Dissolution and Solubility Enhancement of the Highly Lipophilic Drug Phenytoin via Interaction with Poly(N-Isopropylacrylamide-*co*-Vinylpyrrolidone) Excipients

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SUPPORTING INFORMATION

General

Materials: All chemicals were reagent grade and used as received unless otherwise noted. All solvents were HPLC or analytical grade. *N*-isopropylacrylamide (NIPAAm, Aldrich, ≥99.9%), 5,5-Diphenylhydantoin (Phenytoin, Aldrich, ≥99.9%), dimethylformamide (DMF, Aldrich, 99.8%), methanol (fisher), diethyl ether (anhydrous, Fisher), and tetrahydrofuran (THF, Fisher). Fasted simulated intestinal fluid powder (FaSSIF, consisting of 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-Vinyl-2-pyrrolidone (VP, Aldrich, ≥99.9%) was purified by distillation under vacuum and stored at –20 °C refrigerator under nitrogen. 2*,2'*-Azobis(2-methylpropionitrile) (AIBN, Aldrich, 98%) was recrystallized from methanol and stored in a dark, –20 °C refrigerator. Phosphate buffered saline (PBS, pH = 6.5) was prepared from 82 mM sodium chloride (Fisher, ≥99.0%), 20

mM sodium phosphate dibasic heptahydrate (Fisher, 98%), and 47 mM potassium phosphate monobasic (J.T. Baker, ≥99.0%).

Copolymerization procedure

The monomer reactivity ratios, evaluated by ^{1}H NMR experiments for the NIPAAm (M_1) and VP (M_2) pair under similar reaction conditions, are reported in the literature to be r_1-= 0.47 and r_2= 0.5.[1] The method for the synthesis of poly(NIPAAm-*co*-VP) 60:40 are as follows:NIPAAm (2.04 g, 0.018 mol), VP (1.28 mL, 0.012 mol), AIBN (10 mg, 3×10^{-5} mol), and DMF (15 mL) were placed in a round bottom flask. The reaction mixture was flushed with nitrogen gas for at least 30 min and stirred in a thermostated oil bath at 70 °C. The polymerizations were quenched after approximately five hours by cooling to 0 °C and opening the flask to air. The resultant poly(NIPAAm-*co*-VP) was isolated from the reaction mixture by precipitation with diethyl ether followed by filtration under vacuum. The copolymer obtained was re-dissolved in methanol, precipitated and filtered under vacuum, and washed several times with diethyl ether to remove residual monomer and DMF. The solid was dried under vacuum at 40 °C overnight to yield 2.92g (87%) of the copolymer. A similar procedure was used to synthesize all the polymer compositions.

Copolymer Characterization:

Copolymer composition: The chemical compositions of the polymers synthesized were analyzed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy carried out in a Bruker Avance III HD 500 spectrometer at 22 °C. The percentage of each monomer in the polymer was calculated by comparing the characteristic monomer proton integrations of C-(**CH$_3$**)$_2$ (1.1 ppm, 6H) for NIPAAm with C-**CH$_2$** (3.2 ppm, 2H) for VP.

