High–throughput Excipient Discovery Enables Oral Delivery of Poorly Soluble Pharmaceuticals

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# Table of Contents

1. **Materials and Methods**
   1.1 Materials
   1.2 Manual Synthesis
   1.3 Automated Synthesis: Semi–continuous Parallel Polymerization Reactor (ScPPR)
   1.4 Precipitation Inhibition Screening: EVO 200® Liquid Handler
   1.5 Reverse–phase High-performance Liquid Chromatography (HPLC)
   1.6 Size–Exclusion Chromatography with Multi-angle Light Scattering (SEC-MALS)
   1.7 Proton Nuclear Magnetic Resonance (¹H NMR)
   1.8 Two-dimensional Nuclear Overhauser Effect Spectroscopy (2D NOESY)
   1.9 Diffusion Ordered Spectroscopy (DOSY)
   1.10 Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC)
   1.11 UV–Vis Spectroscopy (UV-vis)
   1.12 Temperature Scanning Turbidimetry (TST)
   1.13 Spray Drying
   1.14 Scanning Electron Microscopy (SEM)
   1.15 Powder X–ray Diffraction (PXRD)
   1.16 In vitro Dissolution Testing
   1.17 In vivo Animal Testing
   1.18 Statistical Analysis
   2. **Experimental**
      2.1 **Experiment 1**: Manual and high–throughput RAFT polymerization details.
      2.2 **Experiment 2**: Manual and high–throughput cloud point measurements.
      2.3 **Experiment 3**: Supplemental precipitation inhibition screening.
      2.4 **Experiment 4**: Supplemental 2D NOESY and DOSY NMR experiments.
      2.5 **Experiment 5**: Supplemental solid dispersion characterization.
      2.6 **Experiment 6**: In vitro dissolution testing – NIPAm/DMA composition effects
      2.7 **Experiment 7**: In vitro dissolution testing – AUC₃₆₀₉₈ Enhancement summary.
      2.8 **Experiment 8**: In vivo PK and AUC statistical analysis.
      2.9 **Experiment 9**: In vivo AUC Enhancement summary
      2.10 **Experiment 10**: In vivo ALT/AST enzyme toxicity assay
   3. **Author Roles and Responsibilities**
   4. **References**
1 Materials and Methods

1.1 Materials. All chemicals were used as received (reagent grade) unless otherwise noted; all solvents utilized were HPLC or analytical grade: N-isopropylacrylamide (NIPAm, Aldrich, >99%), N,N-dimethylacrylamide (DMA, Aldrich, 99%), N,N-diethylacrylamide (DEA, Aldrich, 99%), acrylamide (Am, Aldrich, ≥98%), 2-hydroxyethyl-methacrylate (HEMA, Aldrich, ≥99%), isopropyl methacrylate (IPMA, TCI, >98%) 2,2′-azobis(2-methylpropionitrile) (AIBN, 98%), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA, Aldrich, 97%), anisole (Aldrich, ReagentPlus®, 99%), chloroform-d (Aldrich, 99.8 atom % D), methanol-d₄ (MeOD, Aldrich, 99.96 atom % D), dimethyl sulfoxide-d₆ (DMSO-d₆, 99.9 atom % D), trimethylsilyl propanoic acid (TMSP, Aldrich, 98 atom % D), acetone (Aldrich, ≥99.5%), methanol (Aldrich, 99.8%), tetrahydrofuran (Aldrich, anhydrous, ≥99.9%), 1,4-dioxane (Aldrich, ≥99.0%), diethyl ether (Aldrich, ≥99.0%), phenytoin (Aldrich, ≥99%), and nilutamide (Aldrich). DMA, HEMA, and DEA were passed through basic alumina columns to remove trace inhibitors.

HPMCAS (AFFINISOL™ 912G) was supplied by The Dow Chemical Company and used as received. Phosphate buffered saline (PBS, adjusted to pH 6.5 with NaOH) solution was prepared in lab consisting of sodium chloride (82 mM, Fisher, ≥99.0%), sodium phosphate dibasic heptahydrate (20 mM, Fisher, 98%), and potassium phosphate monobasic (47 mM, J.T. Baker, ≥99.0%). Fasted simulated intestinal fluid powder (FaSSIF, with 3 mM sodium taurocholate, 0.2 mM lecithin, 34.8 mM sodium hydroxide, 68.62 mM sodium chloride, and 19.12 mM maleic acid) was purchased from Biorelevant (Surrey, UK).

1.2 Manual Synthesis. All reversible addition-fragmentation chain transfer (RAFT) polymerizations were performed under nitrogen in oven-dried glassware. To a 25 mL round bottom flask, the reagents (monomers, RAFT chain transfer agent, initiator, and solvent) were
combined and allowed to fully dissolve. The solution was then sealed, degassed by bubbling nitrogen for at least 30 min at room temperature, and transferred to a thermostated oil bath at 70 °C under constant stirring. The monomer conversion was monitored through \(^1\)H NMR. These polymers were not used further – see Section 1.3 for the work-up procedure.

1.3 Automated Synthesis: Semi-continuous Parallel Pressure Reactor (ScPPR). All RAFT polymerizations were performed in a Freeslate ScPPR (Sunnyvale, CA) with Library Studio (vers. 8.4) software to specify operating parameters and Automation Studio (vers. 8.4) software to operate the ScPPR. To an array of 24 10 mL test tubes, designated amounts of monomers, initiator AIBN, chain-transfer agent CDTPA, and internal standard anisole (all pre-dissolved in solvent 1,4-dioxane or 1,4-dioxane/DMF that was bubbled for 20 min with nitrogen) were introduced. The molecular weight of RAFT-mediated polymers were targeted using Equation S-1:

\[
M_n = \left( \frac{[M]_0}{[CTA]_0} \right)^x M_0 + M_{CTA}
\]

[S-1]

Here, \([M]_0\) is the initial monomer concentration, \(x\) is the total monomer conversion, \([CTA]_0\) is the initial CTA concentration, \(M_0\) is the monomer molecular weight, and \(M_{CTA}\) is the CTA molecular weight. We aimed to prepare two molecular weight series, denoted as \(M_n\), low (20 kg/mol) and \(M_n\), high (60 kg/mol).

The reactors were pressurized/depressurized with nitrogen 3 times up to 40 psi to remove oxygen prior to each run. After, the reaction flask was heated to and maintained at 70 °C in a thermostated oil bath for 7 h. Subsequently, the reaction mixture was cooled to 0 °C and the flasks in the ScPPR were opened to air.
The crude mixture was diluted with 10 mL solvent (DMF/water mixture (7:3, v/v) for poly(NIPAm-co-Am) or 1,4-dioxane for all other systems) and isolated by precipitation into 300 mL of THF for poly(NIPAm-co-Am), diethyl ether for poly(NIPAm-co-DMA)/poly(NIPAm-co-HEMA), hexane for poly(DEA-co-DMA), and heptane for poly(IPMA-co-DMA). The obtained solid white product was re-dissolved in 5 mL of methanol for poly(NIPAm-co-Am) and THF for poly(NIPAm-co-DMA)/poly(NIPAm-co-HEMA)/poly(DEA-co-DMA)/poly(IPMA-co-DMA), precipitated as before, and filtered under vacuum as set forth above a second time. The resultant product was dried for at least 12 h under vacuum at ~10 mTorr to yield the purified polymer.

1.4 Precipitation Inhibition Screening: EVO® 200 Liquid Handler. Supersaturation testing was performed using high–throughput instrumentation to assess the ability of polymers to inhibit precipitation or crystallization of drugs from supersaturated solutions. 912 µL of PBS containing 0.5 wt % FaSSIF and purified polymers (1 wt % polymer in solution) were robotically delivered into individual 1 mL vials arranged on an aluminum 96 (8 × 12) well array using the Freedom EVO® 200 (Tecan, Switzerland) platform and Evoware 2, Library Studio (vers. 8.4), and Automation Studio (vers. 8.3) software. Samples were set in an isothermal aluminum sample holder at 37 °C.

To begin the experiment, 48 µL of drug solution (20 g/L of drug in methanol) was dispersed into the reaction vials such that the total drug concentration as the dosage was 1000 µg/mL. Drug solutions were delivered such that each respective experimental combination of drug and polymer was run in quadruplicate. The mixtures were agitated via three cycles of aspiration and dispensing using the EVO 200 pipettes. At each time point (30, 90, and 180 min), samples were centrifuged at 3400 g for 4 min, and a 30 µL aliquot was removed and diluted with 150 µL of methanol. The samples were again held at 37 °C until the next time point.
Poly(NIPAm-co-HEMA) and poly(IPMA-co-DMA) were insoluble under ambient conditions and were conducted manually under the same procedure after overnight stirring at 30 °C. The drug concentration in each aliquot was determined by reverse phase high-performance liquid chromatography (HPLC) as described below.

