Supporting Information:

Synthesis and Biological Evaluation of Well Defined Poly(propylene fumarate) Oligomers and their use in 3D Printed Scaffolds

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Large Batch Synthesis (Mn=1.27 kDa) Synthesis of Poly(maleic anhydride-co-propylene oxide)

Maleic anhydride (2.856 mol) and propylene oxide (2.856 mol) were dissolved in toluene (0.4 L) in a 2 L round-bottom flask at ambient temperature under nitrogen. After all monomers were dissolved in toluene with magnetic stirring, $Mg(OEt)_2$ (119 mmol; molar ratio of MAn/ $Mg(OEt)_2$ = 24:1) was added to the mixture and the flask was moved into a silicone oil bath equipped with a water reflux condenser to start polymerization at 80 °C for 40 h. After the designated polymerization time, the system was cooled to

room temperature under nitrogen, evaporated to remove all volatiles and then was diluted with $CHCl_3$, washed with water containing trace amount of HCl to remove the inorganic compound. The organic layer was poured into hexanes after rotary evaporation, and the precipitated polymer mixture was re-dissolved in a minimal amount of $CHCl_3$ that was then concentrated by rotary evaporation. Poly(malic anhydride-co-propylene oxide) was obtained after drying the product under vacuum overnight at room temperature to remove all volatiles, and then the molecular mass and mass distribution properties were characterized by SEC after ^{1}H NMR and ^{13}C NMR characterization (SEC: M_n 1200 Da; ^{1}H NMR please see Figure 1; ^{13}C NMR shown in Figure S1). ^{13}C NMR (300 MHz, Chloroform-*d*) δ (ppm): 164.64, 164.63, 164.35; 130.42, 129.92, 129.78, 129.25; 69.15; 66.37; 16.19.

Isomerization of Poly(maleic anhydride-co-propylene oxide)

Diethylamine (0.15 equivalent) was added to poly(maleic anhydride-co-propylene oxide) after dissolving the polymer in $CHCl_3$ (1 mol/L) in a round-bottomed flask equipped with a water reflux condenser to start isomerization at 55 °C for 20 h under nitrogen. The mixture was then concentrated by rotary evaporation and washed with phosphate buffer saline solution (0.5M, pH = 6) to remove the diethylamine. The organic layer was collected after separation and sodium sulfate was added into the organic layer to remove water. The concentrated organic layer was then precipitated into hexanes several times to remove impurities. The precipitate was collected and kept in vacuum overnight at room temperature to remove all volatiles. ^{1}H NMR and ^{13}C NMR were used for characterization (^{1}H NMR please see Figure 1; ^{13}C NMR shown in Figure S1). ^{13}C NMR (300 MHz, Chloroform-*d*) δ (ppm): 164.36, 164.35, 164.04, 163.98; 134.01, 133.27; 69.26; 66.58; 16.34.

Representative Data Analysis in Intrinsic Viscosity Test of PPF Samples (PPF 1 in Table 1)

Materials and equipments. Thermostated bath, Ubbelohde capillary viscometer (CANNON STATE COLLEGE, PA, 16804, 0016, USA, 50 L79), stopwatch(accuracy: 0.01s), poly(propylene fumarate) (PPF) samples, pure THF solvent, analytical balance, volumetric flasks (10mL), filter(0.45 μ m).

Preparation. Each PPF sample was weighed and diluted in THF in a volumetric flask (10mL). Pure THF was added into the volumetric flask to the 10mL line with a filter and then a stopper was plugged.

Measurement. The capillary viscometer was taken to be rinsed with pure THF firstly, which was then filled with pure THF to an appropriate level by a filter. The thermostated bath was heated to keep the temperature at 35 ^{o}C. The capillary viscometer was kept in the thermostated bath for at least 15 minutes for establishing the thermal equilibrium. An injector was used to make the liquid fill up to more than 1/3 of the top ball of the capillary viscometer and then allowed the liquid to flow down. A stopwatch was

used to record the time when the liquid flew over the first line on the capillary viscometer and stopped recording when the liquid passed the second line on the capillary viscometer. The time of this period was recorded. The flow time was measured for at least 5 times to get 3 times Δt among which no more than 0.2s. Then the THF in the capillary viscometer was poured out. The capillary viscometer was refilled by a filter with 5mL of the solution prepared of PPF and THF. The capillary viscometer was put back into the thermostated bath, allowing about 10 min for equilibration. The flow time was measured and recorded for at least 3 times as the procedures above. Then 5 mL, 3 mL and further 2 mL of pure THF solvent was added into the capillary viscometer by a filter respectively, and the corresponding flow time was measured and recorded for at least 3 times each as the procedures above. The flow times of the solutions with different concentrations (c_0, c_1, c_2, c_3, c_4) were obtained in the experiment. The average values of the flow times and the errors were calculated. The representative data of 700 Da PPF are shown in Table 1.

Table1

Flow times of 700 Da PPF solutions with different concentrations.