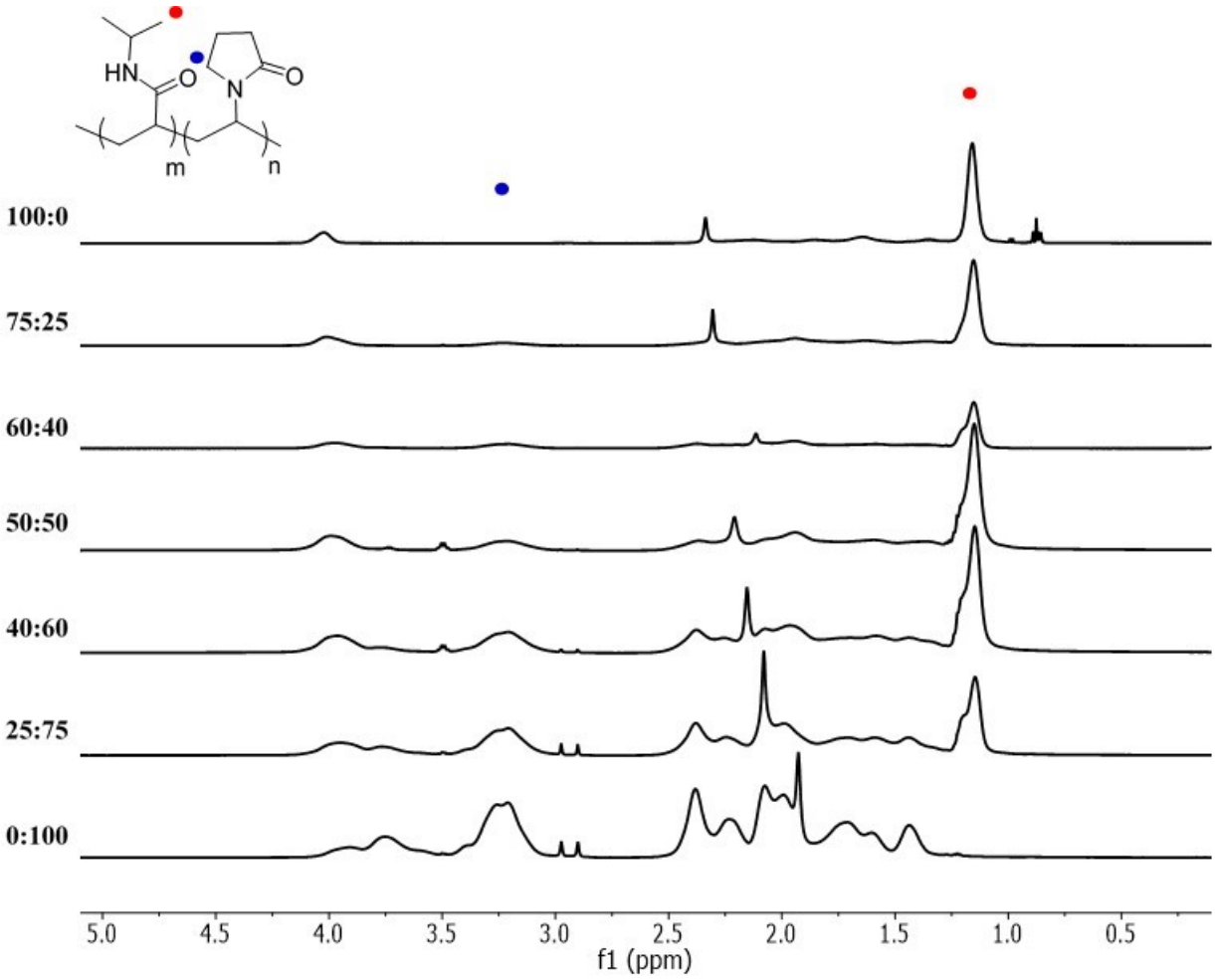

Figure S-1. (top to bottom) ^{1}H NMR spectra of poly(NIPAAm-*co*-VP) 100:0, 75:25, 60:40, 50:50, 40:60, 25:75, and 0:100 in $CDCl_3$. The percentage of each monomer in the polymer chain was calculated by comparing the characteristic monomer proton integrations of C-$(CH_3)_2$ (1.1 ppm, 6H,●) for NIPAAm with C-CH_2 (3.2 ppm, 2H,●) for VP.

The molecular weights and dispersity values were determined by size exclusion chromatography (SEC) conducted on an Agilent 1260 Infinity liquid chromatograph equipped with an Agilent 1260 Infinity Variable Wavelength Detector monitoring at 254 nm (80 Hz data collection frequency), a Wyatt Dawn Heleos II multiangle laser light scattering (MALS) detector

at a laser wavelength of 663.6 nm (18 angles from 10° to 160°), and a Wyatt Optilab T-rEX refractive index detector operating at 658 nm. Tetrahydrofuran (THF) was run as the mobile phase at 1.0 mL/min at 25 °C. The dn/dc values were measured in THF assuming 100% mass recovery. Figure S-2 contains a representative SEC chromatogram of poly(NIPAAm-*co*-VP) 50:50 composition.

