

Water-soluble C₆₀- and C₇₀-PVP polymers for biomaterials with efficient ¹O₂ generation

Sean Oriana,^a Aroua Safwan,^a Xiao-Jing Ma^{a,b} Justus Oliver Butz Söllner,^a Yuko Iwamoto,^a and Yoko Yamakoshi^{*a,c}

Supporting Information

1. General. NMR spectra were recorded on Bruker 400 and Bruker 600 spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). FT-IR spectra were recorded on PerkinElmer Spectrum (PerkinElmer Inc., Waltham, MA, USA). Centrifugation was carried out by Eppendorf Centrifuge 5702R (Eppendorf, Hamburg, Germany). EPR spectra were recorded on Bruker spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a microwave bridge X-band ER 041 XG. All the solvents were purchased from Acros Organic (Thermo Fischer Scientific, Inc., Geel, Belgium). C₆₀ and C₇₀ were purchased from MTR Ltd. and Gd₃N@C₈₀ was from Luna Innovations (Roanoke, VA, USA). All the other reagents were purchased from corresponding suppliers and purified as described when needed.

2. Preparation of fullerene (C₆₀ or C₇₀)-PVP polymers. A solution of fullerene (C₆₀ or C₇₀), azobisisobutyronitrile (AIBN), *N*-vinylpyrrolidone (NVP, distilled) in *o*-dichlorobenzene was degassed four times and was stirred at 60 °C under N₂ until the substance became very viscous. The reaction mixture was then reconstituted into CH₂Cl₂ (initial reaction volume + 2 mL) to provide dark brown solution and then added to Et₂O (100 times of the solution in volume) to precipitate the polymer. The resulting precipitate was separated by centrifugation and dissolved with CH₂Cl₂ and precipitated in Et₂O again (2 times). After drying, the precipitate was dissolved in the minimum required amount of water and then subjected to dialysis in RC membrane (Fisher, 3,500MWCO) followed by lyophilization to dryness.

Table S1. Reaction conditions and product of C₆₀-NVP polymerization

run	Reaction condition					yield [%]**
	C ₆₀ [mg] ([μmol]) eq.	NVP [mg] ([mmol]) eq.	AIBN [equivalent to C ₆₀]	solvent* [mL]	reaction time [h]	
1	50 (69.4) 1	767 (6.9) 100	40	1.74	72	71
2	50 (69.4) 1	1545 (13.9) 200	40	1.74	19	93
3	50 (69.4) 1	2312 (20.8) 300	40	1.74	27	74
4	50 (69.4) 1	3090 (27.8) 400	40	1.74	23	72
5	50 (69.4) 1	3857 (34.7) 500	40	1.74	22	73

* *o*-dichlorobenzene

** Calculated from the weight of polymer.

Table S2. Reaction conditions and product of C₇₀-NVP polymerization

run	Reaction condition					yield [%]**
	C ₇₀ [mg] ([μmol]) eq.	NVP [mg] ([mmol]) eq.	AIBN [eq. to C ₆₀]	solvent* [mL]	reaction time [h]	
1	25 (30) 1	330 (2.97) 100	40	0.65	72	85
2	25 (30) 1	660.17 (5.94) 200	40	0.65	45	73
3	25 (30) 1	990 (8.91) 300	40	0.65	28	87
4	25 (30) 1	1320 (11.88) 400	40	0.75	26	71
5	25 (30) 1	1650 (14.85) 500	40	0.75	26	70