	700 Da PPF	THF	c_1	c_2	c_3	c_4
	c (g/L)	0.00	410.00	205.00	157.69	138.51
t(s)	t1	123.56	693.03	264.97	219.28	201.88
	t2	123.66	692.90	264.82	219.28	201.84
	t3	123.46	695.22	264.92	219.25	201.79
	t_{ave}	123.56	693.72	264.90	219.27	201.84
	σ(s)	0.10	1.30	0.08	0.02	0.05

Based on the data obtained from the experiment, a series of quantities were calculated by using the following equations.

$$\boldsymbol{\eta_r} = \frac{\eta_i}{\eta_0} = \frac{t_i}{t_0} \quad (1)$$

$$\boldsymbol{\eta_{sp}} = \eta_r - 1 \quad (2)$$

$$\boldsymbol{\eta_{inh}} = \frac{\ln\eta_r}{c} \quad (3)$$

$$\boldsymbol{\eta_{red}} = \frac{\eta_{sp}}{c} \quad (4)$$

wherein: η_r is the relative viscosity, η_{sp} is the specific viscosity, η_{inh} is the inherent viscosity, η_{red} is the reduced specific viscosity, η_i is the viscosity of the solution and η_0 is the viscosity of the solvent; t_i is the flow time of the solution and the t_0 is the flow time of the solvent; and c is the concentration of the solution. The results are shown in Table 2.

Table 2

The results of the calculations of 700 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp} = (\eta_r-1)$	η_{sp}/c
solvent	123.56					
c_2	264.90	2.143925	0.762638	0.003720	1.143925	0.005580
c_3	219.27	1.774603	0.573577	0.003637	0.774603	0.004912
c_4	201.84	1.633511	0.490732	0.003543	0.633511	0.004574

The intrinsic viscosity ([η]) may then be obtained by the Huggins equation and the Kraemer Equation where [η] is intrinsic viscosity and k', k'' are constants.

The Huggins equation:

$$\eta_{sp}/c=[\eta]+k'[\eta]^2c \qquad (5)$$

The Kraemer Equation:

$$\ln(\eta_r)/c=[\eta]+k''[\eta]^2c \qquad (6)$$

η_{sp}/c and $\ln(\eta_r)/c$ were both plotted versus c as shown in FIG. 11 by origin 8.0. (See also Table 3, below). For the fitted line of η_{sp}/c versus c on FIG S3, the linear fit was obtained by origin 8.0.

Table 3

Linear fit values of 700 Da PPF solutions

		Value	Standard Error
$(\ln \eta_r)/c$~c	Intercept	0.00322	0.000120255
	Slope	2.49391E-06	7.09936E-07
	Adj. R-Square	0.85008	
		Value	Standard Error
η_{sp}/c~c	Intercept	0.00253	1.34E-04
	Slope	1.49E-05	7.89E-07
	Adj. R-Square	0.99444	

According to FIG S3, relationship between reduced viscosity and concentration is $\eta_{sp}/c = 0.00253 + 0.0000149\ c$. Compared with equation 5, we can get:

$$[\eta] = 0.00253\ \text{L/g}$$

$$\sigma_{[\eta]} = 0.000134\ \text{L/g}$$

$$[\eta]_1 = (0.00253 \pm 0.000134)\ \text{L/g}$$

Similarly, the relationship between intrinsic viscosity and concentration is $\ln\eta_r/c = 0.00322 + 0.0000025c$, and by comparison with equation 6, obtained:

$$[\eta]_2 = (0.00322 \pm 0.00012)\ \text{L/g}$$

The average of [η] is treated as the final result:

$$[\eta]_{THF} = \frac{[\eta]_1 + [\eta]_2}{2} = \frac{0.00322 + 0.00253}{2} = 0.002875$$

$$\sigma_{[\eta]_{THF}} = \frac{1}{2}\sqrt{0.00012^2 + 0.000134^2} = 0.00009$$

$[\eta] = 0.00288 \pm 0.00009$ L/g

Error analysis. The errors can come from many aspects. (1) The concentration of the solutions may not precise; (2) The flow time may not be precise due to the errors in the reaction time of the laboratorian; (3) The temperature in the viscometer may not be equal to that of the thermostated bath.

