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Influence of Chemical Functionality on the Rate of Polymer-Induced Heteronucleation

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Abstract

Polymer-induced heteronucleation can dramatically increase the nucleation rate of pharmaceuticals. However, directly comparing the heteronucleation rates of different polymer functionalities is often convoluted with changing physical or structural aspects of heteronuclei. Here, we report a methodology for comparing nucleation efficiencies of different functionalities on polymer heteronuclei of uniform topology with the goal of identifying those functionalities that best accelerate nucleation of a model pharmaceutical. It was found that the previously employed design for additives to speed acetaminophen crystallization underperforms a modified framework that accounts for the effect of competitive solvent binding. These findings are informed by a survey of interactions from the CSD and not only serve to aid in the controlled crystallization of pharmaceuticals, but also provide insight into the mechanism of heteronucleation.

Graphical Abstract:

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Author Contributions

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](https://pubs.acs.org/doi/10.1021/acs.cgd.7b00593) at DOI: 10.1021/acs.cgd.7b00593.

Experimental methods, Raman spectra of functionalized Merrifield Resin, full listing and discussion of measured crystallization induction times, and CSD search parameters used in calculating average intermolecular bond lengths (PDF)

Notes

The authors declare no competing financial interest.

Crystallization from a supersaturated solution is delayed by significant energetic barrier associated with nucleation of the crystalline state.¹ The addition of a foreign surface, or heteronucleant, speeds nucleation by lowering the surface energy of molecular aggregates in solution.² Polymer-induced heteronucleation (PIHn) is a technique where polymer libraries containing different functionalities in an insoluble resin are added to a solution to induce the formation of crystal polymorphs^{3–6} and multicomponent crystals,⁷ and to tune the rate of crystal nucleation.^{8,9} Polymers as heteronucleants are advantageous due to their stability in a range of solvents and ease of functionalization. Traditionally, to increase the rate of crystallization, polymer heteronuclei are designed to contain the same functionalities^{10,11} as those present on a target molecule for crystallization. Through homomeric interaction, target molecules in solution are believed to form high density aggregates on the surface of heteronuclei and undergo nucleation.^{12–14} Although this method has shown success, the polymerization procedures implemented do not result in heteronuclei with uniform surface area or morphology—as a result, it can be difficult to compare crystallization rates induced by chemically different heteronuclei, and therefore robust structure–property relationships are lacking. Herein we report a robust functionalization method applicable to cross-linked resins to produce uniform heteronucleants with varied functional groups.^{15,16} With such resins, the role of polymer functionality in polymer-induced heteronucleation is probed in the absence of microstructural and topological differences.

Crystallization of acetaminophen from water was investigated because it represents an often employed model system in the crystallization of pharmaceuticals.^{17–21} Heteronuclei used in this study were synthesized via functionalization of Merrifield a significant energetic barrier associated with nucleation Resin (see Scheme 1).²² Merrifield Resin is a cross-linked poly(chloromethylstyrene) resin often used in solid-phase peptide synthesis.²³ Chloromethyl groups on the insoluble polymer can be substituted with functionalized phenols to produce a range of chemistries tethered to the unchanged microstructure of an insoluble network polymer. Successful coupling was verified by Raman spectroscopy, where vibrational bands associated with chloromethyl groups on Merrifield Resin (in particular, a stretch at 676 cm^{–1}) were no longer present.²⁴ Furthermore, peaks corresponding to introduced functionalities appear in the Raman spectra of functionalized resins (see Supporting Information, Figure S1).

Crystallizations were performed in the Technobis Crystal-Breeder. Aqueous acetaminophen (0.28 mL, 136 mM) was pipetted into 0.3 mL vials containing a 5 × 2 mm Teflon stir bar and ~0.5 mg polymer heteronuclei. These vials were hermetically sealed and, to ensure dissolution, stirred (1200 rpm) at 60 °C for 30 min in the CrystalBreeder. Temperature was then lowered to 30 °C to achieve a supersaturation of 1.19.²⁵ Crystallizations are monitored by measuring the transmissivity of an LED through of each vial. Upon nucleation, crystallites of pharmaceutical quickly lead to secondary nucleation and crystallization of the bulk sample, resulting in an identifiable drop in transmissivity. This time between supersaturation and a nucleation event is recorded as the induction time. After measuring transmissivity for 300 min to allow for nucleation to occur, acetaminophen was dissolved back into solution by reheating the vials to 60 °C for 30 min. This dissolution/crystallization cycle was repeated five times with four vials containing the same polymer to give 20 induction time measurements for each sample set. Additionally, the entire process was

repeated three times per resin to give a total of 60 induction time values for each chemically distinct heteronucleant. Vials that did not yield any crystallization over the five cycles were removed from statistical analysis (see Supporting Information for full induction time data and discussion).

