**SUPPORTING INFORMATION**

# PRECISE CONTROL OF DRUG LOADING AND RELEASE OF NSAID-POLYMER CONJUGATE FOR LONG TERM INTRA-ARTICULAR DRUG DELIVERY

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## 1. Materials

Diclofenac was purchased from Beta Pharm Company Ltd. (batch number: 20100628o; assay: 99.12%; Shanghai, China). L-Lysine ethyl ester diisocyanate (ELDI) was obtained from Commonwealth Science and Industrial Research Organization (CSIRO). 4-hydroxybenzoic acid (≥99%), *cis*-1,3-*O*-benzylidene glycerol (97%) poly(ethylene glycol) (PEG, $M_n$ = 200, 400 and 1000 Da), dibutyltin dilaurate (DBTDL) (95%), *N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, ≥98%), ammonium acetate (≥98%), *N,N′*-dicyclohexylcarbodiimide (DCC) (≥99%), 4-(dimethylamino)pyridine (DMAP) (≥99%), acetic anhydride (≥ 98%), *N,N*-dimethylacetamide (99%), triethylamine (≥99%), pyridine (≥99%) lithium bromide (≥99%), hydrochloric acid (37%), acetic acid (glacial, ≥99.85%) were obtained from Sigma Aldrich and used as received. Sodium bicarbonate ($NaHCO_3$) and sodium sulfate ($Na_2SO_4$) were obtained from Ajax Finechem and used as received. Tetrahydrofuran (≥99.9%, LiCrosolv), methanol (≥99.8%, LiCrosolv), dichloromethane (99.8%, Chem Supply), diethyl ether (99.5%, Chem Supply), toluene (EMPURE) and petroleum benzine 60-80 (EMPURE), deuterated chloroform (chloroform-d) (99.8%, Cambridge Isotope Lab Inc.) were used as received.

## 2. Characterizations

$^1$H and $^{13}$C NMR was performed at room T and recorded on Bruker Advance III 400 MHz spectrometer using an external lock and referenced to the deuterated solvent.

Gel permeation chromatography (GPC) analysis of the polymer samples were done on Shimadzu liquid chromatography system equipped with a Shimadzu RID-10A differential refractive index detector (λ = 633 nm) and Shimadzu SPD-20A ultraviolet detector connected to a 5.0μm bead-size guard column (50 × 7.8 mm) followed by three Shodex KF-805L columns (300 × 8 mm, bead size: 10 μm, pore size maximum: 5000 Å) in series operating at 40 °C. The eluent was N,N-dimethylacetamide (HPLC grade, with 0.03% w/v LiBr) and running at 1 mL/min. A molecular weight calibration curve was produced using polystyrene standards with narrow molecular weights distribution ranging from 500 to 2 × $10^6$ Da.

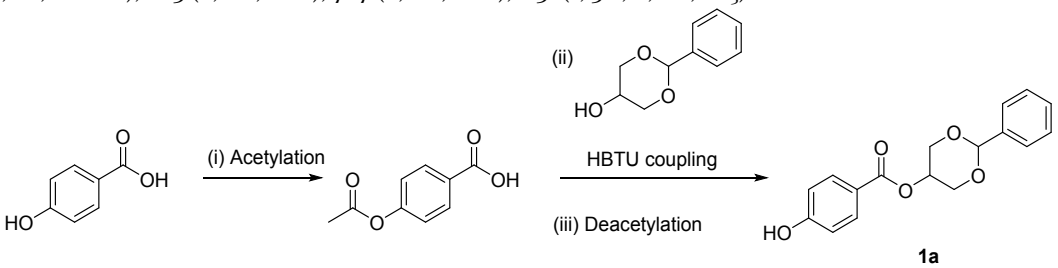
HPLC analysis was performed on Waters HPLC 2690 module using a Water Symmetry C18 guard column and connected to Water Symmetry C18 bonded reversed phase (5 μm particles, 3.9 internal diameter, 150 mm length column). An isocratic flow at 1 ml/min using acetonitrile:water:acetic acid (52:47:1) as mobile phase, using double injections of each sample and standards was utilized. Column oven was set to 30 °C. The injection volume was 10 μL. Quantification of the diclofenac and its corresponding lactam form was performed using a dual wavelength UV detector at 275 and 245 nm respectively. Standards of DCF and lactam were freshly prepared in a dynamic range, from 0.2 to 100 μg/mL. Calibration plots for each were constructed, both showing a correlation coefficient (R2) of 0.9999. The limit of detection (LOD) and quantification (LOQ) for DCF were 0.33 and 1.01 μg/mL respectively, and the LOD and LOQ for the lactam were 0.42 and 1.28 μg/mL.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra were recorded from a Shimadzu IR Tracer-100 Fourier Transform Infrared spectrometer by averaging 128 scans at a resolution of 4 $cm^{-1}$ in the MIR region of 4000-500 $cm^{-1}$.