1. Figures

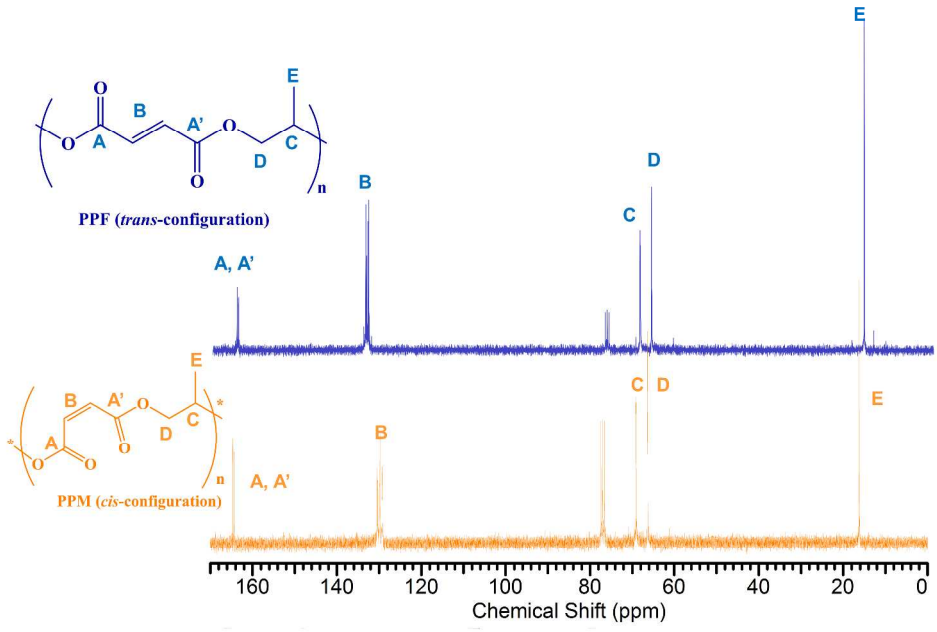

Figure S1. ^{13}C NMR for a poly(propylene malate) (PPM) intermediate and poly(propylene fumarate) (PPF).

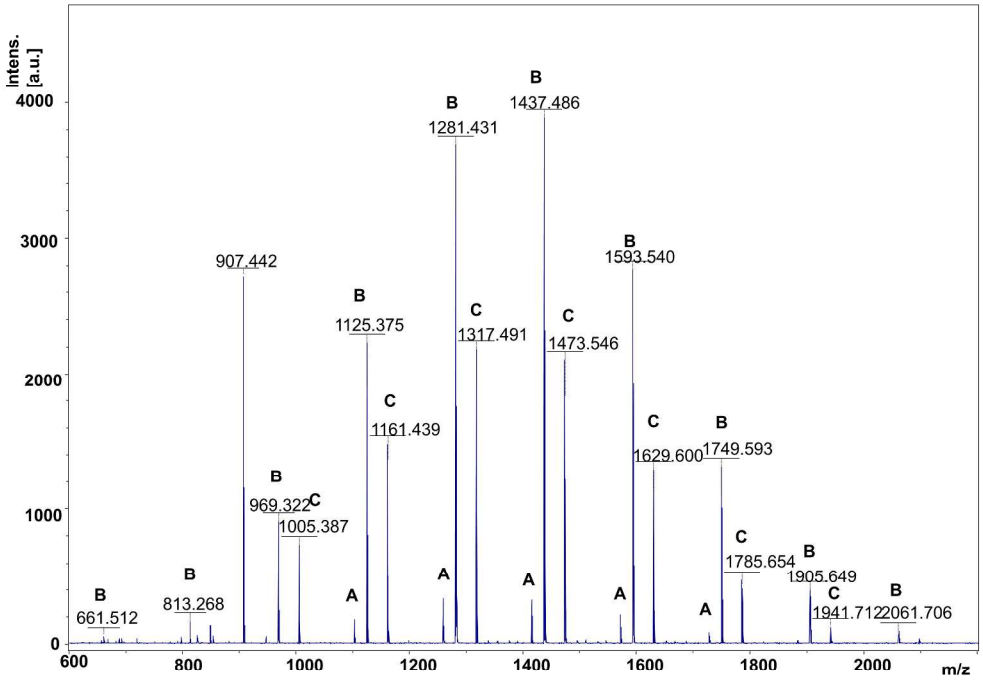

Figure S2. (A) MALDI-TOF mass spectrograph of PPF sample number 3 in Table 1 showing mass distribution in this sample.