1.5 Reverse Phase High-performance Liquid Chromatography (HPLC). Drug concentrations were measured by HPLC for supersaturation and dissolution testing experiments. For supersaturation testing, 2 µL of the respective diluted taken aliquot was injected into an Agilent 1100 HPLC system equipped with a reversed-phase XDB-C8 column (Eclipse, 4.6 × 150 mm, 5.0 µm from Agilent, USA). The mobile phase (2.0 mL/min at 30 °C) consisted of a mixture of acetonitrile/water for phenytoin (60:40, %, v/v) and nilutamide (50:50, %, v/v). The column effluent was analyzed with a diode array detector (1100 DAD from Agilent, USA) at a wavelength of 220 nm. The drug concentrations were determined from the measured elution profile by using a linear calibration curve for the respective drug, which was generated by determining the least squares fit of a straight line that described the relation between the concentration of four solutions of known concentrations (250, 500, 750, and 1000 µg/mL with R² > 0.99) and the respective peak area integral after injection of 2 µL of each drug in methanol. The measured drug concentrations are reported as the mean value of quadruplicate runs.

For dissolution testing, 10 µL of the respective diluted taken aliquot was injected into the HPLC containing a reversed-phase EC-C18 column (Poroshell 120, 4.6 x 50 mm, 2.7 µm from Agilent, USA). The mobile phase (1.0 mL/min at 30 °C) consisted of a mixture of acetonitrile/water for phenytoin (60:40, %, v/v); a mobile phase of acetonitrile/water/methanol was used for nilutamide (18:55:27, %, v/v/v). The column effluent was analyzed with a UV detector (1260 Infinity Multiple Wavelength Detector from Agilent, USA) at a wavelength of
225 nm for phenytoin and 260 nm for nilutamide. The drug concentrations were determined from the measured elution profile by using by using a calibration curves for the respective drug, which was generated by determining the least squares fit of a straight line that described the relation between the concentration of known concentrations (12.5, 31.2, 62.5, 125, 250, and 500 µg/mL for phenytoin, and 25, 50, 100, 500, and 1000 µg/mL for nilutamide with R^2 > 0.99) and the respective peak area integral after injection of 10 µL of each drug in methanol. In the dissolution plots, the area under the dissolution curve (AUC_{360 min}) from 0 to 360 min was calculated with the trapezoidal rule.

1.6 Size–Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS). Polymer absolute molecular weight (M_n) and dispersity (Đ) were characterized using SEC instruments. The dn/dc values for each of the polymers were determined offline with the Optilab rEX refractometer and were used to calculate the absolute M_n and Đ using ASTRA® 6 software.

SEC-MALS experiments for poly(NIPAm-co-DMA), poly(NIPAm-co-HEMA), poly(DEA-co-DMA), and poly(IPMA-co-DMA) were conducted on an Agilent 1260 Infinity high performance liquid chromatography system equipped with one Waters Styragel guard column and three Waters Styragel columns (HR6, HR4, and HR1) with pore sizes suitable for materials with effective molecular weights from 100 to 10,000,000 g/mol; the instrument used an Agilent 1260 Infinity Variable Wavelength Detector set at a monitoring wavelength of 254 nm (80 Hz data collection frequency), a Wyatt Dawn Heleos II multiangle light scattering (MALS) detector operated at a laser wavelength of 663.6 nm (18 angles from 10° to 160°) and a Wyatt Optilab T-rEX refractive index detector operated at a wavelength of 658 nm. About 4-8 mg of these polymers were dissolved in 1 mL THF and filtered through a 0.2 µm membrane filter,
where the solution was then introduced to the chromatography system and eluted with THF at a flow rate of 1.0 mL/min at 25 °C. The run time for the method was 45 min.

SEC-MALS experiments for poly(NIPAm-co-Am) were conducted on an Agilent 1260 high performance liquid chromatography system equipped with Eprogen columns (CATSEC1000 (7 µm, 50 × 4.6 mm), CATSEC100 (5 µm, 250 × 4.6 mm), CATSEC300 (5 µm, 250 × 4.6 mm) and CATSEC1000 (7 µm, 250 × 4.6 mm)) with pore sizes suitable for materials with effective molecular weights from 500 to 1,000,000 g/mol; the instrument used a Wyatt Heleos II light scattering detector operated at a wavelength of 662 nm and an Optilab rEX refractometer operated at a wavelength of 658 nm. About 6-7 mg of the respective polymer were dissolved in 1 mL 0.1 M solution of Na₂SO₄ in aqueous acetic acid (1% v/v) and filtered through a 0.2 µm membrane filter, where the solution was then introduced to the chromatography system and eluted with 0.1 M solution of Na₂SO₄ in aqueous acetic acid (1% v/v) at a flow rate of 0.4 mL/min at 30 °C. The run time for the method was 45 min.

1.7 Proton Nuclear Magnetic Resonance (¹H NMR). In crude mixtures, the conversion of monomers for each reaction was checked with ¹H NMR with anisole as an internal standard. Chemical compositions of prepared polymers were determined using either ¹H NMR or ¹³C NMR analysis.

¹H NMR spectra were acquired on a Bruker Avance III HD 500 spectrometer equipped with a 5 mm Prodigy TCI cryoprobe with z-axis gradients at 22 °C using a 10 second relaxation delay and at least 16 transients without spinning to reduce signal-to-noise ratio in CDCl₃ (for poly(NIPAm-co-DMA) and poly(IPMA-co-DMA)), CD₃OD (for poly(NIPAm-co-Am) and poly(NIPAm-co-HEMA)), and D₂O (for poly(DEA-co-DMA)).
For poly(DEA-co-DMA), $^{13}$C NMR experiments were used to determine molar composition. Analysis solutions were prepared by dissolving polymer (~50 mg) in deuterated chloroform (2.4 mL). $^{13}$C NMR experiments were carried out on either a Bruker AVANCE 400 MHz spectrometer equipped with a 10 mm cryoprobe or a Bruker AVANCE III HD 600 MHz spectrometer equipped with a standard 10 mm probe. Parameters on the 400 MHz cryoprobe instrument were temperature of 25 °C, at least 672 scans, 40 s relaxation delay, 12.1 µs 90° pulse length, spectrum center of 100 ppm, spectral width of 250 ppm, with no sample spinning. Parameters on the 600 MHz standard probe instrument were temperature of 25 °C, at least 16 scans, 40 s relaxation delay, 12.1 µs 90° pulse length, spectrum center of 100 ppm, spectral width of 250 ppm, with no sample spinning. The peaks were referenced to chloroform at 77.2 ppm.

1.8 Two-dimensional Nuclear Overhauser Effect Spectroscopy (2D NOESY). 2D NOESY $^1$H NMR experiments were performed with water suppression. Pre-dissolved drug in DMSO-$d_6$ was solvent shifted into a deuterated PBS solution with polymer, such that the total drug and polymer concentrations were 100 and 500 µg/mL, respectively. All spectra were acquired at 300 K. The NOESY experiments were performed using mixing time of 50 ms and an acquisition time of 0.204 s. The 2D $^1$H/$^1$H NOESY spectra were recorded using 232 $t_1$ increments and 2048 $t_2$ complex points. The experimental data sets were zero-filled in both the $t_1$ and $t_2$ dimensions to form a 1024 × 1024 data matrix. A sine apodization function was applied in both dimensions prior to Fourier transformation. NMR data was processed with TopSpin 3.2 (Bruker, MA, USA).

1.9 Diffusion Ordered Spectroscopy (DOSY). Spectra were recorded on a Bruker Avance III 500 spectrometer, at 11.7 Tesla, at resonating frequency of 500 MHz for $^1$H, using 5 mm
TBO triple resonance PFG probe. The temperature was set at 300 K, and no spinning was applied to the NMR tube. All experiments were conducted in PBS buffer (pH = 6.5) in deuterium oxide with deuterated TMSP as a standard, where 50 µg/mL concentration of phenytoin was created by adding 10 µL of 5 mg/mL drug solution in DMSO-d<sub>6</sub>. The DOSY experiments were performed with a stimulated spin-echo sequence using bipolar gradients and an additional delay just prior to detection for the ring-down of any possible eddy currents. The bipolar gradient duration and the diffusion time were optimized for each sample and were in the range of 0.9 to 1.4 ms and 0.1 to 0.2 s, respectively. The evolution of the pulse-field gradient during DOSY experiments was established in 25 steps, applied linearly from 1 to 45 G cm<sup>-1</sup>. The recycle delay time was set to 10 s and the duration of each NMR diffusion experiment was adjusted to obtain at least 20 in the signal-to-noise ratio. Data was analyzed using TopSpin 3.2 (Bruker, MA, USA).