* *o*-dichlorobenzene

** Calculated from the weight of polymer.

3. Characterization of C₆₀- or C₇₀-PVP polymers

3.1 UV-Vis spectra of polymers

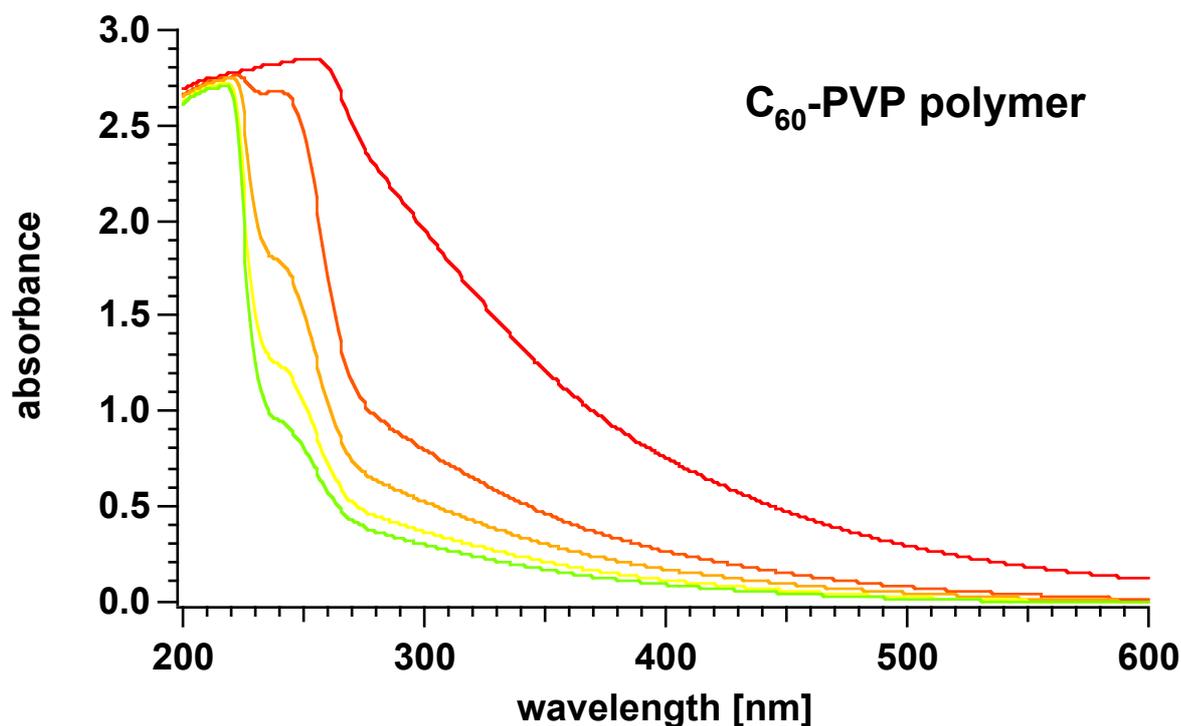


Figure S1. UV-Vis spectra of C₆₀-PVP 2a in water (1 mg/mL). Ratios of subjected C₆₀-NVP: 1: 100 (red), 1:200 (dark orange), 1:300 (orange), 1:400 (yellow), and 1:500 (green).

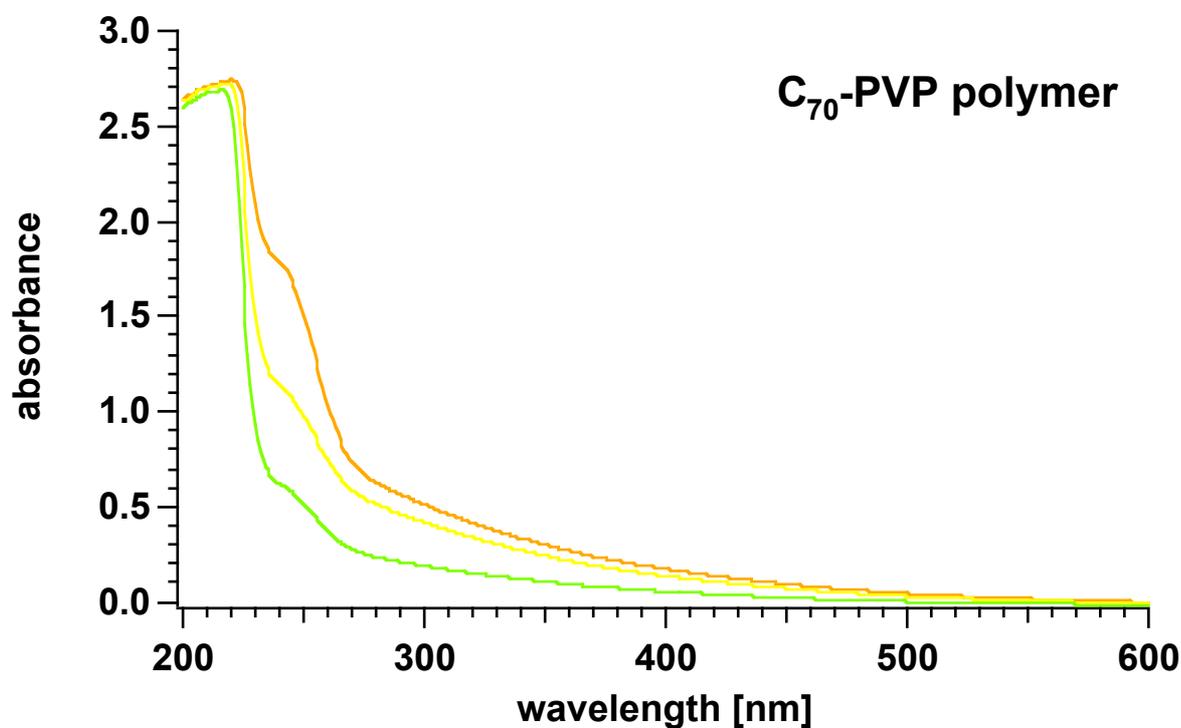


Figure S2. UV-Vis spectra of C₇₀-PVP 2b in water (1 mg/mL). Ratios of subjected C₇₀-NVP: 1:300 (orange), 1:400 (yellow), and 1:500 (green).