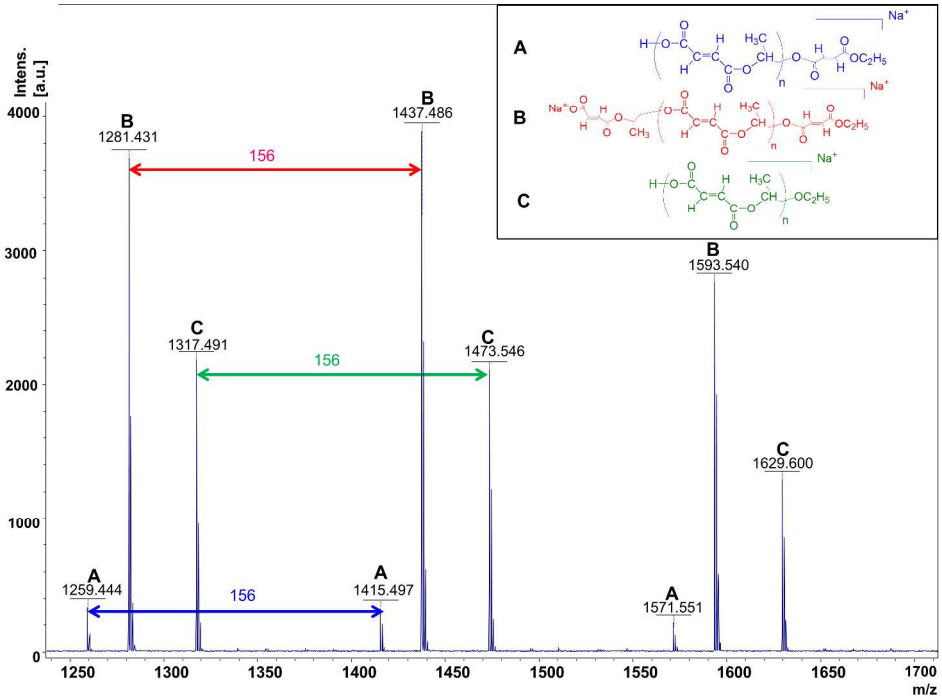

Figure S2. (B) Enlarged portion of a MALDI-TOF mass spectrograph of PPF sample number 2 in Table 1 showing the repeat unit in PPF and the possible end group chemistries which correspond the individual peaks in the distribution depicted in the mass spectrometry data.

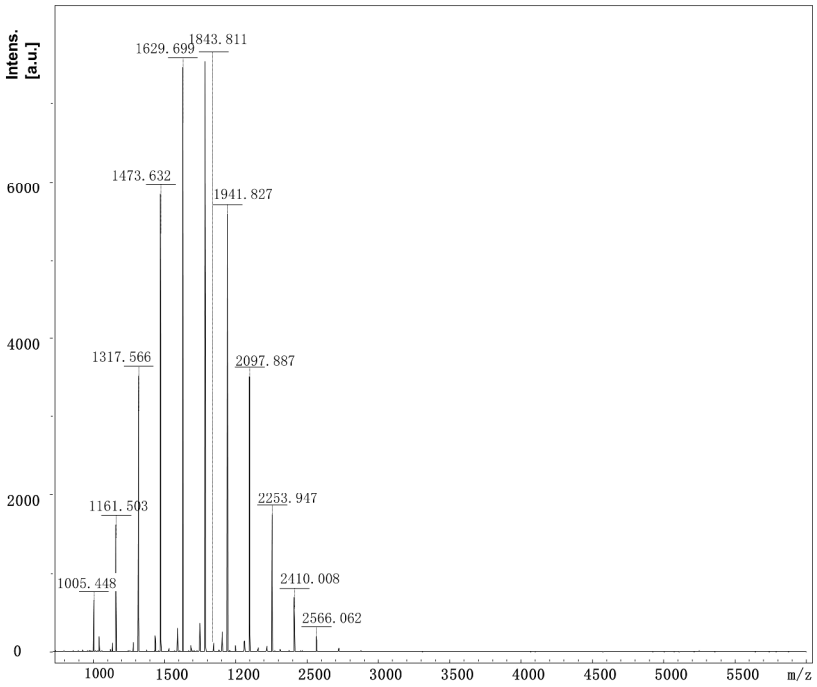

Figure S2. (C) MALDI-TOF mass spectrograph of PPF sample number 3 in Table 1 showing mass distribution in this sample.

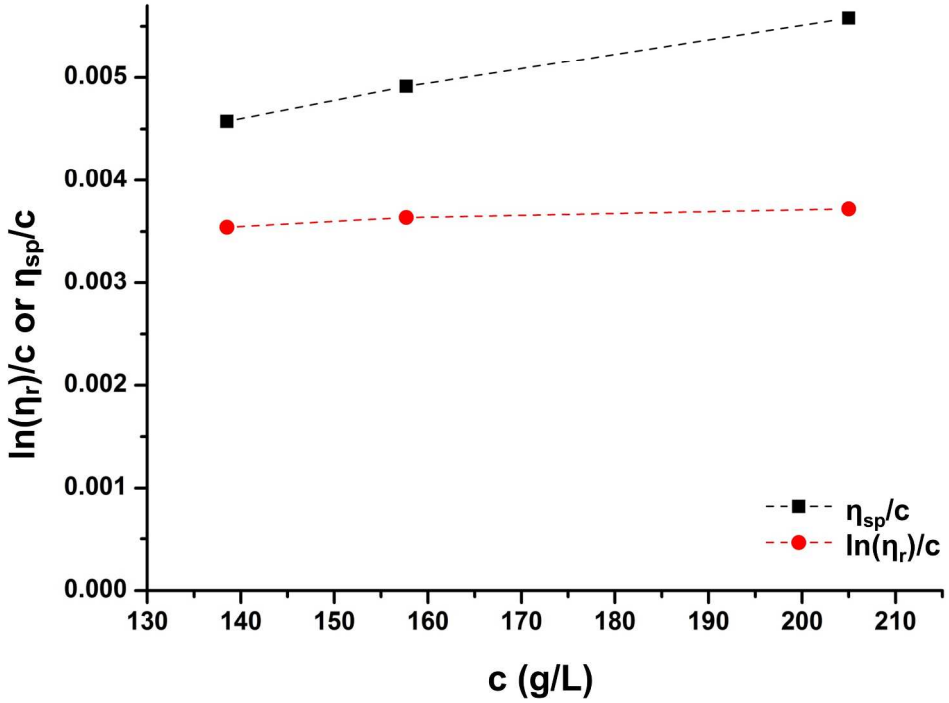

FIG S3. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 1 in Table 1.