Electrospray ionisation mass spectroscopy (ESI-MS) were acquired with a VG Platform mass spectrometer using a cone voltage of 50 V and the source was maintained at 80 °C. The solvent system used was acetonitrile with a flow rate of 0.04 mL min-1

**3. Experimental Methods**

**Synthesis of 4-Acetoxybenzoic acid**. Synthesis of 1a followed the previously reported method.[1] Briefly, (i) 4-hydroxybenzoic acid (1.08 g, 7.8 mmol) was first dissolved in a 5:1 mixture of toluene : THF (50 mL). To this solution pyridine (0.65 mL, 8.1 mmol) and acetic anhydride were added slowly at 0°C. The reaction mixture was then stirred at room temperature for 16 hrs. Solvent was removed *in vacuo*, and the product obtained as a white solid in quantitative yield. The product was thereafter used without further purification. TLC: $R_f$ = 0.50 (silica gel, DCM/MeOH = 15/1, v/v). LC-MS: $M+H^+$=181.0, $M+Na^+$=203.0; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ (ppm) 11.9 (br, 1H, COOH), 8.13 (d, 2H, ArH), 7.17 (d, 2H, ArH), 2.31 (s, 3H, d, 2H,$CH_3$).




**1a**

**Scheme S1**. Synthesis of 2-phenyl-1,3-dioxan-5-yl 4-hydroxybenzoate

**Synthesis of 2-phenyl-1,3-dioxan-5-yl 4-hydroxybenzoate** (1a). (ii) To a stirring suspension of 1a (5.24 g, 29.1 mmol) in anhydrous dichloromethane (120 mL), triethylamine (16 mL, 115.0 mmol), HBTU (11.5 g, 30.3 mmol) and *cis*-1,3- O-benzylidene glycerol (6.31 g, 34.9 mmol) were added. The reaction was stirred at room temperature with exclusion of light for 3 days. The mixture was then washed with saturated $NaHCO_3$ (3 x 100 mL), followed by 1M HCl (3 x 100 ml) and water (3 x 100 mL). The organic layer was dried over $Na_2SO_4$, and filtered through a short plug of silica. Solvent was removed *in vacuo* and the desired product obtained as a white solid in 77% yield. TLC: $R_f$ = 0.60 (silica gel, P.E./EtOAc = 2/1, v/v). LC-MS: $M+Na^+$=365.1; $^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.19 (d, 2H, ArH), 7.40 ~ 7.49 (m, 5H, ArH), 7.17 (d, 2H, ArH), 5.61 (s, 1H, CHPh), 4.41 (m, 1H, $CH(CH_2O)_2$), 4.3~4.1 (m, 4H, $(CH_2O)_2$), 2.31 (s, 3H, d, 2H, $CH_3$). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 168.76, 165.52, 154.55, 137.93, 131.51, 129.12, 128.32, 128.27, 126.08, 121.63, 101.34, 69.07, 66.56, 21.11.

(iii) Deacetylation was performed by adding ammonium acetate (13.8 g, 179.2 mmol) to a solution of 2-phenyl-1,3-dioxan-5-yl 4-acetoxybenzoate (7.63 g, 22.0 mmol) in 75% aqueous methanol (250 mL). The reaction mixture was stirred at room temperature for 16 hrs after which the solvent was removed *in vacuo*. The residue was then extracted with ethyl acetate (3 x 150 mL). The combined organic layer was dried over $Na_2SO_4$ and solvent removed under reduced pressure. Recrystallization from DCM and drying under vacuum gave the desired product (1b) as a white powder in 75% yield. TLC: $R_f$ = 0.40 (silica gel, hexane/EtOAc = 2/1, v/v). LC-MS: $M+Na^+$=323.1; $^1$H NMR (400 MHz, Acetone-$d_6$): $\delta$ (ppm) 7.98 (d, 2H, ArH), 7.30 ~ 7.5 (m, 5H, ArH), 6.96 (d, 2H, ArH), 5.69 (s, 1H, *C*HPh), 4.92 (m, 1H, COO*C*H), 4.4~4.2 (m, 4H, ($CH_2O)_2$).

