expect this approach to be useful for separations of minerals and other crystalline materials (e.g. cocrystals).[26]

MagLev has two useful characteristics for separating mixtures of crystal polymorphs: 1) The separation of crystals (with dimensions of approximately $250 \times 30 \times 30$ μm for the smallest needles; approximately $50 \times 50 \times 50$ μm for the smallest irregular-shaped crystals, prisms, trapezoids) is automated and rapid. Upon introduction into the MagLev device, suspended crystals equilibrate to their equilibrium positions in seconds to minutes. 2) It enables simultaneous identification, separation, and isolation of polymorphs by density. The densities obtained can conveniently be compared with densities estimated by XRD.

MagLev also has limitations as a method for separating crystal polymorphs by density: 1) There is no single best levitation medium for all crystals. Each crystal system may require a different paramagnetic medium (in terms of the

concentrations of paramagnetic and diamagnetic ions). 2) The crystal may be soluble in a particular paramagnetic medium. The rate of dissolution of the crystal in the medium is dependent upon the solubility and surface area of the crystal. The potential for dissolution requires that the crystals separate within the timescale of the experiment. In the experiments summarized in Figure 2, the crystals (by qualitative observation) seemed stable in suspension for periods ranging from a minimum of one hour (forms II and IV of **2**) to three days (form Y of **1**). In addition, there was no apparent color change in the solution and/or of the crystals that suggested coordination of the organic compound with manganese ions. 3) Crystals smaller than about 5 μm in diameter remain dispersed as a result of Brownian motion, and do not localize at a well-defined equilibrium levitation height.

MagLev can make separating and isolating polymorphs for seeding more straightforward than existing methods, even in situations when the desired crystals cannot be separated manually and where the presence of multiple polymorphs may not be obvious.

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