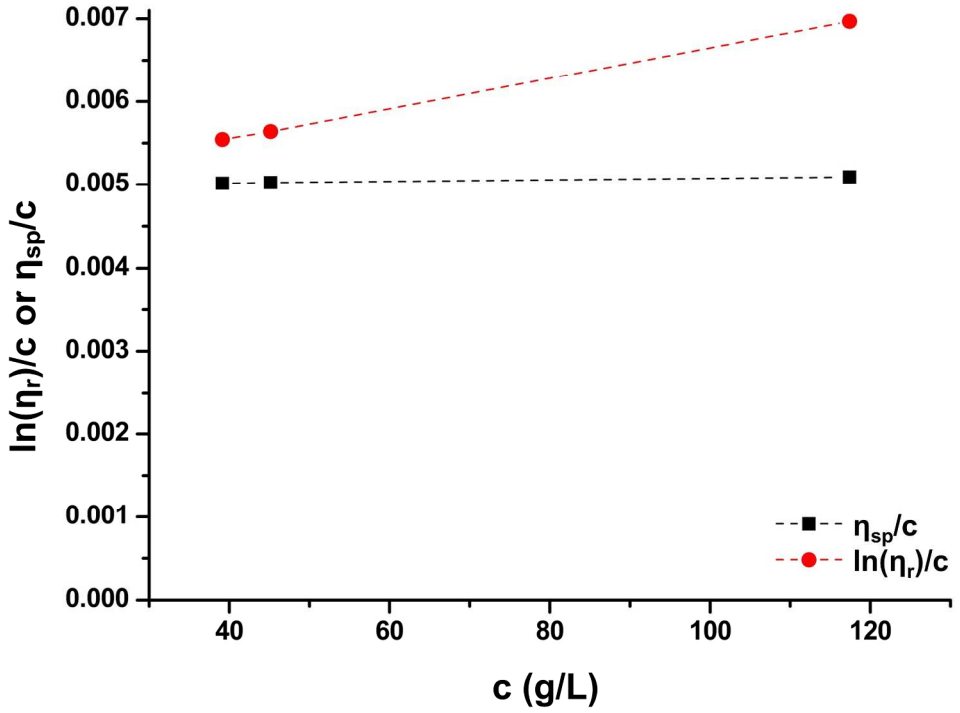

FIG S4. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 2 in Table 1.

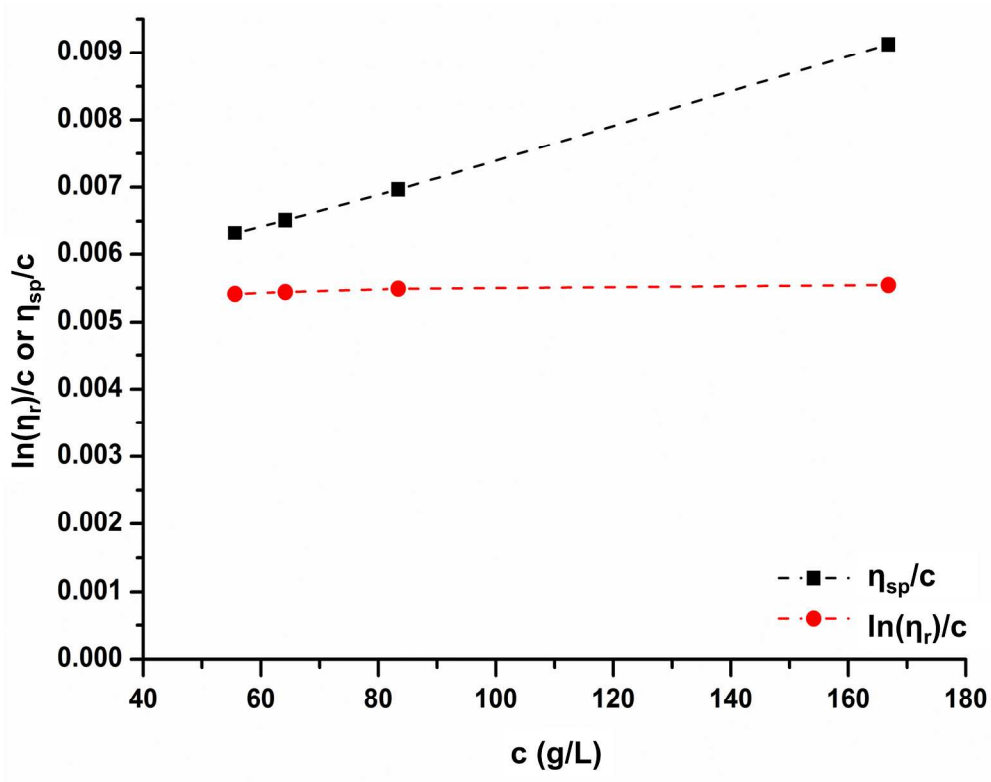

FIG S5. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 3 in Table 1.