3.2 NMR spectra of polymers.

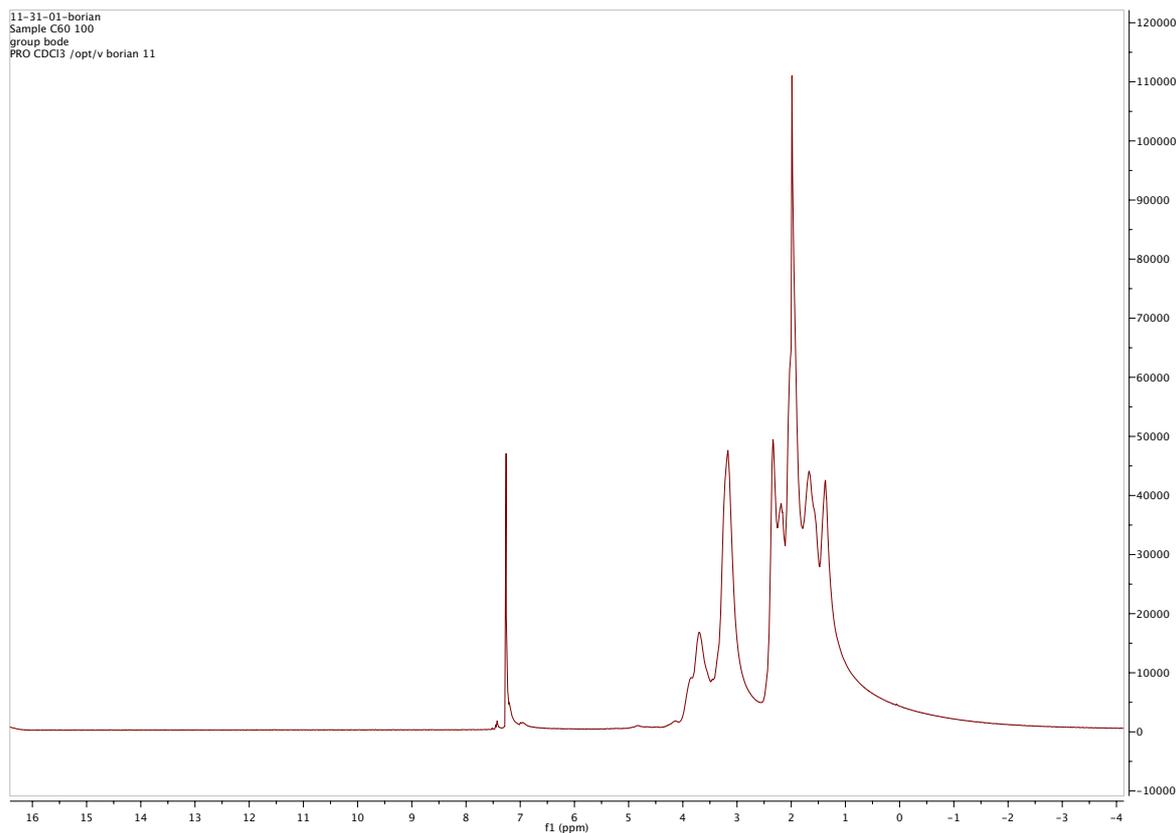


Figure S3. ¹H-NMR spectrum of C₆₀-PVP copolymer **2a** (1:100) (in CDCl₃, 300 MHz).

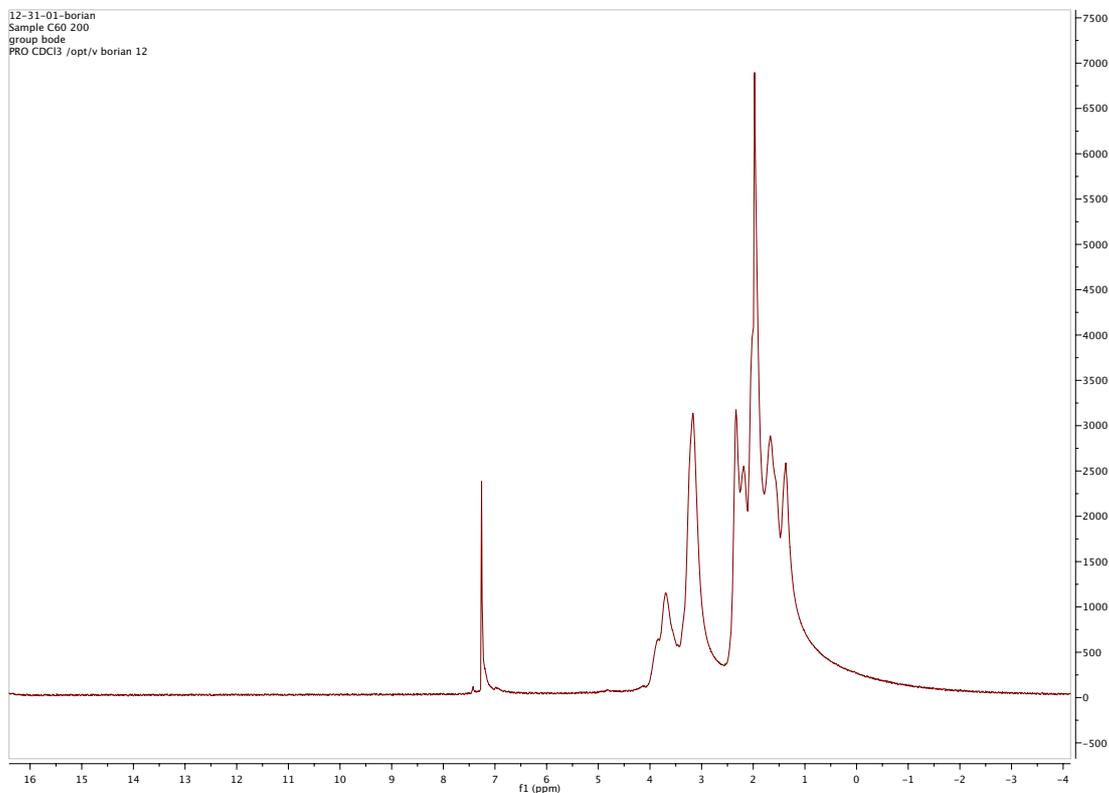


Figure S4. ¹H-NMR spectrum of C₆₀-PVP copolymer **2a** (1:200) (in CDCl₃, 300 MHz).