To quantify the heteronucleation effects of functionalized resins, a probability distribution of induction times was graphed for each resin (Figure 1). This plot allows for a direct comparison of how different functionalities act as heteronucleants for acetaminophen crystallization. Data in black represent acetaminophen crystallization without added polymer and colored traces correspond to crystallization in the presence of the polymer heteronuclei depicted below the graph in the same color. The right-hand inset shows the probability of crystallization occurring in the first 25 min. A potent heteronucleant is characterized by its ability to reduce the induction time to crystallization. As expected, adding insoluble polymer speeds nucleation rates by heteronuclear seeding. Resins without hydrogen bonding functionalities (PH-MFR and MFR) less quickly nucleate acetaminophen than those that can participate in hydrogen bonding (ACM-MFR, AN-MFR, AP-MFR, MACM-MFR) with the pharmaceutical. Potent heteronuclei display much steeper slopes early in the crystallization process—meaning many vials are nucleating quickly—and this is reflected in a far lower median induction time (Figure 2).

Two of the primary factors that dictate the rate of polymer-induced heteronucleation are intermolecular pharmaceutical/ heteronuclei interaction strength and solvent effects. In previous work, insoluble polymers to accelerate nucleation were designed to optimize intermolecular interaction by mimicking drug functionality on heteronuclei.⁹ One method to estimate the interaction energy between two functionalities is by surveying crystalline materials in the Cambridge Structural Database (CSD). The average distance between two functionalities approximates their strength of interaction, where a shorter average bond length indicates a stronger intermolecular interaction between functionalities and longer bonds indicate a weaker interaction. To estimate the affinity of acetaminophen to a given heteronucleant, the average bond length between a phenolic hydroxy group, an analogue for acetaminophen, and hydrogen bond accepting functionalities was calculated (see Supporting Information for motivation to use this particular interaction). Figure 3 shows the average bond length between a phenolic hydroxy group and heteronuclei functionalities as found in the CSD. If one only considers the length of the hydroxy/functionality interaction, it is true that mimicking acetaminophen functionality on a resin produces heteronuclei with short intermolecular bonds and presumably strong binding to the pharmaceutical. ACM-MFR and MACM-MFR—resins mimicking acetaminophen—contain the shortest average intermolecular interactions to the phenolic hydroxy group. These resins outperform AP-MFR to accelerate nucleation, which by contrast has the longest average intermolecular bond length to the phenol. If we allow these bond lengths to serve as a gauge for intermolecular interaction energies, it follows that strong binding of acetaminophen to a heteronucleus results in fast heteronucleation. However, for a pharmaceutical to nucleate, it must outcompete water to bind and aggregate on a polymer. This second parameter, competitive binding over solvent, plays an unexplored role in dictating the ability of an insoluble polymer to impact heteronucleation rates. The affinity of water to bind to a functionalized resin can be approximated by the average intermolecular bond distance