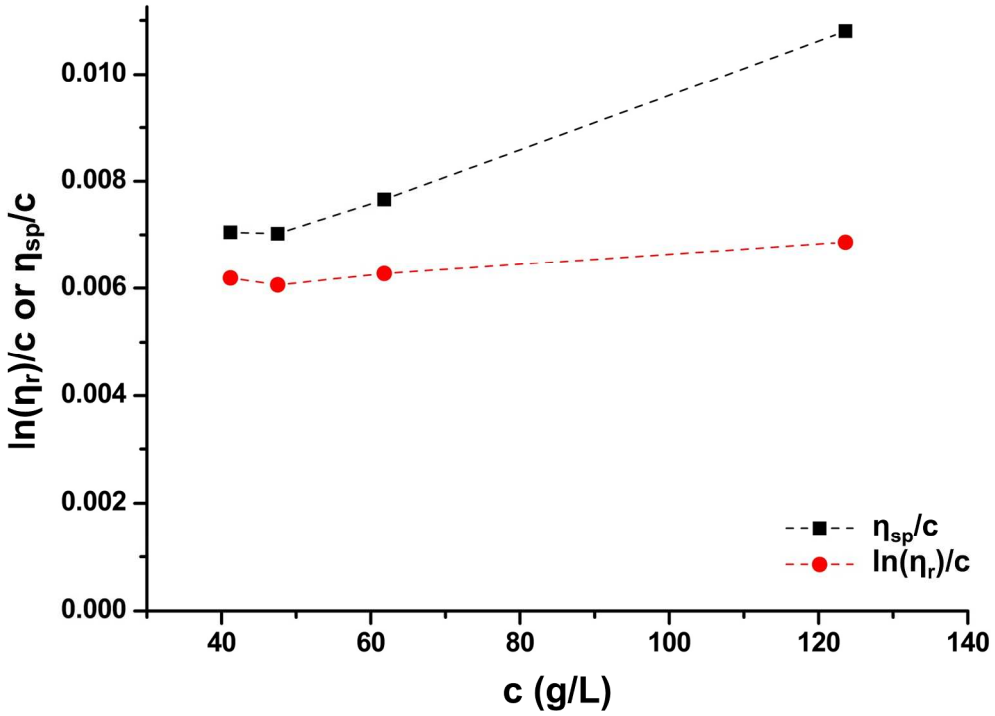

FIG S6. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 4 in Table 1.

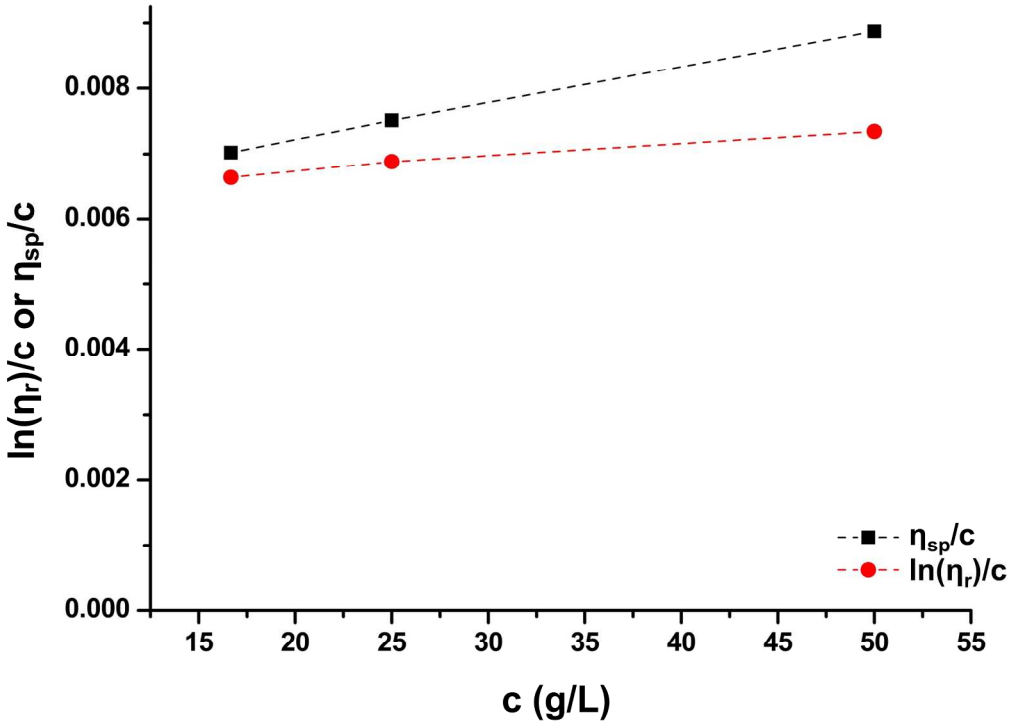

FIG S7. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 5 in Table 1.