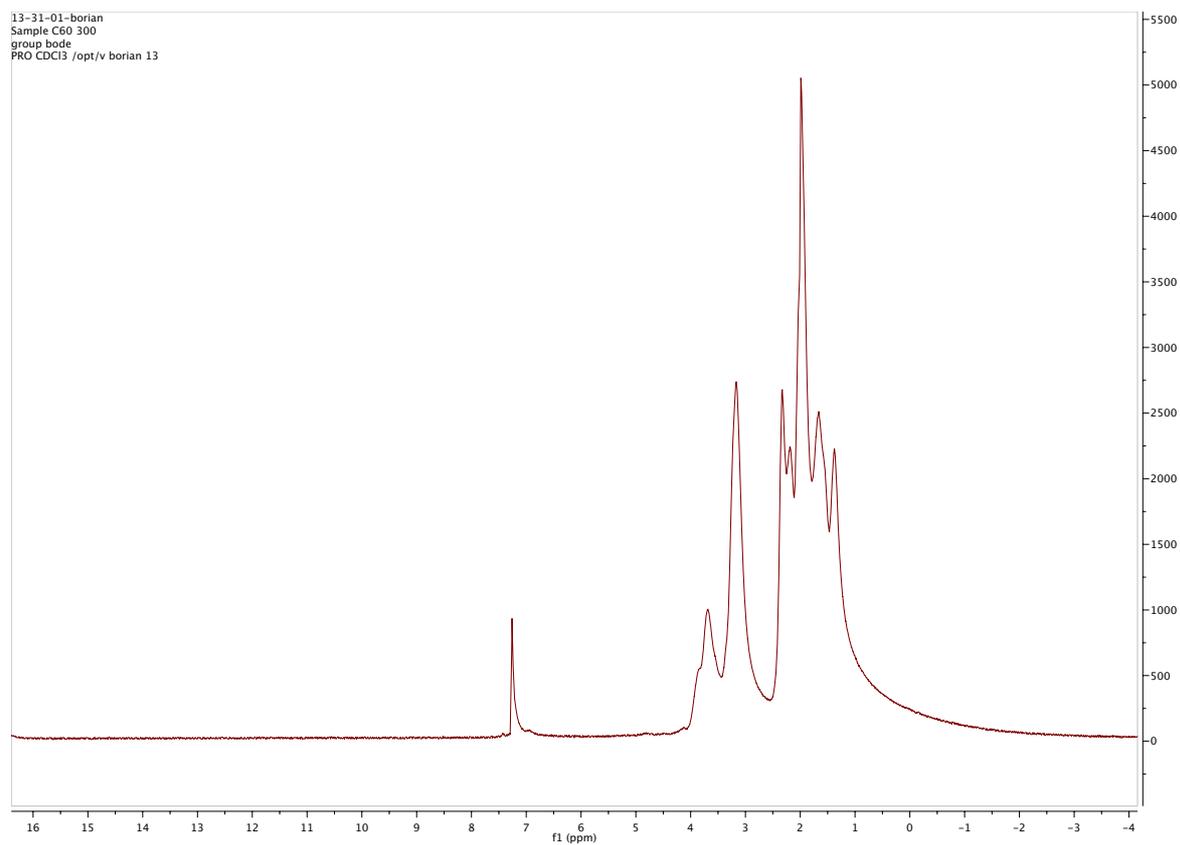


Figure S5. ^1H -NMR spectrum of C_{60} -PVP copolymer **2a** (1:300) (in CDCl_3 , 300 MHz).