between a water molecule and the resin functionality; Figure 3 shows the average bond distance between water molecules and the resin functionalities as found in crystalline hydrates. The extent of water solvation to each resin helps to explain the observed ranking of functionalities to accelerate nucleation. Although ACM-MFR contains short intermolecular interactions with phenolic hydroxy groups, it also favorably binds to water, as exemplified by a short water/acetamide bond length ($3.06 \pm 0.02 \text{ \AA}$). The aniline on AN-MFR shows comparatively longer hydrogen bonds to phenolic hydroxy groups, yet very long intermolecular bonds with water ($3.21 \pm 0.001 \text{ \AA}$). Considering these values, we propose that acetaminophen outcompetes water to interact with the AN-MFR polymer and undergo rapid nucleation whereas ACM-MFR is slower to heteronucleate due to competitive binding by water molecules. In the case of the methylated MACM-MFR resin, it would seem that the polymer does not substantially bind to acetaminophen over water given the bond length values in Figure 3. We might expect the resin to have roughly the same heteronucleation ability as ACMMFR, contradictory to experimentally measured induction times. However, hydrophobicity plays a crucial role, as steric hindrance prevents water from interacting with the nitrogen atom in the tertiary acetamide of MACM-MFR ($N_{\text{acetamide}}\text{---}O_{\text{water}}$ bond length of $3.63 \pm 0.05 \text{ \AA}$) compared to the secondary acetamide in ACM-MFR ($N_{\text{acetamide}}\text{---}O_{\text{water}}$ bond length of $3.17 \pm 0.02 \text{ \AA}$). We hypothesize that methylation of MACM-MFR decreases intermolecular bond strength between the polymer and water molecules. Without water strongly bound to the acetamide functionality, acetaminophen would be comparatively unhindered to donate into a hydrogen bond with MACM-MFR over ACM-MFR. This model suggests that, rather than a change in intermolecular pharmaceutical/ heteronuclei interaction, the increased hydrophobicity of MACM-MFR results in faster heteronucleation compared to ACM-MFR, as evidenced by its lower average induction time. The improvement in heteronucleation rate by MACM-MFR over ACM-MFR highlights the subtle yet potent effects of solvent on the interplay between dissolved acetaminophen and a heteronucleus. Heteronucleants designed to mimic a target pharmaceutical to crystallize (a homomeric interaction) run the risk of solvation competing with heteronucleation. By imparting hydrophobicity to hydrogen bond acceptors, a heteromeric interaction between acetaminophen and polymer surface has been shown to outperform the previously employed homomeric design for potent heteronuclei.

A versatile methodology has been employed for the uniform synthesis of polymer heteronuclei to compare the ability of various functionalities to reduce induction times for crystallization. A comparison of functionalities acting as heteronuclei, combined with statistical data extracted from the CSD, elucidates the role of solvent effects in the design of potent nucleation accelerators. These updated design requirements for potent heteronuclei can be applied to the accelerated nucleation of large, lipophilic molecules generally slow to nucleate. Further work will investigate the heteronucleation of difficult-to-crystallize pharmaceuticals with the vision that this heteronucleation technology will aid in the structural determination and processing of molecules resistant to crystallization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Induction time data of polymer induced heteronucleation of acetaminophen crystallization in water (136 mM at 30 °C). The plot in black represents acetaminophen crystallization without added heteronucleant and colored lines depict crystallization in the presence of the functionalized resins (color coded and shown underneath). The inset in the bottom right shows probability of crystallization in the initial 25 min.

Figure 2. Statistical analysis of induction time data for polymerinduced heteronucleation. The median induction time is shown on the left in a lighter color, and the time at which 50% vials have undergone crystallization is shown in a darker color on the right.

	AP-MFR	ACM-MFR	AN-MFR	MACM-MFR
Average Induction Time (minutes)	14 ± 3.7	8.0 ± 1.9	3.7 ± 0.3	3.1 ± 0.3
Average bond distance to phenol (Å)	3.32 ± 0.01	3.09 ± 0.03	3.13 ± 0.02	3.05 ± 0.04
Average bond distance to water (Å)	3.09 ± 0.02	3.06 ± 0.02	3.21 ± 0.001	3.04 ± 0.03

Figure 3.

Average induction time is presented with the standard error of measurement for the four functionalized polymers which induced crystallization in all vials before 300 min. Below are the average intermolecular bond distances between a phenolic hydroxy functionality and hydrogen bond accepting functionalities (hydroxy to carbonyl oxygen atom for AP-MFR, ACM-MFR, and MACM-MFR; hydroxy to aniline nitrogen for AN-MFR) as averaged over materials in the Cambridge Structural Database (CSD) containing intermolecular bonds between the relevant functionalities (see Supporting Information for additional details). Also listed is the average bond length to a water molecule from these functionalities as found in crystalline hydrates.

Scheme 1. Coupling between Merrifield Resin and Phenols to Synthesize Heteronuclei^a

^aThe R group indicates the position of functional group variation that was controlled to build the structure–property relationships in this study.