**Synthesis of diclofenac *p*-hydroxybenzoic acid-1,3-dihydroxy glycerol ester (DCF-PHB-MG (2))**. *(i)* To a mixture of diclofenac (4.15 g, 14.0 mmol), DMAP (0.191 g, 1.6 mmol) and 1b (4.24 g, 14.1 mmol) in anhydrous dichloromethane (250 mL), a solution of DCC (3.71 g, 17.9 mmol) in anhydrous dichloromethane (20 mL) was added dropwise at 0°C over 30 minutes. The reaction mixture was stirred at 0°C for 2 hours. The solid by-product was removed by filtration through a thin bed of celite, and solvent was removed under reduced pressure. The crude material was purified via column chromatography on silica gel (20% ethyl acetate/hexanes, followed by 30% ethyl acetate / hexanes as eluents) to give 2-phenyl-1,3-dioxan-5-yl 4-(2-(2-((2,6-dichlorophenyl)amino)phenyl) acetoxy) benzoate as a white solid in 62% yield.$^1$H NMR (400 MHz, $CDCl_3$) δ 8.32 – 8.05 (m, 2H), 7.64 – 7.47 (m, 2H), 7.47 – 7.28 (m, 6H), 7.28 – 7.11 (m, 4H), 7.11 – 6.88 (m, 2H), 6.78 – 6.49 (m, 2H), 5.62 (s, 1H), 5.05 – 4.84 (m, 1H), 4.52 – 4.16 (m, 4H), 4.08 (s, 2H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 170.05, 165.47, 154.41, 142.67, 137.68, 131.55, 131.03, 129.45, 129.12, 128.85, 128.32, 126.07, 124.17, 123.56, 122.37, 121.63, 118.62, 101.37, 69.08, 66.58, 38.56. ESI-MS: *m/z* 581 (1%, $M^+$, $C_{31}H_{25}{}^{37}Cl_2NO_6$), 579 (4%, $M^+$, $C_{31}H_{25}{}^{37}Cl^{35}ClNO_6$), 577 (6%, $M^+$, $C_{31}H_{25}{}^{35}Cl_2NO_6$), 543 (3), 541 (5), 280 (14), 279 (24), 277 (32), 214 (68), 121 (100). 105 (33). IR $\nu_{max}$ ($cm^{-1}$): 3388, 3361, 2857, 1720, 1453, 1275, 1231, 1162, 1081, 1013, 767, 742.

*(ii)* To remove the benzylidene protecting group, 2-phenyl-1,3-dioxan-5-yl 4-(2-(2-((2,6-dichlorophenyl) amino)phenyl)acetoxy) benzoate (3.21 g, 5.50 mmol) was dissolved in 80% acetic acid in $H_2O$ and stirred at 60°C for 6 hours. The solution as cooled down to room temperature and stirred for a further 18 hours. The solution was dried *in vacuo* and then purified further *via* column chromatography on silica gel (gradient method using hexane/ethyl acetate 0 to 100%) to give the desired product DCF-PHB-MG, 2, in 70% yield as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.26 – 7.86 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 3H), 7.23 – 7.09 (m, 3H),

7.07 – 6.88 (m, 2H), 6.70 – 6.48 (m, 2H), 5.14 (p, $J$ = 4.7 Hz, 1H), 4.07 (s, 2H), 3.94 (d, $J$ = 4.7 Hz, 4H), 2.50 (bs, 2H). $^{13}$C NMR (50 MHz, $CDCl_3$) δ 170.10, 154.43, 142.66, 137.63, 131.38, 131.03, 129.44, 128.85, 128.41, 127.54, 124.21, 123.48, 122.37, 121.71, 118.59, 75.81, 62.43, 38.53. ESI-MS: $m/z$ 493 (2%, $M^+$, $C_{24}H_{21}{}^{37}Cl_2NO_6$), 491 (6%, $M^+$, $C_{24}H_{21}{}^{37}Cl^{35}ClNO_6$), 489 (8%, $M^+$, $C_{24}H_{21}{}^{35}Cl_2NO_6$), 280 (22), 279 (36), 278 (34), 277 (49), 250 (20), 216 (40), 214 (100), 121 (83). IR $\nu_{max}$ ($cm^{-1}$): 3541, 3326, 2981, 1741, 1703, 1504, 1453, 1302, 1230, 1121, 750.