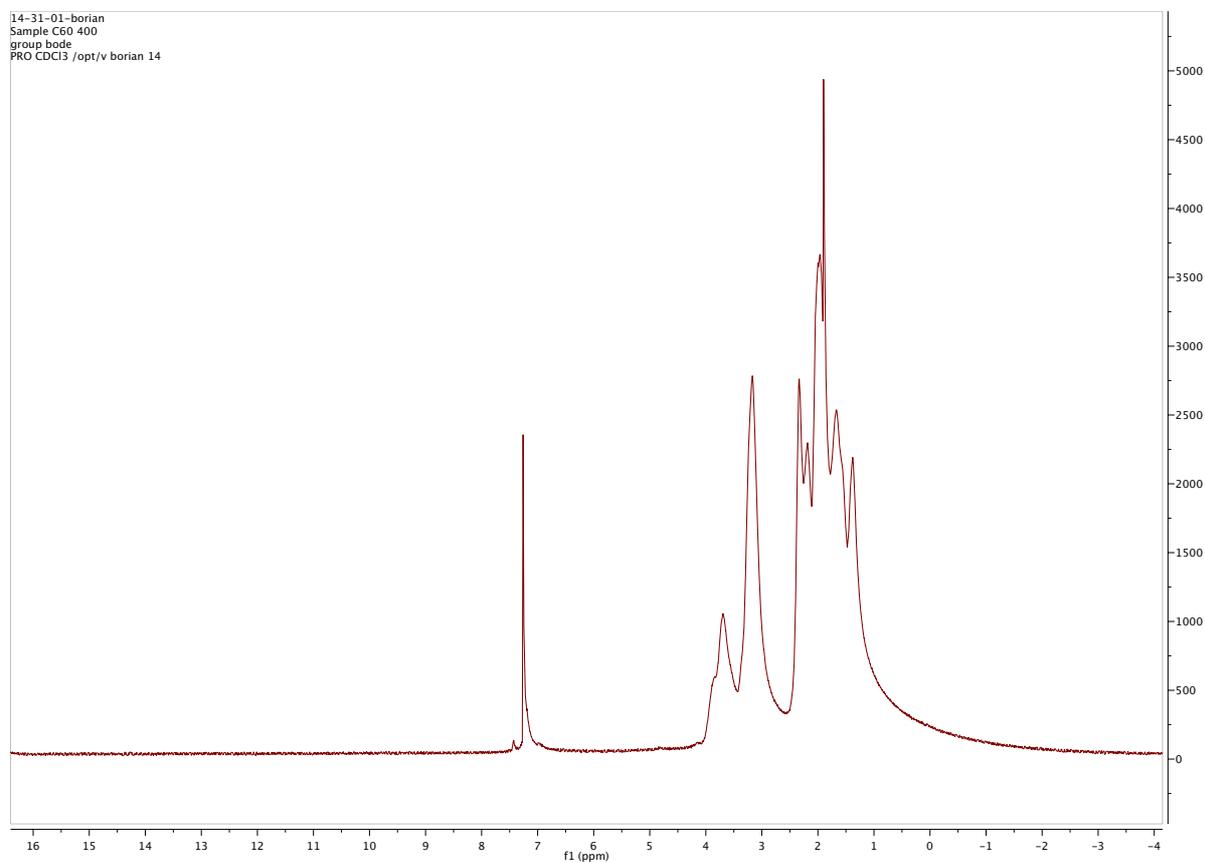


Figure S6. ^1H -NMR spectrum of C_{60} -PVP copolymer **2a** (1:400) (in CDCl_3 , 300 MHz).

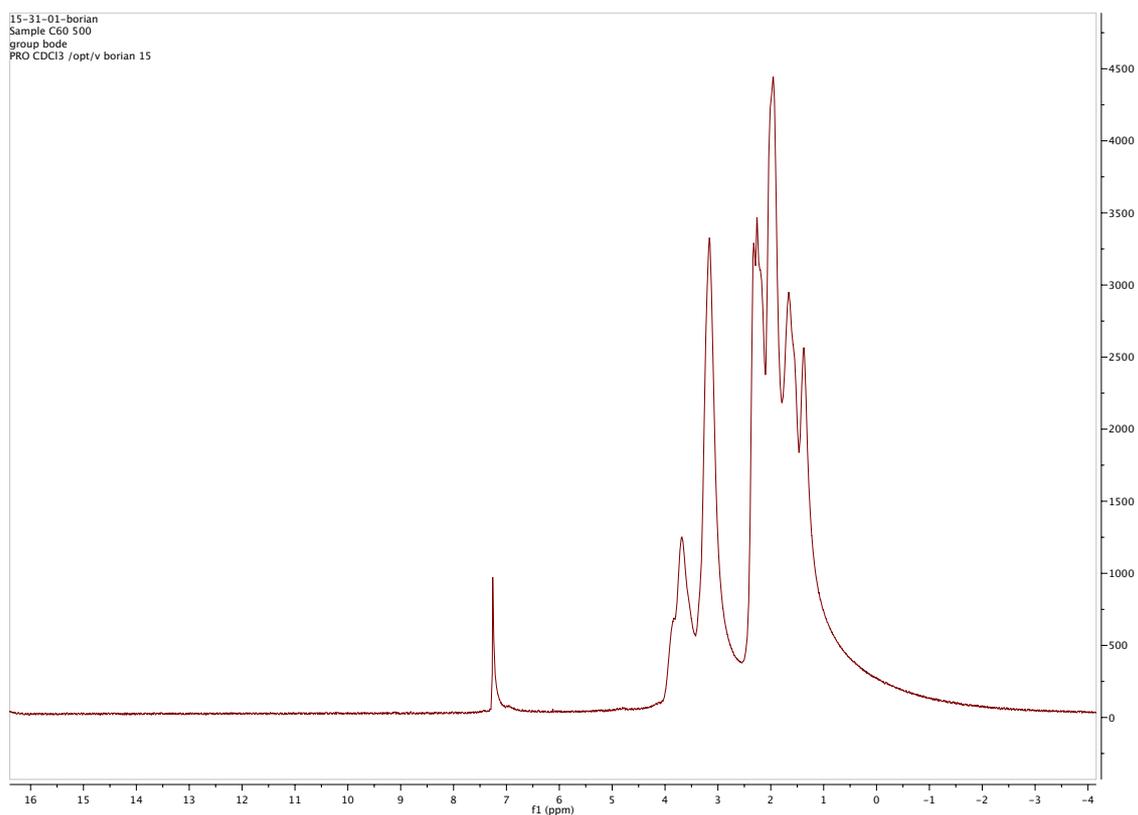


Figure S7. $^1\text{H-NMR}$ spectrum of C_{60} -PVP copolymer **2a** (1:500) (in CDCl_3 , 300 MHz).