After collecting DOSY data, three peaks were analyzed: 7.5 to 7.3 ppm that corresponds to the phenytoin phenyl ring, 1.2 to 0.9 ppm that corresponds to a polymer backbone, and 0.1 to −0.1 ppm that corresponds to methyl group of a standard. Peaks area dependence on magnetic field gradient was collected. To determine the diffusion constant $D$, a linear regression was applied to experimental data according to Equation S-2:

$$\ln \left( \frac{I}{I_0} \right) = -D (\gamma g \delta)^2 (\Delta - \delta/3) \quad [S-2]$$

Here, $I$ is the observed intensity, $I_0$ is the reference intensity, $\gamma$ is the gyro magnetic ratio of the encoded nucleus, $g$ is the gradient strength, $\Delta$ is the diffusion time, and $\delta$ is the length of the gradient pulse. The binding between polymer and drug ($K_b = k_1/k_{-1}$) can be interpreted as a second order, reversible reaction:
Here, [drug]₀ is the free drug concentration, [mon. inhibitor] is the inhibitor monomer concentration, [drug] bound is the bound drug concentration.

Assuming that the exchange between free and bound drug is fast on the relevant time scale for each magnetic field gradient measurement, the mean diffusion constant $\langle D \rangle$ can be deconstructed as a linear molar combination of the diffusion constants of free drug $D_{\text{drug,0}}$ and polymer $D_{\text{polymer}}$ as shown in Equation S-4:

$$\langle D \rangle = x_{\text{drug,free}} D_{\text{drug,0}} + (1 - x_{\text{drug,free}}) D_{\text{polymer}}$$ [S-4]

Here, $x_{\text{drug,free}}$ is the mole fraction of free drug in solution. Finally, applying the same quasi steady-state approximation as before, Equation S-5 can be rearranged into Equation S-6. Combination with Equation S-4 allows $K_b$ to be directly calculated from all measurements at all polymer concentrations in Equation S-7.

$$k_1 [\text{drug}]_0 [\text{mon. inhibitor}] = k_{-1} [\text{drug}]_{\text{bound}}$$ [S-5]

$$K_b = \frac{k_1}{k_{-1}} = \frac{[\text{drug}]_{\text{bound}}}{[\text{drug}]_0 [\text{mon. inhibitor}]}$$ [S-6]

$$K_b = \frac{1}{x_{\text{drug,free}} - 1} \frac{1}{[\text{mon. inhibitor}]} \left( \frac{\langle D \rangle - D_{\text{drug,0}}}{D_{\text{polymer}} - \langle D \rangle} \right)$$ [S-7]
1.10 Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC). Samples of known mass (2–10 mg) were weighed, hermetically crimped in T-zero aluminum pans, and analyzed on a TA Instruments Discovery DSC. To determine the glass transition temperature \( T_g \) of a polymer, the temperature was ramped from 20 to 120 °C at a rate of 10 °C/min and then cooled down to 22 °C. In second heating cycle, the temperature was ramped to 180 °C at a rate of 2.5 °C/min, and again cooled down to 22 °C at a rate of 10 °C/min. The \( T_g \) was determined using the second heating scan at the midpoint of the change in heat capacity upon glass transition. For spray-dried dispersions, first heating \( T_g \) measurements were collected with modulated DSC with the following settings: ±1 °C amplitude every 40 s from 0 to 150 °C at a rate of 1 °C/min. Residual methanol in select spray-dried samples was analyzed with a Pyris Diamond (PerkinElmer) Thermogravimetric Analyzer model TGA7. Nitrogen (10 mL/min) was used as a purge gas at a heating rate of 10 °C/min. All analyses were carried out using TA TRIOS software (Version 2.2).

1.11 UV–Vis Spectroscopy (UV–vis). The cloud point temperatures of manually-synthesized polymers were determined using a UV–Vis spectrophotometer coupled with temperature controller in both water and PBS solution. Cloud point temperatures were measured from the transmittance at 450 nm. The samples were prepared by dissolving 18 mg polymer in 1.8 mL of water or PBS solution. The heating rate was 0.2 °C/min.

1.12 Temperature Scanning Turbidimetry (TST). TST experiments were performed using an in-house built apparatus comprising of a heated stage with 51 sample wells mounted on top of a vertical, oscillating stage for agitation, a QImaging QICAM Fast 1394 camera, and internally-developed software for analyzing captured images. A flat uniform white light-emitting panel was positioned behind the vial-holding panel for contrast; a specified region within a circular optical
window was defined in the software for each vial. Solutions containing 1 wt % polymer in deionized water or PBS (0.7 mL) were heated from 24-97 °C at a rate of 0.5 °C/min. Herein, the camera recorded an image at an interval of 1 min. The cloud point temperature was defined as the temperature where the optical transmittance dropped below 95% of the optical transmittance of the fully dissolved solution within the region of interest at 24 °C.

1.13 Spray Drying. Spray drying was performed on a laboratory scale using a Bend Research Mini Spray Dryer (Bend, OR). A solution consisting of 1 wt % of polymer plus drug at 10 and 25 wt % of drug loading in methanol (1:1, v/v) was prepared, with the following as an example: 30 mg of phenytoin and 270 mg of polymer were combined with 29.7 g methanol to prepare 10 wt % phenytoin spray-dried dispersions. Prior to spray drying, the polymer-drug solution was well-mixed for at least 30 min and transferred to a 20.0 mL syringe for spray drying at the following processing parameters: solution feed rate = 0.65 mL/min, inlet temperature = 90 °C, nitrogen feed rate = 12.8 standard liter per minute (SLPM). The outlet temperature ranged from 25-30 °C. Particles were collected from the 1.5” Whatman filter paper, dried under vacuum (10 mTorr) overnight for at least 12 h, and stored in a vacuum desiccator at 22 °C.

1.14 Scanning Electron Microscopy (SEM). Samples were transferred onto carbon tape and sputter-coated with a 100 Å thick layer of conductive gold/palladium (60:40, %, w/w) using a Denton DV-502A high vacuum deposition system. SEM images were obtained on a Hitachi S-900 SEM equipped with a backscattering detector with Autrata modified YAG (yttrium aluminum garnet, cerium doped) crystal. Images were taken at an accelerating voltage of 2.0 kV.

1.15 Powder X-ray Diffraction (PXRD). Powder samples (50-70 mg) were transferred into a 0.5 mm deep zero-background glass frame and placed on a Bruker-AXS D5005 (Siemens) diffractometer. The X-ray source (2.2 kW sealed Cu, λ = 1.54 Å) was run at a current of 40 mA
and a voltage of 45 kV. Data were collected from 5 to 40° (2θ) with a step size of 0.02° and a scan rate of 1 s/step.

1.16 **In vitro Dissolution Testing.** Dissolution tests were performed using a microcentrifuge dissolution test method under non–sink conditions (13). Weighed samples were transferred in duplicate into 2.0 mL plastic conical microcentrifuge tubes. Measured amounts of phosphate buffer saline (PBS, pH 6.5) with 0.5 wt % fasted intestinal fluid powder (FaSSIF) at 37 °C were added to begin the experiment. Dosages were chosen to target a total drug concentration of 1000 µg/mL (e.g., 7.2 mg solid dispersion solute loaded with 25 wt % phenytoin, comprising 1.8 mg phenytoin and 5.4 mg polymer, was diluted with 1.8 mL PBS/FaSSIF solvent). Samples were immediately vortexed for ~30 s on a Vortex Genie 2 equipped with a Scientific Industries V524 Vertical Microtube Holder and incubated in a VWR Digital Heatblock at 37 °C. At each time point (4, 10, 20, 40, 90, 180, and 360 min), samples were centrifuged for 1 min at 13,000 RPM in an Eppendorf Centrifuge 5414R, and a 50 µL aliquot was taken and diluted with 250 µL methanol for subsequent HPLC analysis (described above). Finally, samples were vortexed for ~30 s and transferred to the heating block at 37 °C until the next time point.

1.17 **In vivo Animal Testing.** The animal work for this study was performed by Washington Biotechnology Inc. (Baltimore, MD, USA) following a protocol approved by an Institutional Animal Care and Use Committee (IACUC) representative. Fifteen intact female Sprague-Dawley rats weighing 160-180 g were received, inspected for clinical stress or injury, quarantined individually for 72 h, and fed a commercially balanced diet with water *ad libitum*. During this time, animals were housed in a temperature- and humidity-controlled (68-79 °F, 30-70% RH) room with 12 h light/dark cycles. Once per day animals were examined for signs of clinical distress, disease, or injury. Animals were then transferred to routine maintenance in regularly
cleaned, vented cage racks and housed at three rats per cage. Prior to dosing, animals were fasted for 12 h. The animals were randomly divided into five groups of three so that body weight means were approximately equal; each group received a formulation treatment through oral (P.O.) gavage administration.

The administered drug dose was 40 mg/kg-body weight over 24 h. Blood collection through the orbital sinus (~0.2-0.6 mL/draw) were withdrawn by capillary action under anesthetic at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post dosing. After each volume was collected, gauze was held over the eye until bleeding stopped, and a small amount of triple antibiotic was applied to the eye to prevent infection. At the end of the experiment, animals were euthanized using carbon dioxide inhalation.