**Synthesis of diclofenac-polymer conjugate (Po-19).** DCF-PHB-MG (**2**) (80.0 mg, 0.136 mmol) and different amount of PEG (no PEG for Po, PEG200 for P1-6, PEG400 for P7-13 and PEG1000 for P14-P19) were added into a 4 mL vial. ELDI was added into the mixture and the amount was adjusted such that there was an equimolar ratio of isocyanates to the hydroxyl groups. For example, to synthesize P2, add **2** (80.0 mg, 0.163 mmol) and PEG200 (10.3 mg, 0.052 mmol) into a 4 mL vial followed by the addition of ELDI (48.6 mg, 0.215 mmol). The mixture was solubilized using 0.400 mL of anhydrous DCM to give a clear, homogenous solution. Subsequently, 13.6 µL of dibutyltin dilaurate (DBTDL) stock solution in anhydrous DCM (100 mg/mL) (1.36 mg, 0.002 mmol) was added to the solution to start the polymerisation. The vial was sealed and heated to 35°C and the reaction was allowed to proceed for 24 hours and the solution turned into a clear pale yellow at the end of reaction. The polymer was isolated by precipitation in 30 mL diethyl ether/hexane (2/1 v/v%) twice to give an off white brittle solid when dried (75% yield, 104 mg). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.01 – 8.09 (m, 2H), 7.30 – 7.36 (m, 3H), 7.12 – 7.22 (m, 3H), 6.95 – 7.05 (m, 2H), 6.66 (s, 1H), 6.57 (d, $J$ = 6.58 Hz, 1H), 4.9-5.8 (m, 2H), 4.0-4.45 (m, 6H), 3.55-3.75 (m, PEG H backbone), 3.0-3.2 (m, 2H), 1.15-1.9 (m, 4H). GPC results are summarized in Table S1.

**Available Drug Assay.** 10 mg of polymer was weighed into a 20-mL scintillation glass vial and 15 mL of 10.0 M HCl solution was added. The vial was placed into a water bath at 37 °C with magnetic stirring at 300 rpm in order to dissolve the polymers completely. After 24 hours, 100 µL of sample was withdrawn, diluted half with phase and analysed by HPLC to determine the amount of diclofenac lactam present.

**In vitro Drug Release.** An accurately weighed sample of polymer (10 mg) was placed in a 20-mL scintillation glass vial. 15 mL of PBS pH 7.4 was then added and the vial placed in water bath at 37°C and the solution was gently stirred at 300 rpm to commence the release experiment. At designated pre-determined time points, 100 µL of sample solution was withdrawn, replaced with an equal volume of fresh medium and the samples were analysed directly by HPLC using the conditions described for the available drug assay above. The amount of the DCF released was determined using the DCF calibration curve..

**Table S1. Molecular weight and compositional characterization of linear DCF- polymer synthesized with various amount of PEG200, PEG400 and PEG1000 in the backbone**