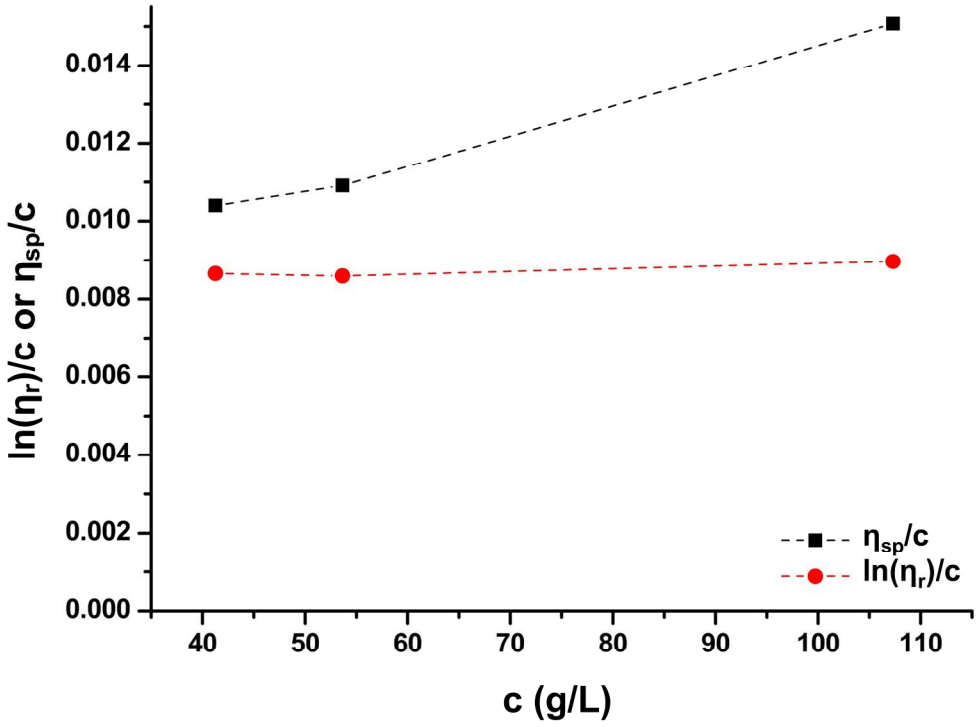

FIG S8. A graph showing η_{sp}/c and $\ln(\eta_r)/c$ versus c for PPF sample number 6 in Table 1.

2. Tables

Table 4

Flow times of 1270 Da PPF solutions with different concentrations.

	1270 Da PPF	THF	c_1	c_2	c_3	c_4
	c (g/L)	0.00	117.4	58.7	45.15	39.13
t(s)	t_1	123.56	224.75	165.41	155.07	150.37
	t_2	123.66	224.56	165.48	154.94	150.28
	t_3	123.46	224.63	165.5	155.06	150.43
	t_{ave}	123.56	224.65	165.46	155.02	150.36
	σ(s)	0.1	0.10	0.05	0.07	0.07

Table 5

The results of the calculations of 1270 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp} = (\eta_r - 1)$	η_{sp}/c
solvent	123.56	NA	NA	NA	NA	NA
c_1	224.65	1.818118	0.597802	0.005092	0.818118	0.006969
c_3	155.02	1.254640	0.226849	0.005024	0.254640	0.005639
c_4	150.36	1.216899	0.196306	0.005016	0.216899	0.005543

Table 6

Linear fit values of 1270 Da PPF solutions

$(\ln \eta_r)/c \sim c$		Value	Standard Error
	Intercept	0.00322	0.000120255
	Slope	2.49391E-06	7.09936E-07
	Adj. R-Square	0.85008	
		Value	Standard Error
$\eta_{sp}/c \sim c$	Intercept	0.00253	1.34E-04
	Slope	1.49E-05	7.89E-07
	Adj. R-Square	0.99444	

Table 7

Flow times of 1500 Da PPF solutions with different concentrations.

	1500 Da PPF	THF	c_1	c_2	c_3	c_4
	c (g/L)	0.00	166.84	83.42	64.17	55.61
t(s)	t_1	123.56	311.63	195.38	175.17	167.02
	t_2	123.46	311.66	195.29	175.30	166.99
	t_3	123.66	311.58	195.44	175.12	166.91
	t_{ave}	123.56	311.62	195.37	175.20	166.97
	σ (s)	0.10	0.04	0.08	0.09	0.06

Table 8

The results of the calculations of 1500 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp}=(\eta_r-1)$	η_{sp}/c
solvent	123.56	NA	NA	NA	NA	NA
c_1	311.62	2.522014	0.925058	0.005545	1.522014	0.009123
c_2	195.37	1.581148	0.458151	0.005492	0.581148	0.006967
c_3	175.20	1.417908	0.349182	0.005442	0.417908	0.006513
c_4	166.97	1.351354	0.301107	0.005414	0.351354	0.006318

Table 9

Linear fit values of 1500 Da PPF solutions

$(\ln \eta_r)/c \sim c$		Value	Standard Error
	Intercept	0.00538	0.0000292
	Slope	1.055E-06	2.85E-07
	Adj. R-Square	0.809	
		Value	Standard Error
$\eta_{sp}/c \sim c$	Intercept	0.00488	7.71E-19
	Slope	2.54E-05	6.65E-21
	Adj. R-Square	1	

Table 10

Flow times of 1860 Da PPF solutions with different concentrations.