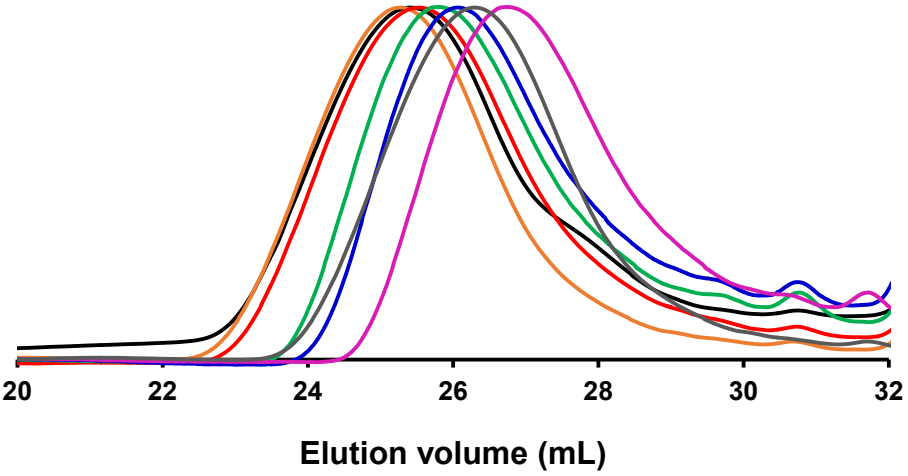

Figure S-2. SEC chromatogram of poly(NIPAAm-*co*-VP) 100:0 (●), 75:25 (●), 60:40 (●), 50:50 (●), 40:60 (●), 25:75 (●) and 0:100 (●).

Thermal behavior: The glass transition temperature of the polymers was determined using differential scanning calorimetry (DSC) using a TA Instruments Discovery DSC. All samples (~4−6 mg) were placed in T-zero aluminum pans. The temperature was ramped between 0 °C and 180 °C at a rate of 10 °C/min and the Tg values were analyzed using TA TRIOS software version 2.2. The second heating scans are reported.

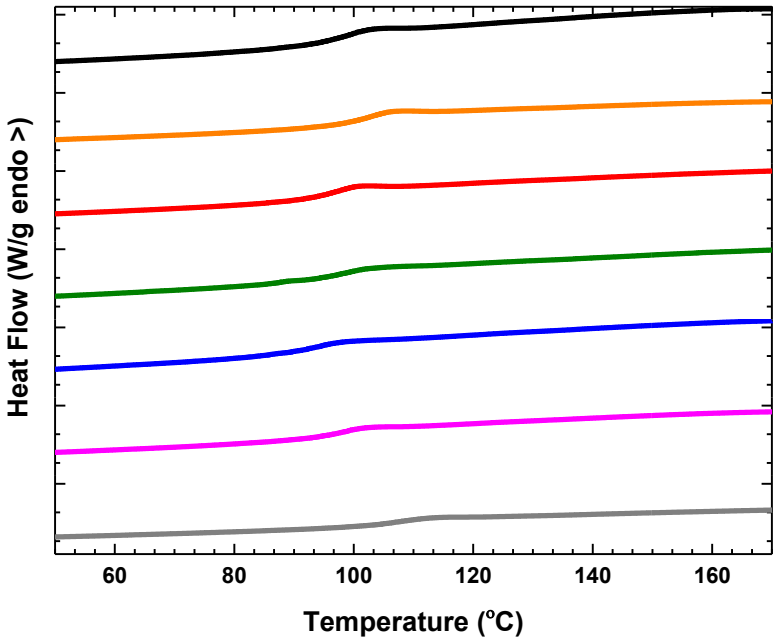

Figure S-3. DSC traces of total heat flow for poly(NIPAAm-*co*-VP) 100:0 (●), 75:25 (●), 60:40 (●), 50:50 (●), 40:60 (●), 25:75 (●) and 0:100 (●) polymers. Samples were analyzed between 0 °C and 180 °C with a ramp of 10 °C/min. Second heating scans are shown.