| | Polymer | Feed Ratio | | | $^1$H NMR analysis (mol %) | | | PEG (w/w%) | GPC | | | Drug loading (DCF) (w/w%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ELDI | DCF-PHB-MG | PEG | ELDI | DCF-PHB-MG | PEG | | Mn (kDa) | Mw (kDa) | Đ | $^1$H NMR | HPLC |
| PEG200 | P1 | 50 | 44 | 6 | 51.9 | 42.2 | 5.9 | 3.5 | 9.62 | 19.1 | 1.98 | 36.9 | 33.7 |
| | P2 | 50 | 38 | 12 | 48 | 41 | 11 | 6.6 | 11.8 | 28.1 | 2.37 | 36.6 | 27.1 |
| | P3 | 50 | 32 | 18 | 51.8 | 28.6 | 19.5 | 13.1 | 10 | 23.9 | 2.38 | 28.6 | 25.2 |
| | P4 | 50 | 26 | 24 | 51 | 23.3 | 25.8 | 18.4 | 11.3 | 28.1 | 2.48 | 24.5 | 20.5 |
| | P5 | 50 | 20 | 30 | 50.6 | 17.9 | 31.5 | 23.6 | 10.9 | 29.6 | 2.69 | 20.4 | 17.7 |
| | P6 | 50 | 10 | 40 | 48.2 | 9.4 | 42.4 | 35.8 | 13.5 | 32.3 | 2.39 | 11.6 | 10.2 |
| PEG400 | P7 | 50 | 47 | 3 | 52.2 | 45.0 | 2.9 | 3.3 | 8.34 | 14.3 | 1.71 | 38.2 | 35.7 |
| | P8 | 50 | 44 | 6 | 51.0 | 44.4 | 4.7 | 5.3 | 6.74 | 9.29 | 1.35 | 37.6 | 24.9 |
| | P9 | 50 | 41 | 9 | 53.3 | 39.1 | 7.6 | 8.9 | 9.6 | 17.4 | 1.85 | 33.8 | 31.5 |
| | P10 | 50 | 38 | 12 | 53 | 37.1 | 9.9 | 11.7 | 9.44 | 15.9 | 1.68 | 32.1 | 26.1 |
| | P11 | 50 | 35 | 15 | 53 | 33.3 | 13.7 | 16.2 | 9.6 | 18.2 | 1.89 | 29.2 | 22.1 |
| | P12 | 50 | 30 | 20 | 53.2 | 28.1 | 18.7 | 22.4 | 9.95 | 19.9 | 2.01 | 25.0 | 18.3 |
| | P13 | 50 | 25 | 25 | 53.7 | 23.1 | 23.2 | 28.3 | 9.16 | 17.4 | 1.9 | 20.9 | 20.7 |
| PEG1000 | P14 | 50 | 47.6 | 2.4 | 53.8 | 43.5 | 2.7 | 7.2 | 5.05 | 11.4 | 2.26 | 36.4 | 32.0 |
| | P15 | 50 | 46.4 | 3.6 | 52.0 | 44 | 4.0 | 10.3 | 5.33 | 13.6 | 2.55 | 35.1 | 34.8 |
| | P16 | 50 | 45.2 | 4.8 | 52.3 | 42.5 | 5.2 | 13.8 | 5.27 | 12.9 | 2.46 | 33.2 | 30.2 |
| | P17 | 50 | 44 | 6 | 52.2 | 41.3 | 6.5 | 16.2 | 5.11 | 14.1 | 2.76 | 32.2 | 27.6 |
| | P18 | 50 | 42 | 8 | 51.3 | 39.5 | 9.2 | 22.3 | 6.26 | 22.2 | 3.56 | 29.3 | 25.3 |
| | P19 | 50 | 40 | 10 | 52.6 | 36.5 | 10.8 | 26.1 | 6.52 | 22.1 | 3.38 | 26.9 | 25.0 |