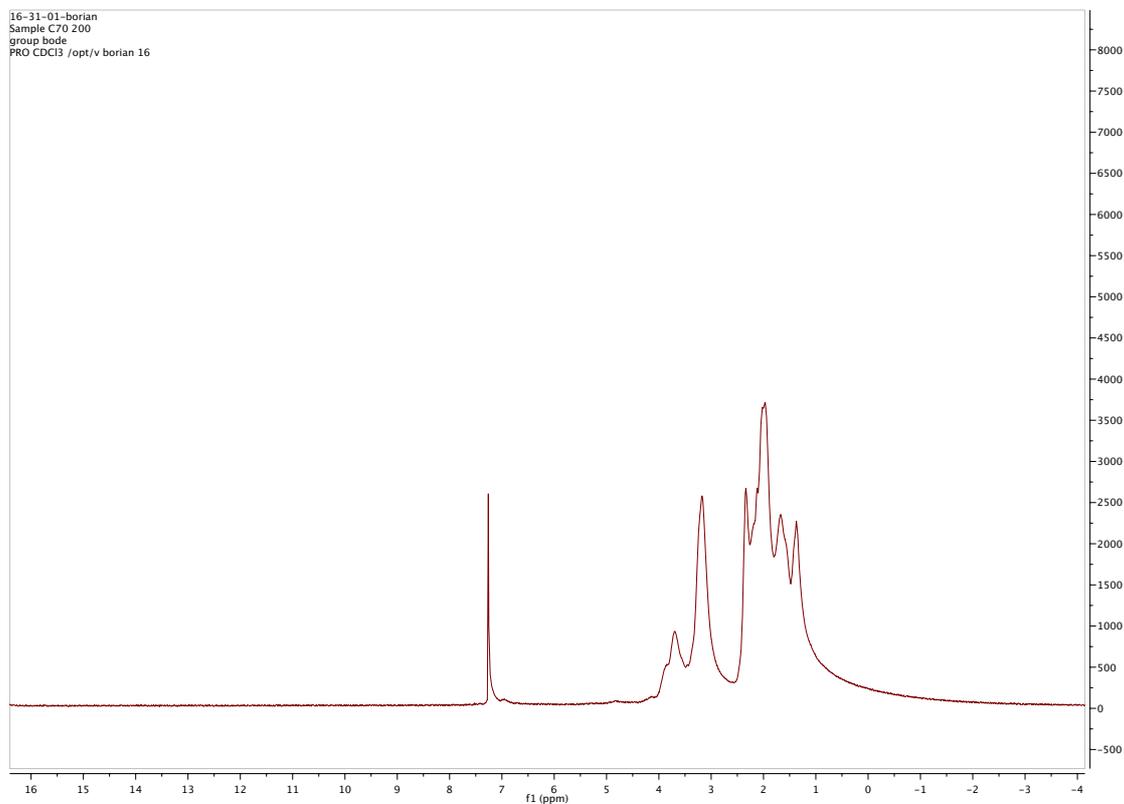


Figure S8. $^1\text{H-NMR}$ spectrum of C_{70} -PVP copolymer **2b** (1:200) (in CDCl_3 , 300 MHz).

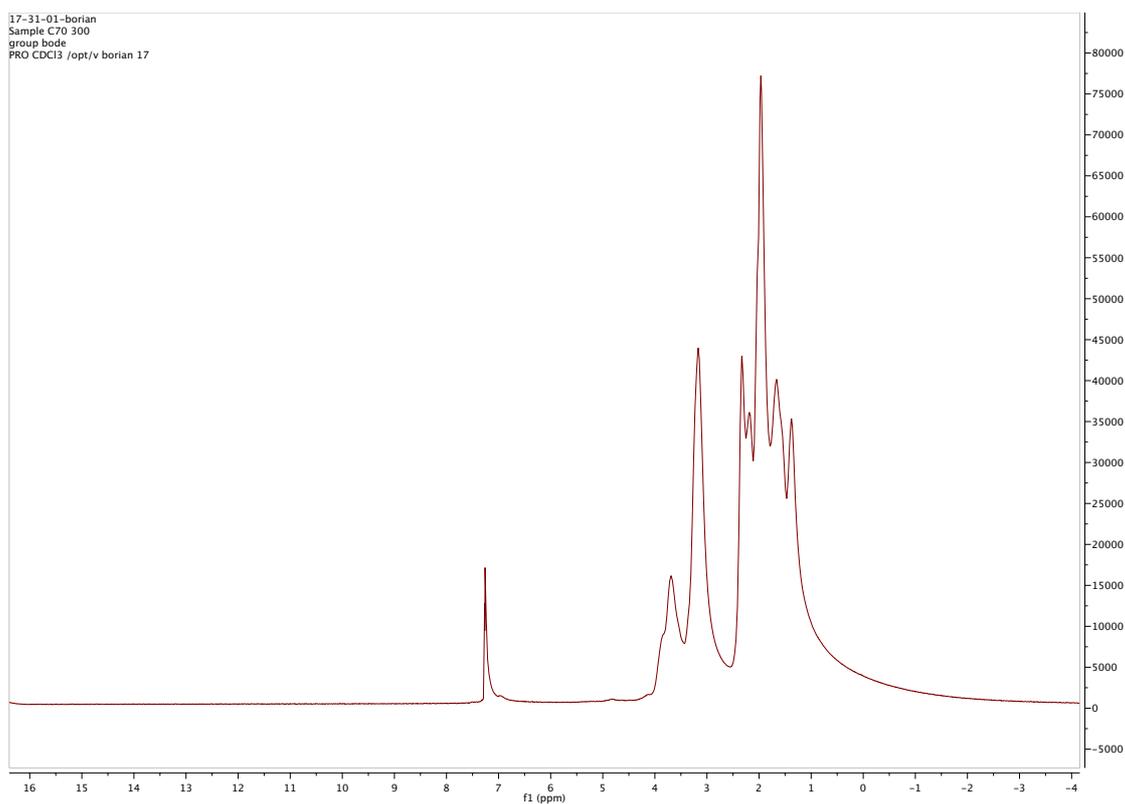


Figure S9. ¹H-NMR spectrum of C₇₀-PVP copolymer **2b** (1:300) (in CDCl₃, 300 MHz).