Lower critical solution temperature (LCST) measurements: LCST measurements were performed on a UV-Vis spectrophotometer equipped with a heating system and temperature control unit. The temperature of the polymer solutions (1.0 wt.%) in phosphate buffered saline (as described in *Materials*) supplemented with 0.5 wt % FaSSIF at pH 6.5 was increased at a rate of 0.2 °C/min starting from 25 °C to 70 °C. The % transmittance of the solutions at a wavelength of 450 nm was recorded at 0.2 °C intervals. The LCST was taken as the midpoint between the onset and end set of the transmittance (%) decay.

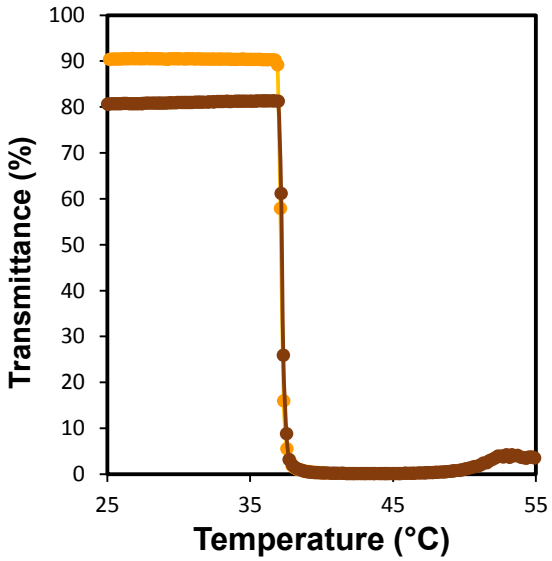

Figure S-4: Copolymer composition-LCST behavior relationship for poly(NIPAAm-*co*-VP) 75:25 free polymer (●), and spray dried polymer (●) dissolved in PBS + FaSSIF to make a final concentration of 1wt% of polymer. The LCST of the free polymer is 37 °C and does not change upon spray drying.

Polymer solubility measurements: For each polymer, approximately 20 mg was weighed into a glass vial and the weight was recorded. Aliquots of phosphate buffered saline (as described in *Materials*) supplemented with 0.5 wt % FaSSIF at pH 6.5 was added to each vial followed by vortexing for 1-2 minutes until the polymer is completely dissolved. Solubility was calculated using the amount of polymer and the volume of dissolution media used.

SDD preparation by spray drying. Samples of phenytoin in combination with different polymer excipients at 10 and 25 wt% were dissolved in a suitable amount of THF. The solvent was subsequently removed by spray drying to obtain the amorphous material. The spray drying was carried out using Bend Research Mini Spray Drier under the following conditions: inlet temperature of 68 °C, nitrogen flow rate of 28.6 SLPM, a 1.3 mL/min syringe flow rate, and

collected on a 4“ Whatman filter. Unless otherwise noted, the total solute content spray dried was always 1 wt %.

Solid-state Characterization of SDDs

Powder X-Ray Diffraction (PXRD) Analysis: PXRD experiments were carried out on a Bruker-AXS (Siemens) D5005 diffractometer. Samples were packed into standard glass holders with zero background. Data for each sample was collected from 5° to 40° on the 2θ scale over approximately 30 minutes at a scan step of 0.5 seconds and a step size of 0.02°/s. The x-ray source (KCuα, λ = 1.54 Å) was operated at a voltage of 45 kV and a current of 40 mA.