Whole blood samples were processed to serum/plasma through microcentrifugation: samples diluted in 3 volumes of ice-cold internal standard (acetonitrile containing 25 ng/mL diclofenac) were allowed to clot for ~15-30 min at room temperature before centrifuging samples at 6000 x g for 30 min. The serum/plasma supernatant was decanted and stored at -80 °C for subsequent analysis. All samples were transferred to an autosampler plate and diluted with 3 volumes of water. The plasma concentration of phenytoin was determined by liquid chromatography with tandem mass spectrometric (LC–MS) detection and electrospray ionization (ESI). 20 µL of the respective diluted taken aliquot was injected into a LC (Shimadzu VP Series 10 System) containing a Higgins Analytical column (Proto 200 C18, 20 x 2 mm). The mobile phase consisted of a gradient mixture of water and methanol at 25 °C as shown in Table S-1:
Table S-1. Shimadzu LC mobile phase conditions for phenytoin plasma analysis.

| Time (min) | Flow Rate (mL/min) | Mobile Phase (Water / Methanol, % v/v) |
|-----------|--------------------|--------------------------------------|
| 0         | 1.2                | 95 / 5                               |
| 0.25      | 0.8                | 95 / 5                               |
| 1.50      | 0.8                | 5 / 95                               |
| 1.70      | 0.8                | 5 / 95                               |
| 1.75      | 0.8                | 95 / 5                               |
| 2.00      | 1.2                | 95 / 5                               |

The column effluent was analyzed with a mass spectrometer system (Applied Biosystems / MDS SCIEX API 4000 MS/MS) with Analyst (v1.5) software. Matrices were obtained from BioreclamationIVT (Westbury, NY). TurbolonSpray was used as the ESI with negative ion conditions at a source temperature of 400 °C. Transitions of phenytoin (analyte) and diclofenac (internal standard) were measured at 250.9/101.9 and 293.9/249.9 m/z, respectively. A power regression with a standard curve spanning 15-50,000 ng/mL was used with an acceptance criterion of ±25%, 30% at the lower limit of quantification. Pharmacokinetic metrics were evaluated by direct inspection of the drug plasma concentration–time profiles.

1.18 Statistical Analysis. Phenytoin pharmacokinetic parameter values were compared using one-way analysis of variance (ANOVA), Welch, Tukey’s honest significant difference (HSD), and Student–Newman–Keuls (SNK) tests with significance assigned at \( p = 0.05 \). These statistical tests were performed using Igor Pro (v6.37).
2 Experimental

2.1 Experiment 1: Manual and high-throughput RAFT polymerization details. The following describes experiments related to the synthesis and characterization of polymer libraries using reversible addition-fragmentation chain transfer (RAFT) chemistry, a reversible–deactivation radical polymerization technique capable of preparing uniform polymer lengths with low dispersity. We prepared 5 sets of RAFT polymerization reactions (Fig. S-1) using semi–continuous parallel pressure reactor (ScPPR) systems as part of the high-throughput instrumentation suite for excipient development.

**Polymer Microstructure.** Hierarchical polymer microstructure has been previously shown to affect the solution-state and dissolution efficacy of solid dispersion systems \(^1\). In order to quantify the predicted microstructure of our five copolymer systems, the reactivity ratio pairings for each polymer were taken from experimentally-measured literature references \(^2\)–\(^6\), summarized in Table S-2. By definition, the reactivity ratio is the relative probabilities of monomer self-propagation (monomer \(n\) to \(n\)) to cross-propagation (monomer \(n\) to \(m\)); based on the product of the two pairwise reactivity ratios \(r_1\) and \(r_2\), the microstructural character of the copolymer produced can be anticipated. The \(r_1 \times r_2\) value for poly(NIPAm-co-DMA), poly(NIPAm-co-Am) and poly(DEA-co-DMA) approach unity, indicative of an “ideal” polymerization in which both monomers have equivalent relative tendencies to add to both radicals and yield random copolymers. Meanwhile, the product of \(r_1\) and \(r_2\) for poly(NIPAm-co-HEMA) approaches zero, suggesting a high tendency towards alternation as growing NIPAm monomers have a low probability to add to another NIPAm monomer. Finally, the poly(IPMA-co-DMA) \(r_1 \times r_2\) value exceeds unity. This means that the polymer architecture likely contains long blocky sequences of IPMA and DMA repeat units.
Figure S-1. Schematic representation of RAFT polymerization reactions using RAFT chain transfer agent 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA) and radical initiator 2,2′-azobis(2-methylpropionitrile) (AIBN). Prepared polymer systems include (A) poly(NIPAm-co-DMA), (B) poly(NIPAm-co-HEMA), (C) poly(NIPAm-co-Am), (D) poly(DEA-co-DMA), and (E) poly(IPMA-co-DMA).
Table S-2. Literature reactivity ratios for investigated systems.

| Reference          | System (poly(1-co-2)) | r₁   | r₂   | T (°C) | r₁ x r₂ | Microstructure   |
|--------------------|-----------------------|------|------|--------|---------|------------------|
| Bauri et al. ²     | poly(NIPAm-co-DMA)    | 0.84 | 1.11 | 70     | 0.93    | Ideal / random   |
| Mumick et al. ³    | poly(NIPAm-co-Am)     | 1.04 | 0.95 | 70     | 0.99    | Ideal / random   |
| Fares et al. ⁴     | poly(NIPAm-co-HEMA)   | 0.05 | 0.43 | 30     | 0.02    | Alternating      |
| Pandiyarajan ⁵     | poly(DEA-co-HEMA)     | 1.15 | 0.86 | 60     | 0.99    | Ideal / random   |
| Srinivasulu et al. ⁶ | poly(IPMA-co-DMA)   | 2.76 | 0.58 | 60     | 1.60    | Blocky           |

Furthermore, the chemical microstructure of copolymerization processes can be gauged with the Skeist equation; we have previously employed this approach in the context of microstructural predictions for multicomponent polymeric systems.⁷ By extension, we computed the theoretical compositional profiles versus conversion with arbitrary feed monomer mole fractions of M₁ = 0.25, 0.50, and 0.75 for all systems (Fig. S-2). The predicted cumulant polymer compositional profiles reaffirm the \( r₁ \times r₂ \) predictions: all ideal/random systems show little compositional drift from targeted polymer compositions, while poly(NIPAm-co-HEMA) and poly(IPMA-co-DMA) exhibited composition dependency on monomer feed and conversion.
Figure S-2. Predicted cumulative polymer compositions as a function of total monomer conversion for the copolymerization of $M_1$ (solid lines) and $M_2$ (dashed lines), based on given reactivity ratios. Three initial $M_1$ values were arbitrarily chosen to illustrate composition drift.
Polymer Composition. The total monomer conversion was checked with $^1$H NMR on the crude (prior to polymer precipitation) mixture (Fig. S-3). The molar composition of copolymers was calculated from the ratio of integrated peak areas assigned to certain structural elements.

**Figure S-3.** Representative $^1$H NMR spectrum for crude poly(NIPAm61-co-HEMA39) with anisole as an internal standard in CD$_3$OD.

For poly(NIPAm-co-DMA) (Fig. S-4A), the molar composition was calculated by comparing the characteristic proton peaks of –N-CH– at about 4.0 ppm for NIPAm (1 H) and –N-(CH$_3$)$_2$ at about 2.8-3.3 ppm for DMA (6 H). For poly(NIPAm-co-Am) (Fig. S-4B), the molar composition was determined by relating the integrated area of the peak of –N-CH– at about 4.0 ppm for NIPAm (1 H) to the integrated area of all peaks related to the –CH$_2$-CH– backbone and –CH-(CH$_3$)$_2$ NIPAm units from 0.9-2.5 ppm for NIPAm backbone (3 H), Am backbone (3 H), and NIPAm methyl groups (6 H). For poly(IPMA-co-DMA) (Fig. S-4C), the molar composition was determined by comparing the integrated area of the peak of –O-CH– at about 4.8 ppm for
IPMA (1 H) and the integrated area of all peaks related to the –CH\(_2\)-CH– backbone and –CH-(CH\(_3\))\(_2\) IPMA units from 0.5-3.25 ppm for IPMA backbone (3 H), DMA backbone (3 H), and NIPAm methyl groups (6 H). For poly(NIPAm-co-HEMA) (Fig. S-4D), the molar composition was calculated by comparing the integrated area of the peak of –CH\(_2\)-OH at about 3.7 ppm for HEMA (2 H) in relation to the integrated area of all peaks related to the –CH\(_2\)-CH– backbone and –CH-(CH\(_3\))\(_2\) NIPAm units in the range between about 0.6-2.4 ppm for NIPAm backbone (3 H), Am backbone (3 H), and NIPAm methyl groups (6 H).

Figure S-4. Representative \(^1\)H NMR spectrum for (A) poly(NIPAm-co-DMA) in CDCl\(_3\), (B) poly(NIPAm-co-Am) in CD\(_3\)OD, (C) poly(IPMA-co-DMA) in CDCl\(_3\), and (D) poly(NIPAm-co-HEMA) in CD\(_3\)OD.
Polymer Molecular Weight and Dispersity. The number-average molecular weight ($M_n$) and dispersity (D) of the purified polymers were determined using size–exclusion chromatography with multiangle light scattering (SEC-MALS). Absolute $M_n$ values were reported by first measuring the $dn$/$dc$ of each sample. Fig. S-5 shows representative SEC traces for investigated polymer systems at various chemical compositions. Elution volumes of a single peak were consistent across chemical compositional windows. Figs. S-6–S-8 show $M_n$ and D measurements for select polymer series where we explored the molecular weight and chemical composition state space. In general, automated RAFT polymerization yielded targetable molecular weights with low D values.