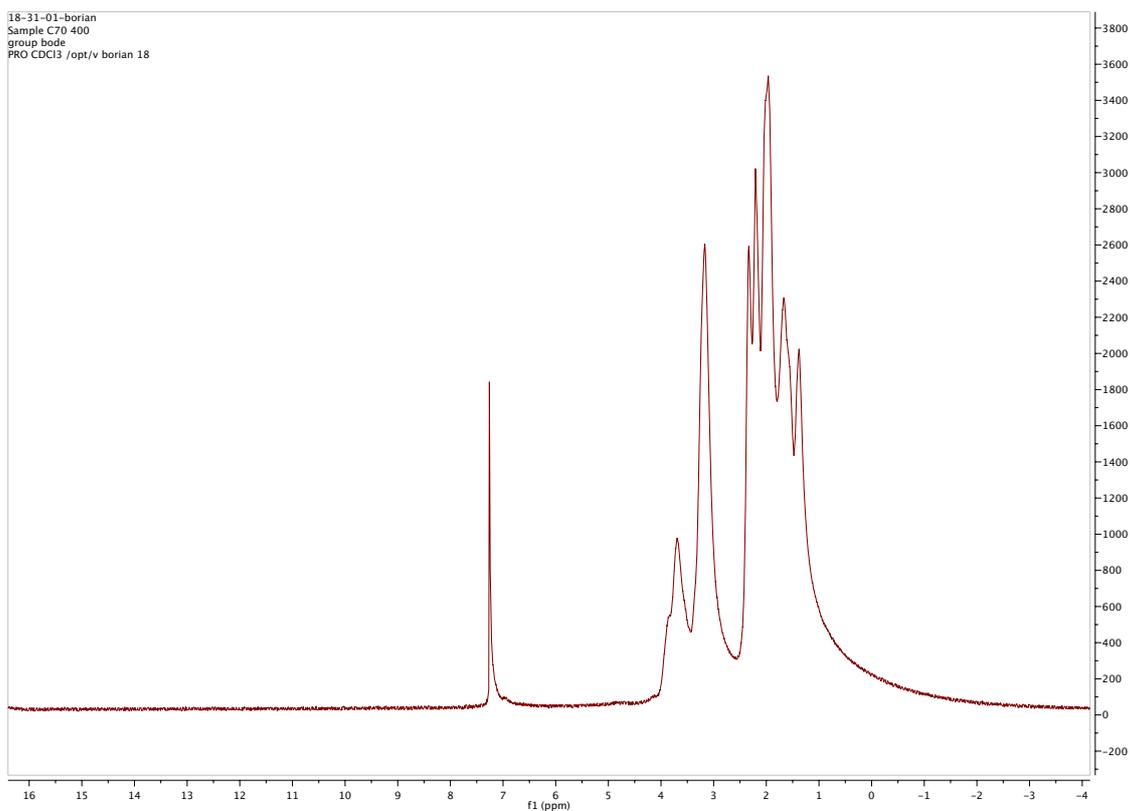


Figure S10. ¹H-NMR spectrum of C₇₀-PVP copolymer **2b** (1:400) (in CDCl₃, 300 MHz).

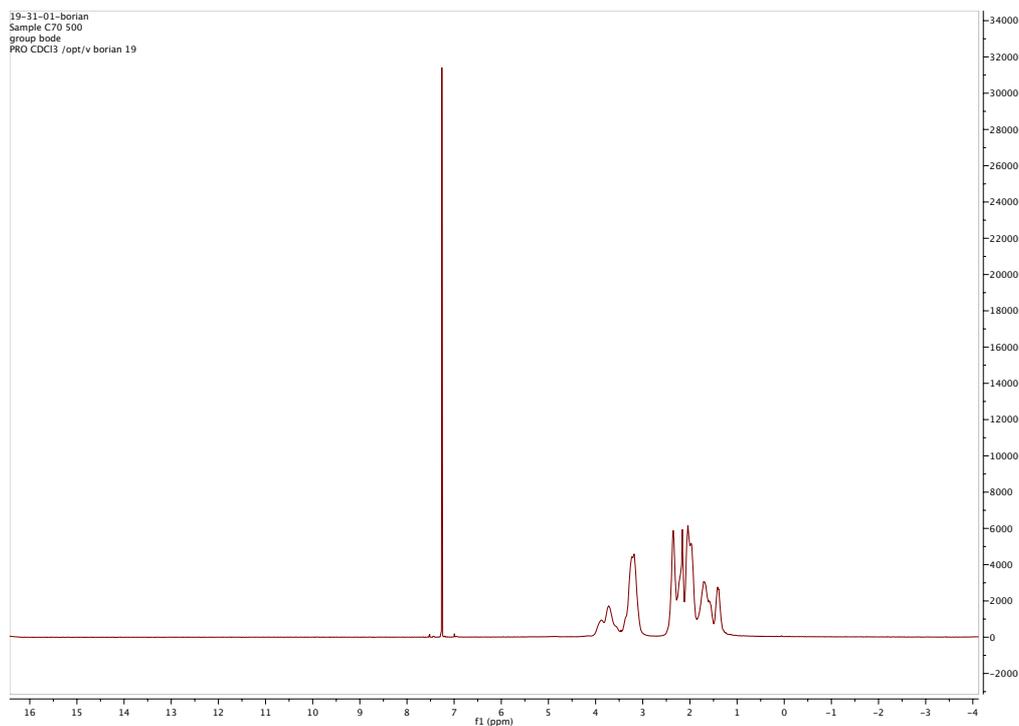


Figure S11. ^1H -NMR spectrum of C_{70} -PVP copolymer **2b** (1:500) (in CDCl_3 , 300 MHz).