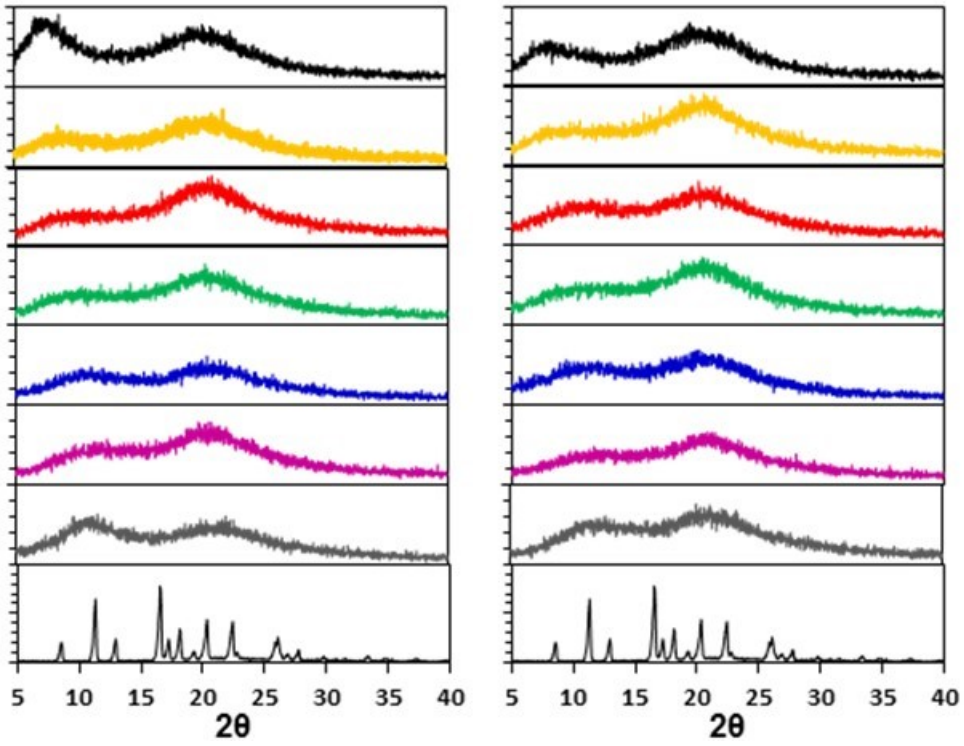

Figure S-5. PXRD patterns for SDDs with phenytoin loadings of 10 wt % (a) and 25 wt % (b) comparing poly(NIPAAm-*co*-VP) 100:0 (●), 75:25 (●), 60:40 (●), 50:50 (●), 40:60 (●), 25:75 (●) and 0:100 (●). PXRD pattern for crystalline phenytoin is shown for comparison. All SDDs were found to be amorphous.

In Vitro dissolution testing. Dissolution testing was performed on the SDDs, a drug-polymer physical mixture, and crystalline drug to determine the amount of soluble drug and the supersaturation maintenance profile. Dissolution testing medium consisted of phosphate buffered saline (as described in *Materials*) supplemented with 0.5 wt % FaSSIF adjusted to pH 6.5.

Each sample was accurately weighed into 2.0 mL microcentrifuge tubes to give an ultimate total drug concentration of 1,000 mg/mL if fully dissolved. To begin the testing, 1.8 mL of PBS + FaSSIF medium (pH 6.5, 37 °C) was added to the tubes, which were then vortexed for one minute and placed into an aluminum heating block held at 37 °C. At 4, 10, 20, 40, 90, 180, and 360 minutes the tubes were centrifuged at 13 000 rpm, 37 °C for 1 minute, then 50 µL of supernatant was aliquotted into HPLC vials. The samples were then vortexed for 30 seconds and placed back into the 37 °C block until the next time point. The supernatant in the HPLC vials was then diluted with 250 µL of methanol and analyzed for drug via HPLC.

The HPLC consisted of a reversed-phase EC-C18 column (Poroshell 120, 4.6 x 50 mm, 2.7 µm, Agilent, USA). For phenytoin detection, a mobile phase of 40:60 (v/v) acetonitrile:water was pumped at a flow rate of 1.0 mL/min at 30 °C. A 10 µL aliquot of sample was injected, and the column effluent was detected at 241 nm with a UV detector (1260 Infinity Multiple Wavelength Detector, Agilent). Phenytoin was detected at 215 nm and the sample concentration was determined using a calibration curve of 0.1 – 500 µg/mL concentrations.