![Representative SEC traces for various chemical compositions](image)

Figure S-5. Representative size–exclusion chromatography (SEC) chromatograms of prepared polymer systems. Plotted curves are normalized from the SEC refractive index detector. SEC curves at varying chemical compositions were vertically shifted.
Figure S-6. Characterization summary of poly(NIPAm-co-DMA) polymers prepared with automated polymer syntheses, consisting of a series of copolymers (A) varying chemical composition at targeted constant molecular weight, and (B) varying molecular weight at targeted constant chemical composition.

Figure S-7. Characterization summary of poly(NIPAm-co-DMA) polymers prepared with automated polymer syntheses, consisting of a series of copolymers at targeted (A) low and (B) high molecular weights to span the chemical composition window.
Figure S-8. Characterization summary of poly(NIPAm-co-Am) polymers prepared with automated polymer syntheses, consisting of a series of copolymers at targeted (A) low and (B) high molecular weights to span the chemical composition window.

**Polymer Glass Transition.** For long–term stability of solid dispersions in the powdered state, the glass transition temperature ($T_g$) of the excipient is a conventional metric of comparison as it influences the inhibition of drug molecule molecular mobility. This is important to the viability of solid dispersions since recrystallization negates the solubility advantage in solution.

Figs. S-9 and S-10 show $T_g$ measurements for select polymer series spanning molecular weight and chemical composition windows. For the poly(NIPAm-co-DMA) series, we observed increasing $T_g$ values as NIPAm incorporation increased. At a constant targeted chemical composition (66 mol % NIPAm), copolymers exhibited relatively uniform $T_g$ values. Minor discrepancies can be attributed to variability in chemical composition. In general, we have shown that we generated high $T_g$ polymers that are viable excipient candidates for spray drying applications.
Figure S-9. Characterization summary of glass transition temperature ($T_g$) values of the poly(NIPAm-co-DMA) system for the (A) low $M_n$ series, (B) high $M_n$ series, and (C) targeted 66 mol % NIPAm composition series. $T_g$ measurements were determined from second heating differential scanning calorimetry experiments at a 2.5 °C/min heating rate.

Figure S-10. Characterization summary of glass transition temperature ($T_g$) values of the (A) poly(NIPAm-co-HEMA) and (B) poly(NIPAm-co-Am) series spanning the chemical composition window. $T_g$ measurements were determined from second heating differential scanning calorimetry experiments at a 2.5 °C/min heating rate.
2.2 Experiment 2: Manual and high–throughput cloud point measurements.

Beforehand, solutions containing 1 wt % polymer were visually examined for solubility at 25 °C. Copolymers poly(NIPAm-co-DMA), poly(NIPAm-co-Am), and poly(DEA-co-DMA) exhibited good aqueous solubility at all compositions, while the poly(NIPAm-co-HEMA) and poly(IPMA-co-DMA) systems varied from cloudy or insoluble and were not studied further.

For our manually prepared polymers, we used UV–vis spectrophotometry in deionized water and PBS solution. Fig. S-11 shows the cloud point curves, and Table S-3 summarizes the corresponding cloud point temperature measurements of poly(NIPAm-co-DMA) from UV-vis spectrophotometry.

![Figure S-11](image)

**Figure S-11.** Cloud point curves of poly(NIPAm-co-DMA) at 1 wt % concentration in deionized water and PBS solution. Measurements were collected at a heating rate of 0.2 °C/min.
Table S-3. Cloud point temperatures for poly(NIPAm-co-DMA).

| NIPAm Comp. (mol%) | DMA Comp. (mol%) | Cloud Point, water (°C) | Cloud Point, PBS (°C) |
|-------------------|-----------------|-------------------------|-----------------------|
| 0                 | 100             | N/A                     | N/A                   |
| 23                | 77              | >90                     | >90                   |
| 46                | 54              | 71                      | 54                    |
| 70                | 30              | 55                      | 37                    |
| 84                | 16              | 30                      | N/A                   |
| 100               | 0               | 28                      | N/A                   |

Automation was achieved using an in–house temperature scanning turbidimetry (TST) instrument, shown schematically in Fig. S-12. Tables S-4–S-6 show the cloud points measured by TST experiments for the poly(NIPAm-co-Am) and poly(DEA-co-DMA) series with corresponding curves in Fig. S-13.

Figure S-12. Schematic of the temperature scanning turbidimetry instrumentation. All solutions contained 1 wt % polymer. The cloud point was optically detected by the camera (where the transmittance drops below 95% of the initial state).
Table S-4. Cloud point temperatures for poly(NIPAm-co-Am), Low $M_n$.

| NIPAm Comp. (mol%) | Am Comp. (mol%) | Cloud Point, water ($^\circ$C) | Cloud Point, PBS ($^\circ$C) |
|--------------------|----------------|-------------------------------|--------------------------|
| 0                  | 100            | N/A                           | N/A                      |
| 27                 | 73             | >100                          | >100                     |
| 36                 | 64             | >100                          | >100                     |
| 45                 | 55             | >100                          | >100                     |
| 57                 | 43             | >100                          | 73                       |
| 65                 | 35             | 75                            | 56                       |
| 73                 | 27             | 50                            | 45                       |

Table S-5. Cloud point temperatures for poly(NIPAm-co-Am), High $M_n$.

| NIPAm Comp. (mol%) | Am Comp. (mol%) | Cloud Point, water ($^\circ$C) | Cloud Point, PBS ($^\circ$C) |
|--------------------|----------------|-------------------------------|--------------------------|
| 0                  | 100            | N/A                           | N/A                      |
| 28                 | 72             | >100                          | >100                     |
| 38                 | 62             | >100                          | >100                     |
| 47                 | 53             | >100                          | >100                     |
| 57                 | 43             | >100                          | >100                     |
| 65                 | 35             | >100                          | 67                       |
| 76                 | 24             | >100                          | 51                       |

Table S-6. Cloud point temperatures for poly(DEA-co-DMA).

| DEA Comp. (mol%) | DMA Comp. (mol%) | Cloud Point, water ($^\circ$C) | Cloud Point, PBS ($^\circ$C) |
|------------------|------------------|-------------------------------|--------------------------|
| 20               | 80               | N/A                           | N/A                      |
| 40               | 60               | 77                            | 67                       |
| 50               | 50               | 61                            | 57                       |
| 60               | 40               | 55                            | 48                       |
| 65               | 35               | 50                            | 45                       |
| 69               | 31               | 46                            | 43                       |
| 81               | 19               | 40                            | 37                       |
| 100              | 0                | N/A                           | N/A                      |
Figure S-13. Cloud point curves of (A) low $M_n$ poly(NIPAm-co-Am), (B) high $M_n$ poly(NIPAm-co-Am), and (C) poly(DEA-co-DMA) series at 1 wt % concentration in deionized water and PBS solution using TST at a heating rate of 0.5 °C/min.

2.3 Experiment 3: Supplemental precipitation inhibition screening. The quantitative high–throughput supersaturation screening of phenytoin and nilutamide using the polymer excipients that we have developed is shown in Figs. S-14 and S-15. Additional representative heat maps examining poly(NIPAm-co-DMA) at $M_n, \text{low}$ and $M_n, \text{high}$ with phenytoin and griseofulvin are shown in Figs. S-16.
Figure S-14. High–throughput precipitation inhibition screening of phenytoin. Supersaturation profiles of (A) NIPAm–containing systems with hydrophilic comonomers and (B) DMA–containing systems with inhibitor comonomers were monitored over 3 h. Experiments were prepared with a total nilutamide concentration of 1000 µg/mL. Circles represent the mean of $N = 3$ runs.
Figure S-15. High–throughput precipitation inhibition screening of nilutamide. Supersaturation profiles of (A) NIPAm–containing systems with hydrophilic comonomers and (B) DMA–containing systems with inhibitor comonomers were monitored over 3 h. Experiments were prepared with a total nilutamide concentration of 1000 µg/mL. Circles represent the mean of $N = 3$ runs.
Figure S-16. High-throughput precipitation inhibition screening, spanning across all polymer chemical compositions of poly(NIPam-co-DMA). Heat map array plots represent supersaturated drug concentrations of (A) phenytoin and (B) griseofulvin in PBS solution at 37 °C over 180 min. Experiments were prepared with a total drug concentration of 1000 µg/mL. Each box represents the mean of $N = 3$ runs.