	1860 Da PPF	THF	c_1	c_2	c_3	c_4
	c (g/L)	0.00	123.60	61.80	47.54	41.20
t(s)	t_1	123.56	288.66	182.10	164.82	159.47
	t_2	123.66	288.46	182.09	164.81	159.40
	t_3	123.46	288.50	181.94	164.78	159.44
	t_{ave}	123.56	288.54	182.04	164.80	159.44
	σ(s)	0.10	0.11	0.09	0.02	0.04

Table 11

The results of the calculations of 1860 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp} = (\eta_r - 1)$	η_{sp}/c
solvent	123.56	NA	NA	NA	NA	NA
c_1	224.65	1.818118	0.597802	0.005092	0.818118	0.006969
c_3	155.02	1.254640	0.226849	0.005024	0.254640	0.005639
c_4	150.36	1.216899	0.196306	0.005016	0.216899	0.005543

Table 12

Linear fit values of 1860 Da PPF solutions

		Value	Standard Error
$(\ln \eta_r)/c \sim c$	Intercept	0.00322	0.000120255
	Slope	2.49391E-06	7.09936E-07
	Adj. R-Square	0.85008	
		Value	Standard Error
$\eta_{sp}/c \sim c$	Intercept	0.00253	1.34E-04
	Slope	1.49E-05	7.89E-07
	Adj. R-Square	0.99444	

Table 13

Flow times of 2450 Da PPF solutions with different concentrations.

	2450 Da PPF	THF	c_1	c_2	c_3	c_4
	c (g/L)	0.00	50.00	25.00	19.23	16.67
t(s)	t_1	123.56	178.47	146.75	141.22	137.97
	t_2	123.66	178.34	146.69	141.16	137.97
	t_3	123.46	178.31	146.84	141.17	138.12
	t_{ave}	123.56	178.37	146.76	141.18	138.02
	σ(s)	0.00	50.00	25.00	19.23	16.67

Table 14

The results of the calculations of 2450 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp} = (\eta_r-1)$	η_{sp}/c
solvent	123.56	NA	NA	NA	NA	NA
c_1	178.37	1.443617	0.367152	0.007343	0.443617	0.008872
c_2	146.76	1.187763	0.172072	0.006883	0.187763	0.007511
c_4	138.02	1.117028	0.110672	0.006640	0.117028	0.007022

Table 15

Linear fit values of 2450 Da PPF solution

$(\ln \eta_r)/c \sim c$		Value	Standard Error
	Intercept	0.00633	7.20E-05
	Slope	2.05E-05	2.14E-06
	Adj. R-Square	0.97839	
		Value	Standard Error
$\eta_{sp}/c \sim c$	Intercept	0.00611	2.82E-05
	Slope	5.53E-05	8.37E-07
	Adj. R-Square	0.99954	

Table 16

Flow times of 3160 Da PPF solutions with different concentrations.

	3160Da PPF	THF	c_1	c_2	c_3
	c (g/L)	0.00	107.30	53.65	41.27
t(s)	t_1	123.56	323.32	195.81	176.50
	t_2	123.66	323.32	195.94	176.66
	t_3	123.46	323.50	195.97	176.60
	t_{ave}	123.56	323.38	195.91	176.59
	σ (s)	0.10	0.10	0.09	0.08

Table 17

The results of the calculations of 3160 Da PPF.

solutions	t_{ave} (s)	$\eta_r = t/t_0$	$\ln\eta_r$	$(\ln\eta_r)/c$	$\eta_{sp}=(\eta_r-1)$	η_{sp}/c
solvent	123.56	NA	NA	NA	NA	NA
c_1	323.38	2.617190	0.962101	0.008966	1.617190	0.015072
c_2	195.91	1.585519	0.460911	0.008591	0.585519	0.010914
c_3	176.59	1.429157	0.357085	0.008653	0.429157	0.010399

Table 18

Linear fit values of 3160 Da PPF solutions

$(\ln \eta_r)/c \sim c$		Value	Standard Error
	Intercept	0.00837	1.36E-04
	Slope	5.43E-06	1.86E-06
	Adj. R-Square	0.78926	
		Value	Standard Error
$\eta_{sp}/c \sim c$	Intercept	0.00722	4.10E-04
	Slope	7.28E-05	5.59E-06
	Adj. R-Square	0.98826	