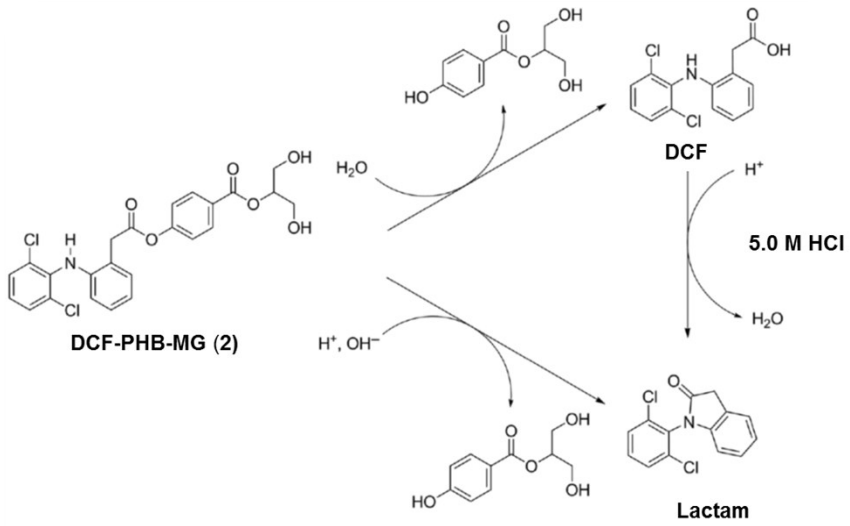



**Figure S1. Formation of lactam from DCF-PHB-MG (2), adapted from Wang et al.[2]**

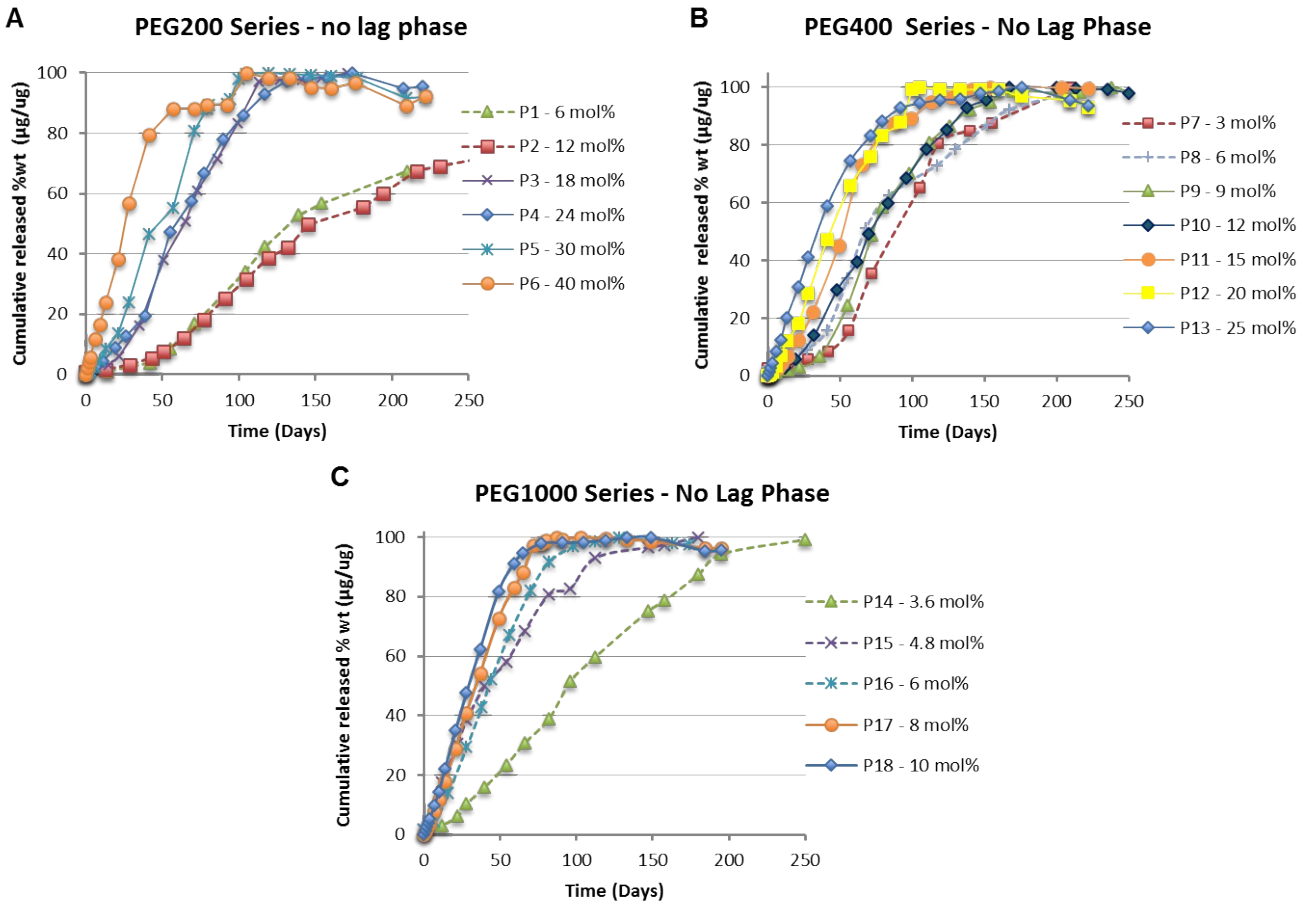



**Figure S2. Modified cumulative release of DCF from DCF-polymer having the lag phase removed containing various mol% of (A) PEG200, (B) PEG400 and (C) PEG1000 PEG in the backbone. The dotted line indicates polymers with lag phase.**

**References**


**(1)** D'Souza A. M.; Donohue A. C.; Tait R. J.; Graichen, F. H. M.; Ng S.; Sulistio A., Polymer–NSAID conjugate. Patent WO2014000033 A1, **2014**

(2) Wang, F.; Finnin, J.; Tait, C.; Quirk, S.; Chekhtman, I.; Donohue, A. C.; Ng, S.; D'Souza, A.; Tait, R.; Prankerd, R.; *J. Pharm. Sci.*, **2016**, 105, 773 – 785