2.4 Experiment 4: Supplemental 2D NOESY and DOSY NMR experiments. The nuclear Overhauser effect (NOE) strongly depends on the internuclear distance between neighboring molecules. Thus, this technique can provide conformational analysis and potentially pinpoint the proximity of amorphized drug molecules to distinct monomeric units within 5-6 Å in sensitivity. For phenytoin, the phenyl peaks (7.5–7.3 ppm) were used as the reference for intermolecular interactions between drug and polymer. First, to check for confounding spin diffusion effects from NOE buildup, we evaluated the relative phenytoin NOESY peak intensity as a function of experimental mixing time from 0.25 to 1.5 s. As seen in Fig. S-17, the intensity has not plateaued at a mixing time of 1.5 s. The sharper increase in intensity from 0.25 to 0.5 s was attributed to transient NOE. From 0.5 to 1.5 s the proton intensity continued to increase, indicating that spin diffusion effects were not induced for our systems. Thus, we maintained a mixing time of 1.5 s
for the following experiments to identify specific polymer–drug interactions for leading candidates from the supersaturation screening assay.

**Figure S-17.** Relative NOESY phenytoin peak intensity versus experimental NMR mixing time. The relative peak intensity values were calculated by taking the ratio of the cross peak intensity to the diagonal peak intensity at 7.5 to 7.3 ppm in the $^1$H NMR spectra.

Fig. S-18 shows the NOESY spectrum of poly(NIPAm70-co-DMA30) and phenytoin. Strong cross peaks were present between the drug phenyl protons and the NIPAm isopropyl protons at 3.9–3.8 and 1.3–1.1 ppm. A 1D $^1$H NMR spectrum from slicing the NOESY spectrum at 7.4 ppm showed peaks that closely corresponded to the positive NOESY peaks in NIPAm only. Therefore, we concluded that the NIPAm units indeed act as inhibitors of desupersaturation.
Figure S-18. (A) 2D $^1$H NOESY NMR spectrum of phenytoin solvent–shifted (600 µg/mL, DMSO-$d_6$, 1% v/v) into a deuterated PBS solution containing pre-dissolved poly(NIPAm70-co-DMA30) (900 µg/mL) at a mixing time of 1.5 s. The red circle denotes the drug phenyl peaks (7.5–7.3 ppm). (B) Representative 1D spectrum sliced at 7.4 ppm (red dashed line) from the NOESY spectrum.

Fig. S-19 shows the NOESY spectrum of poly(NIPAm73-co-Am27) and phenytoin. Interestingly, no cross peaks were observed between the drug phenyl protons and monomer constituents. We speculate that because the hydrophilic monomer Am acts as a hydrogen bond acceptor and donor, NIPAm units were unable to complex as strongly as in the case of a NIPAm/DMA combination. Investigating this remains the subject of ongoing work.
Figure S-19. 2D $^1$H NOESY NMR spectrum of phenytoin solvent–shifted (600 µg/mL, DMSO-$d_6$, 1% v/v) into a deuterated PBS solution containing pre-dissolved poly(NIPAm73-co-Am27) (900 µg/mL) at a mixing time of 1.5 s. The red circle denotes the drug phenyl peaks (7.5–7.3 ppm).

Fig. S-20 shows the NOESY spectrum of poly(NIPAm78-co-HEMA22) and phenytoin. Again, no drug–polymer cross peaks were observed. Instead, we report cross peaks between NIPAm and HEMA units, suggesting that intramolecular interactions prevailed for this particular system. Additionally, from the visual solubility tests we noted that the NIPAm/HEMA series were relatively insoluble in aqueous settings, which may have facilitated rapid desupersaturation.
Figure S-20. 2D $^1$H NOESY NMR spectrum of phenytoin solvent–shifted (600 µg/mL, DMSO-$d_6$, 1% v/v) into a deuterated PBS solution containing pre-dissolved poly(NIPAm78-co-HEMA22) (900 µg/mL) at a mixing time of 1.5 s. The red circle denotes the drug phenyl peaks (7.5–7.3 ppm).

Fig. S-21 shows the NOESY spectrum of poly(DEA65-co-DMA35) and phenytoin. Strong cross peaks were present between the drug phenyl protons and the DEA methyl protons at 1.3–1.1 ppm. Thus, DMA again appears to only act to increase the hydrophilicity of the polymer chains, whereas DEA interacts with drug molecules through hydrophobic effects or van der Waals interactions.
Figure S-21. 2D $^1$H NOESY NMR spectrum of phenytoin solvent–shifted (600 µg/mL, DMSO-$d_6$, 1% v/v) into a deuterated PBS solution containing pre-dissolved poly(DEA65-co-DMA35) (900 µg/mL) at a mixing time of 1.5 s. The red circle denotes the drug phenyl peaks (7.5–7.3 ppm).

Fig. S-22 shows the NOESY spectrum of poly(IPMA16-co-DMA84) and phenytoin. No cross peaks were observed in this experiment. We note that NIPAm and IPMA both share isopropyl groups, which promotes hydrophobic-hydrophobic interactions. However, the low supersaturation levels were observed in the precipitation inhibition assay, and from the absence
of strong NOESY cross-correlations, we strongly suspect hydrogen bonding to be the prevailing stabilization mechanism between NIPAm units and phenytoin.

**Figure S-22.** 2D $^1$H NOESY NMR spectrum of phenytoin solvent–shifted (600 µg/mL, DMSO-$d_6$, 1% v/v) into a deuterated PBS solution containing pre-dissolved poly(IPMA16-co-DMA84) (900 µg/mL) at a mixing time of 1.5 s. The red circle denotes the drug phenyl peaks (7.5–7.3 ppm).

In addition to NOESY experiments, we conducted DOSY NMR runs for all leading samples. In DOSY experiments, regular pulse sequences establish gradients, allowing molecules to be spatially labeled. After a specified diffusion time, the resultant gradient enables
quantification of molecular movement in solution. Thus, parameters such as the translational diffusion coefficient \( (D_0) \) or binding strength \( (K_b) \) in solution can be calculated.

Trimethylsilyl propanoic acid (TMSP) was used as an internal standard. In the absence of polymer, the translational diffusion constant \( (D_0) \) for phenytoin and TMSP were determined to be \((4.457 \pm .025) \times 10^{-10}\) m\(^2\)/s and \((4.803 \pm .007) \times 10^{-10}\) m\(^2\)/s, respectively (Fig. S-23). The diffusivity of TMSP remained independent of polymer concentration in all cases, so viscous effects did not interfere with DOSY measurements.

**Figure S-23.** Reduced pulse field gradient intensity for the trimethylsilyl propanoic acid (TMSP) standard (gray diamond) and phenytoin (red circle) as a function of DOSY parameters. The gray and red dashed lines represent linear regression fittings for TMSP standard \( (r^2 = 0.99995) \) and phenytoin \( (r^2 = 0.99927) \), respectively.

Fig. S-24 shows the DOSY results for all five systems. In the leading poly(NIPAm70-co-DMA30) system (Fig. S-24A), there was a steady decrease in the reduced diffusion coefficient at increasing polymer concentration. Moreover, its predicted diffusivity (from the calculated \( K_b = \)
44 ± 11 L mol⁻¹ using Equation S-7) agreed closely with the experimental measurements. The same result was seen for the poly(NIPAm78-co-HEMA22) (Fig. S-24C) and poly(IPMA16-co-DMA84) (Fig. S-24E) systems. However, the calculated $K_b$ values (17 ± 8 and 19 ± 10 L/mol, respectively) were about less than half as strong as that of the NIPAm/DMA system.

**Figure S-24.** Reduced diffusion coefficients for TMSP standard (gray diamond) and phenytoin (red circle) versus polymer concentration for (A) poly(NIPAm70-co-DMA30), (B) poly(NIPAm73-co-Am27), and (C) poly(NIPAm78-co-HEMA22), (D) poly(DEA65-co-DMA35), and (E) poly(IPMA16-co-DMA84). Dashed red lines show predicted phenytoin behavior from a calculated $K_b$ of (A) 44 ± 11 L/mol, (C) 17 ± 8 L/mol, and (E) 19 ± 10 L/mol.

2.5 **Experiment 5: Supplemental solid dispersion characterization.** Scanning electron microscopy (SEM) revealed the particle size–reduction of crystalline phenytoin as received
through spray drying. Fig. S-25 shows select images of phenytoin spray–dried with poly(NIPAm70-co-DMA30) at 10 and 25 wt % drug loading. The collapsed morphology is characteristic of spray-dried dispersions containing high–$T_g$ polymers.$^9$

Figure S-25. Representative scanning electron microscopy (SEM) images of (A) crystalline phenytoin, (B) crystalline nilutamide, (C) spray–dried poly(NIPAm70-co-DMA30) with 10 wt % phenytoin, and (D) spray–dried poly(NIPAm70-co-DMA30) with 25 wt % phenytoin. Note the scale bars are different in each image.
Thermogravimetric analysis indicated less than 2.5 wt % residual solvent remained in the solid dispersion particles at both drug loadings (Fig. S-26). The pilot spray dryer used in this work did not have control of the outlet temperature, which we expect to assist in driving solvent evaporation for scale up operations.