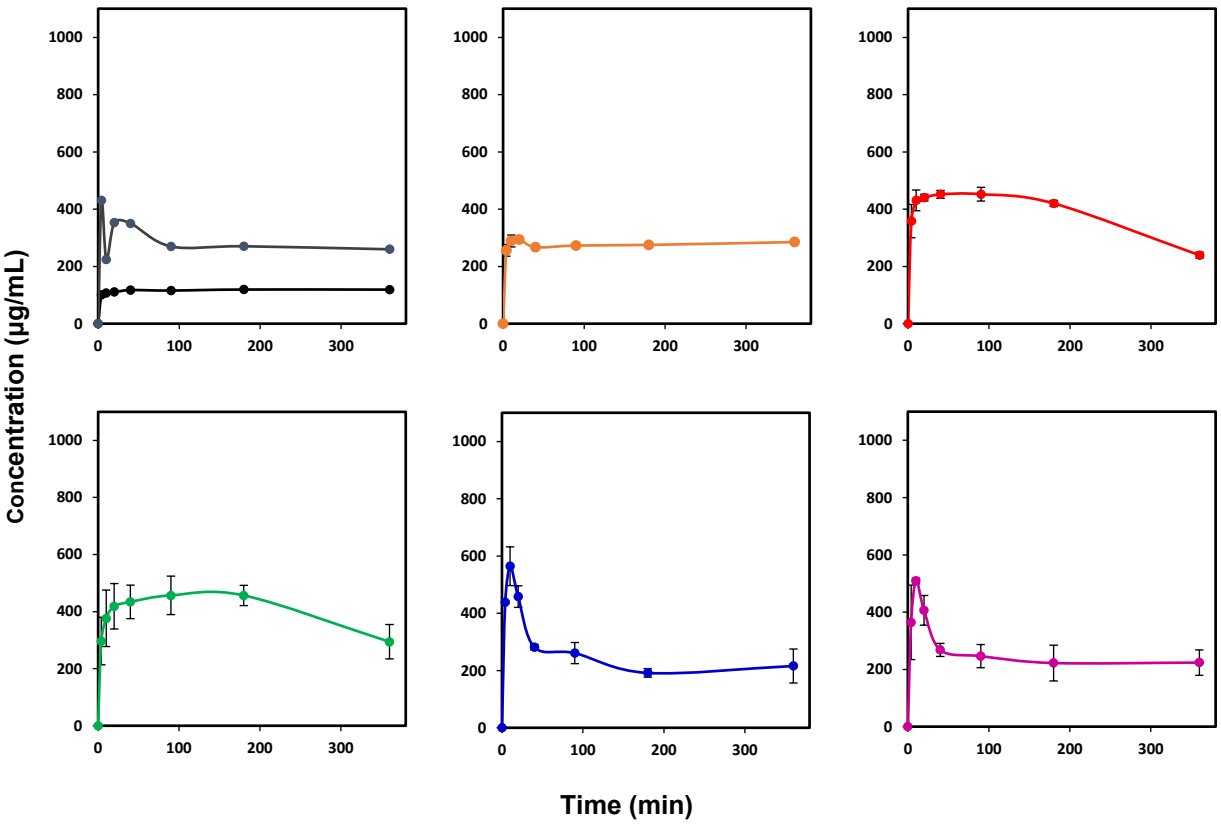

Figure S-6. Dissolution data of the SDDs with 25 wt % phenytoin loading at 37 °C. Polymer compositions used are, poly(NIPAAm-*co*-VP) 100:0 (●), 75:25 (●), 60:40 (●), 50:50 (●), 40:60 (●), 25:75 (●) and 0:100 (●). The target concentration of phenytoin was 1000 µg/mL. Error bars represent the standard deviation where n=2.

1. Dincer, S.; Rzaev, Z.; Piskin, E. Synthesis and characterization of stimuli-responsive poly(N-isopropylacrylamide-*co*-N-vinyl-2-pyrrolidone). *J. Polym. Res.* **2006,** *13*, 121-131.