Figure S-26. Representative thermogravimetric analysis curves for poly(NIPAm70-co-DMA30) at (A) 10 wt % loading phenytoin and (B) 25 wt % loading phenytoin. Samples were heated at 10 °C/min with purging nitrogen gas at a flow rate of 10 mL/min.

The amorphicity of our solid dispersions was assessed through powder X–ray diffraction (PXRD). Fig. S-27 shows representative PXRD patterns of select poly(NIPAm-co-DMA) solid dispersions at 10 and 25 wt % phenytoin loading. No sharp crystalline peaks were detected, indicating that phenytoin was maintained in the amorphous state. Additionally, MDSC experiments were performed to measure the $T_g$ of representative spray-dried dispersions (Table S-7). The values were comparable to high–$T_g$ systems in literature examples.9
Figure S-27. Representative powder X-ray diffraction (PXRD) patterns of select poly(NIPAm-co-DMA) systems at (A) 10 wt % loading phenytoin and (B) 25 wt % loading phenytoin. PXRD curves at increasing NIPAm composition were vertically shifted.

Table S-7. Representative $T_g$ measurements with MDSC.

| System                  | Drug Loading (wt %) | $T_g$ (°C) |
|-------------------------|---------------------|------------|
| Poly(NIPAm46-co-DMA54)  | 10                  | 89.0       |
|                         | 25                  | 89.5       |
| Poly(NIPAm70-co-DMA30)  | 10                  | 84.5       |
|                         | 25                  | 83.9       |

2.6 Experiment 6: In vitro dissolution testing – NIPAm/DMA composition effects. Fig. S-28 shows the drug dissolution profiles of spray–dried dispersions sampling different compositions of the poly(NIPAm-co-DMA) series. Here, the delicate balance between inhibiting NIPAm and hydrophilic DMA (as identified by the supersaturation screening experiments) is evident. Increasing the composition of NIPAm from 23 to 70 mol % resulted in higher maximum drug concentrations reached and greater supersaturation stabilization for both 10 and 25 wt % drug loading. However, for the 84 mol % NIPAm system, particles were not as visually soluble,
and we suspect that the low DMA incorporation did not promote rapid dissolution of the solid dispersion into solution.

**Figure S-28.** In vitro drug dissolution testing for leading excipient poly(NIPAM-co-DMA) at 10 and 25 wt % phenytoin loading for systems containing (A) 20 mol % NIPAm, (B) 46 mol % NIPAm, (C) 70 mol % NIPAm, and (D) 84 mol % NIPAm. All experiments were prepared with a total phenytoin concentration of 1000 µg/mL. Error bars represent the range of collected data.

### 2.7 Experiment 7: In vitro dissolution testing – AUC$_{360\ min}$ Enhancement summary

The area–under–the–dissolution–curve for 6 h (AUC$_{360\ min}$) Enhancement provides a measure of the effectiveness of the solid dispersion (Equation S-8). As an example of its physical meaning, an AUC$_{360\ min}$ Enhancement of 5 means that the solid dispersion formulation resulted in a five–fold increase in the area under the dissolve curve metric over the crystalline drug only.

\[
\text{AUC}_{360\ min} \text{ Enhancement} = \frac{\text{AUC}_{\text{solid dispersion for 360\ min}}}{\text{AUC}_{\text{drug only for 360\ min}}} \quad [S-8]
\]
We spray-dried polymers that were (i) the leading compositional hit from our high-throughput supersaturation screening experiments and (ii) an arbitrarily selected nonideal system. Fig. S-29 summarizes the $AUC_{360\text{ min}}$ Enhancement of these select solid dispersions at 10 and 25 wt % phenytoin loading. In general, the solubilization advantage reinforces the results of the supersaturation screening runs and shows the importance of optimizing excipient design.

**Figure S-29.** Area–under–the–dissolution–curve enhancement over 360 min ($AUC_{360\text{ min}}$ Enhancement) for select spray–dried dispersion systems at 10 and 25 wt % phenytoin loading. Within each set of polymer systems (separated by the dashed purple lines), the first group is the leading excipient identified by the supersaturation screening runs; the second (if shown) group is an off-composition from the screening. Error bars denote the range of the measured data.
2.8 Experiment 8: In vivo PK and AUC statistical analysis. Fig. S-30 graphically illustrates the formulation groups used for in vivo pharmacokinetic (PK) animal studies. Crystalline phenytoin and hydroxypropyl methylcellulose acetate succinate (HPMCAS) served as negative and positive controls, respectively. The remaining three poly(NIPAm-co-DMA) groups enabled us to study chemical compositional and drug loading effects centered on the leading excipient from supersaturation screening.

Figure S-30. Formulation groups used for in vivo rat studies ($N = 3$), consisting of crystalline phenytoin control, HPMCAS spray–dried with 10 wt % phenytoin control, poly(NIPAm46-co-DMA54) spray-dried with 10 wt % phenytoin, poly(NIPAm70-co-DMA30) spray dried with 10 wt % phenytoin, and poly(NIPAm70-co-DMA30) spray dried with 25 wt % phenytoin.

We performed statistical tests on the calculated area–under–the–curve (AUC) values at 6 h. A one-way between subjects ANOVA was conducted to compare the effect of drug
formulation on AUC in various excipient conditions; there was a significant effect of formulation on AUC at the p < 0.05 level for the five conditions (F(4, 10) = 6.252, p = 0.00870). We also performed a Welch test to examine potential unequal variance effects; results confirmed that there was a significant effect at the p < 0.05 level (F(4, 4) = 42.89, p = 0.00153). Because we found a statistically significant result, we performed a Tukey post hoc test, designed to compare each of our conditions to every other conditions. Post hoc pairwise comparisons using the Tukey’s honest significant difference test indicated that the mean of the NIPAm70 (10 wt %) condition was statistically different from that of the phenytoin condition (q = 6.863 > q_c = 4.654) and the HPMCAS (10 wt %) condition (q = 4.827 > q_c = 4.654). Furthermore, the Student–Newman–Keuls test showed that the poly(NIPAm70-co-DMA30) + Drug (10 wt %) is statistically different from all other groups. Tables S-8–S-11 summarize these results.

**Table S-8. Area–under–the–curve values (µg/mL-time) of each animal for 6 h.**

| Formulation Group | Animal 1 | Animal 2 | Animal 3 |
|-------------------|----------|----------|----------|
| Crystalline Phenytoin | 178  | 78  | 101  |
| HPMCAS + Drug (10 wt %) | 1748 | 398 | 589 |
| Poly(NIPAm46-co-DMA54) + Drug (10 wt %) | 1089 | 1137 | 1069 |
| Poly(NIPAm70-co-DMA30) + Drug (10 wt %) | 2504 | 3388 | 2486 |
| Poly(NIPAm70-co-DMA30) + Drug (25 wt %) | 468  | 2651 | 641  |

**Table S-9. ANOVA test for area–under–the–curve values (µg/mL-time) for 6 h.**

| Degree of Freedom | Sum of Squares | Mean Sum of Squares | F    | F_c  | p    |
|------------------|----------------|---------------------|------|------|------|
| Groups           | 4              | 1.14 × 10^7         | 2.85 × 10^6 | 6.252 | 0.00870 |
| Error            | 10             | 4.55 × 10^6         | 4.55 × 10^5 |      |      |
| Total            | 14             | 1.59 × 10^7         | 1.14 × 10^6 |      |      |
Table S-10. Welch test for area–under–the–curve values (µg/mL-time) for 6 h.

| Degree of Freedom $N_1$ | Degree of Freedom $N_2$ | $F$  | $F_c$ | $p$  |
|-------------------------|-------------------------|------|-------|------|
| 4                       | 4                       | 42.886 | 6.388 | 0.00153 |

Table S-11. Tukey test for area–under–the–curve values (µg/mL-time) for 6 h.

| Pairing* | Difference of Means | Standard Error | $q$  | $q_c$ |
|----------|---------------------|----------------|------|-------|
| 4 vs. 1  | 2674                | 390            | 6.863 | 4.654 |
| 4 vs. 2  | 1880                | 390            | 4.827 | 4.654 |
| 4 vs. 3  | 1694                | 390            | 4.349 | 4.654 |
| 4 vs. 5  | 1539                | 390            | 3.951 | 4.654 |
| 5 vs. 1  | 1134                | 390            | 2.912 | 4.654 |
| 5 vs. 2  | 341                 | 390            | 0.876 | 4.654 |
| 5 vs. 3  | 155                 | 390            | 0.397 | 4.654 |
| 3 vs. 1  | 980                 | 390            | 2.515 | 4.654 |
| 3 vs. 2  | 186                 | 390            | 0.478 | 4.654 |
| 2 vs. 1  | 793                 | 390            | 2.036 | 4.654 |

* For the pairings: (1) Crystalline Phenytoin, (2) HPMCAS + Drug (10 wt %), (3) Poly(NIPAm46-co-DMA54) + Drug (10 wt %), (4) Poly(NIPAm70-co-DMA30) + Drug (10 wt %), and (5) Poly(NIPAm70-co-DMA30) + Drug (25 wt %).

We then repeated this analysis for the AUC values after 24 h. A one-way between subjects ANOVA was conducted to compare the effect of drug formulation on AUC in various excipient conditions; there was a significant effect of formulation on AUC at the $p < 0.05$ level for the five conditions ($F(4, 10) = 3.564$, $p = 0.0469$). We also performed a Welch test to examine potential unequal variance effects; results confirmed that there was a significant effect at the $p < 0.05$ level ($F(4, 4) = 7.306$, $p = 0.0400$). Because we found a statistically significant result, we performed a Tukey post hoc test, designed to compare each of our conditions to every other condition. Post hoc pairwise comparisons using the Tukey’s honest significant difference test indicated that the mean of the poly(NIPAm70-co-DMA30) + Drug (10 wt %) condition was statistically different from that of the phenytoin condition ($q = 5.238 > q_c = 4.654$). Tables S-12–S-15 summarize these results.
Table S-12. Area–under–the–curve values (µg/mL-time) for 24 h.

| Formulation Group | Animal 1 | Animal 2 | Animal 3 |
|-------------------|----------|----------|----------|
| Crystalline Phenytoin | 231 | 259 | 197 |
| HPMCAS + Drug (10 wt %) | 3932 | 1225 | 1915 |
| Poly(NIPAm46-co-DMA54) + Drug (10 wt %) | 2755 | 2472 | 2038 |
| Poly(NIPAm70-co-DMA30) + Drug (10 wt %) | 3519 | 6377 | 2695 |
| Poly(NIPAm70-co-DMA30) + Drug (25 wt %) | 687 | 3633 | 829 |

Table S-13. ANOVA test for area–under–the–curve values (µg/mL-time) for 24 h.

| Degree of Freedom | Sum of Squares | Mean Sum of Squares | F | Fc | p |
|-------------------|----------------|---------------------|---|----|---|
| Groups            | 4              | 2.45 × 10^7         | 6.13 × 10^6 |     |    |
| Error             | 10             | 1.72 × 10^7         | 1.72 × 10^6 |     |    |
| Total             | 14             | 4.17 × 10^7         | 2.98 × 10^6 | 3.564 | 3.478 | 0.0469 |

Table S-14. Welch test for area–under–the–curve values (µg/mL-time) for 24 h.

| Degree of Freedom N₁ | Degree of Freedom N₂ | F | Fc | p |
|----------------------|----------------------|---|----|---|
| 4                    | 4                    | 7.306 | 6.388 | 0.0400 |

Table S-15. Tukey test for area–under–the–curve values (µg/mL-time) for 24 h.

| Pairing | Difference of Means | Standard Error | q | qc |
|---------|---------------------|----------------|---|----|
| 4 vs. 1 | 3968 | 757 | 5.238 | 4.654 |
| 4 vs. 5 | 2481 | 757 | 3.275 | 4.654 |
| 4 vs. 2 | 1839 | 757 | 2.428 | 4.654 |
| 4 vs. 3 | 1775 | 757 | 2.344 | 4.654 |
| 3 vs. 1 | 2193 | 757 | 2.895 | 4.654 |
| 3 vs. 5 | 706 | 757 | 0.932 | 4.654 |
| 3 vs. 2 | 64 | 757 | 0.084 | 4.654 |
| 2 vs. 1 | 2129 | 757 | 2.810 | 4.654 |
| 2 vs. 5 | 642 | 757 | 0.847 | 4.654 |
| 5 vs. 1 | 1487 | 757 | 1.963 | 4.654 |

* For the pairings: (1) Crystalline Phenytoin, (2) HPMCAS + Drug (10 wt %), (3) Poly(NIPAm46-co-DMA54) + Drug (10 wt %), (4) Poly(NIPAm70-co-DMA30) + Drug (10 wt %), and (5) Poly(NIPAm70-co-DMA30) + Drug (25 wt %).

2.9 Experiment 9: In vivo AUC Enhancement summary. We applied a similar analysis of the Area–under–the–curve (AUC) Enhancement for the in vivo results at both 6 and 24 h, shown in Equation S-9. Fig. S-31 shows a comparison of the AUC Enhancement values. Qualitatively,
we show that the microcentrifuge dissolution test provides good in vitro – in vivo correlation in predicting oral bioavailability.

\[ \text{AUC}_t \text{ Enhancement} = \frac{\text{AUC}_{\text{solid dispersion for time } t}}{\text{AUC}_{\text{drug only for time } t}} \]  

[S-9]

**Figure S-31.** Area–under–the–curve (AUC) Enhancement for the pharmacokinetic profiles of formulations tested in vivo. The in vitro AUC\textsubscript{6h} Enhancement values (solid pink) and error bars denote the mean and range for the measured data. The in vivo AUC\textsubscript{6h} Enhancement and AUC\textsubscript{24h} Enhancement values (dashed purple and yellow, respectively) and error bars denote the mean and propagated standard error of the mean for \(N = 3\).

2.10 **Experiment 10: In vivo ALT/AST enzyme toxicity assay.** Tables S-16–S-19 show results of the statistical analysis of the in vivo ALT/AST enzyme toxicity assay.
Table S-16. AST/ALT ratio values.

| Formulation Group                        | Animal 1 | Animal 2 | Animal 3 |
|------------------------------------------|----------|----------|----------|
| Crystalline Phenytoin                    | 1.52     | 2.22     | 1.91     |
| HPMCAS + Drug (10 wt %)                  | 1.89     | 1.29     | 1.94     |
| Poly(NIPAm46-co-DMA54) + Drug (10 wt %)  | 1.24     | 1.86     | 2.80     |
| Poly(NIPAm70-co-DMA30) + Drug (10 wt %)  | 1.53     | 1.61     | 1.60     |
| Poly(NIPAm70-co-DMA30) + Drug (25 wt %)  | 1.77     | 2.00     | 1.40     |

Table S-17. ANOVA test for AST/ALT ratio values.

|                              | Degree of Freedom | Sum of Squares | Mean Sum of Squares | F      | Fc     | p     |
|------------------------------|-------------------|----------------|---------------------|--------|--------|-------|
| Groups                       | 4                 | 0              | 0                   |        |        |       |
| Error                        | 10                | 2              | 0                   |        |        |       |
| Total                        | 14                | 2              | 0                   | 0.365  | 3.478  | 0.828 |

Table S-18. Welch test for AST/ALT ratio values.

| Degree of Freedom N₁ | Degree of Freedom N₂ | F     | Fc    | p     |
|----------------------|----------------------|-------|-------|-------|
| 4                    | 4                    | 0.215 | 6.388 | 0.917 |

Table S-19. Tukey test for AST/ALT ratio values.

| Pairing | Difference of Means | Standard Error | q | q<sub>c</sub> |
|---------|---------------------|----------------|---|---------------|
| 3 vs. 4 | 0.39                | 0.25           | 1.523 | 4.654 |
| 3 vs. 2 | 0.26                | 0.25           | 1.030 | 4.654 |
| 3 vs. 5 | 0.24                | 0.25           | 0.952 | 4.654 |
| 3 vs. 1 | 0.08                | 0.25           | 0.324 | 4.654 |
| 1 vs. 4 | 0.30                | 0.25           | 1.199 | 4.654 |
| 1 vs. 2 | 0.18                | 0.25           | 0.706 | 4.654 |
| 1 vs. 5 | 0.16                | 0.25           | 0.628 | 4.654 |
| 5 vs. 4 | 0.14                | 0.25           | 0.571 | 4.654 |
| 5 vs. 2 | 0.02                | 0.25           | 0.078 | 4.654 |
| 2 vs. 4 | 0.13                | 0.25           | 0.493 | 4.654 |

* For the pairings: (1) Crystalline Phenytoin, (2) HPMCAS + Drug (10 wt %), (3) Poly(NIPAm46-co-DMA54) + Drug (10 wt %), (4) Poly(NIPAm70-co-DMA30) + Drug (10 wt %), and (5) Poly(NIPAm70-co-DMA30) + Drug (25 wt %).

3 Author Roles and Responsibilities

J.M.T. led the design, data acquisition, interpretation, and writing of the reported polymer synthesis/characterization, screening, in vitro, and in vivo experiments. S.T. participated in the
design, data acquisition, interpretation, and drafting of the polymer synthesis/characterization, screening, and in vitro experiments. A.A.P. participated in the design, data acquisition, and interpretation of the screening, NMR, and in vitro experiments. S.D.J. contributed to the data acquisition of the polymer synthesis/characterization, screening, and in vitro experiments. L.W. and Z.P.T. contributed to the design of the polymer synthesis/characterization. L.G. and S.J.G. contributed to the design, data acquisition, interpretation, and writing of the high-throughput synthesis/characterization. F.S.B. was the co-principal investigator of the project and directed the research, managed the project, and contributed to the data analyses and interpretation of the polymer synthesis/characterization, screening, and in vitro experiments. T.M.R was the co-principle investigator of the project and directed the research, managed the project, and contributed to the design, data analyses, and interpretation of the polymer synthesis/characterization, screening, in vitro, and in vivo experiments.

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