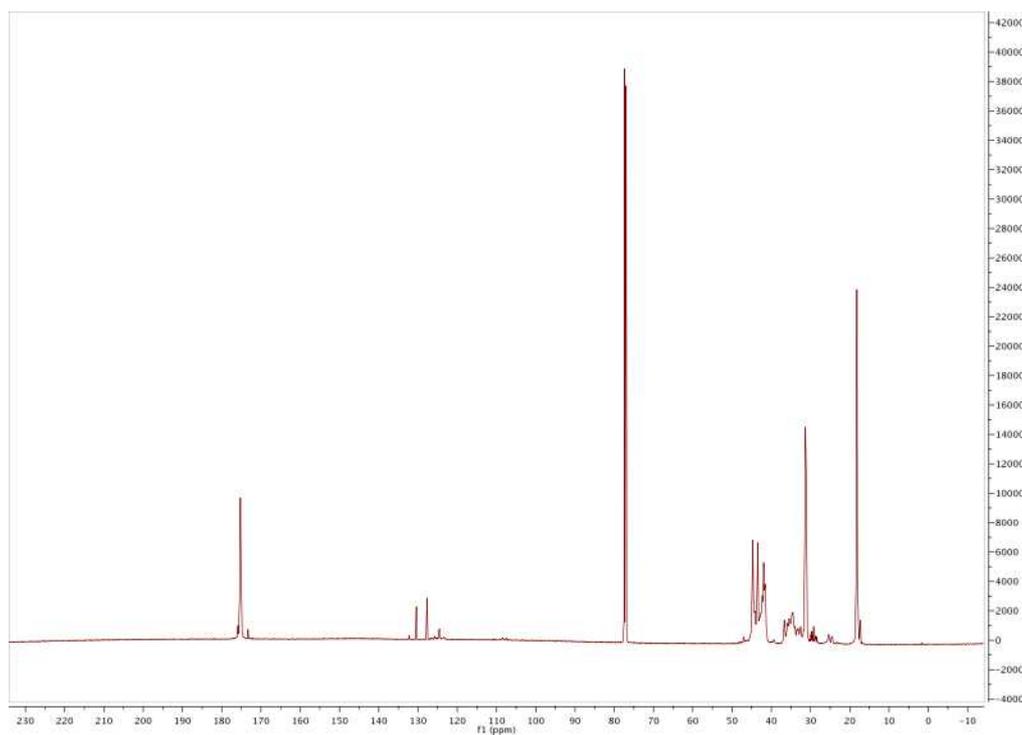


Figure S12. ^{13}C -NMR spectrum of C_{60} -PVP copolymer **2a** (1:100) (in CDCl_3 , 600 MHz).

3.2 FT-IR spectra of polymers.

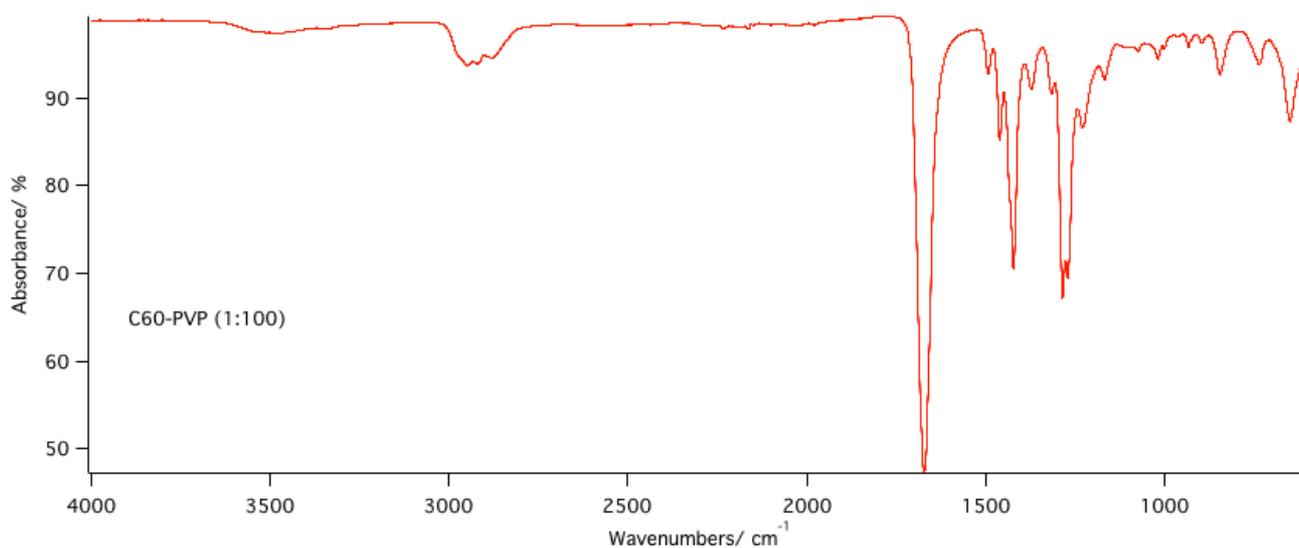


Figure S13. IR Spectrum of C₆₀-PVP copolymer **2a** (1:100) (neat).

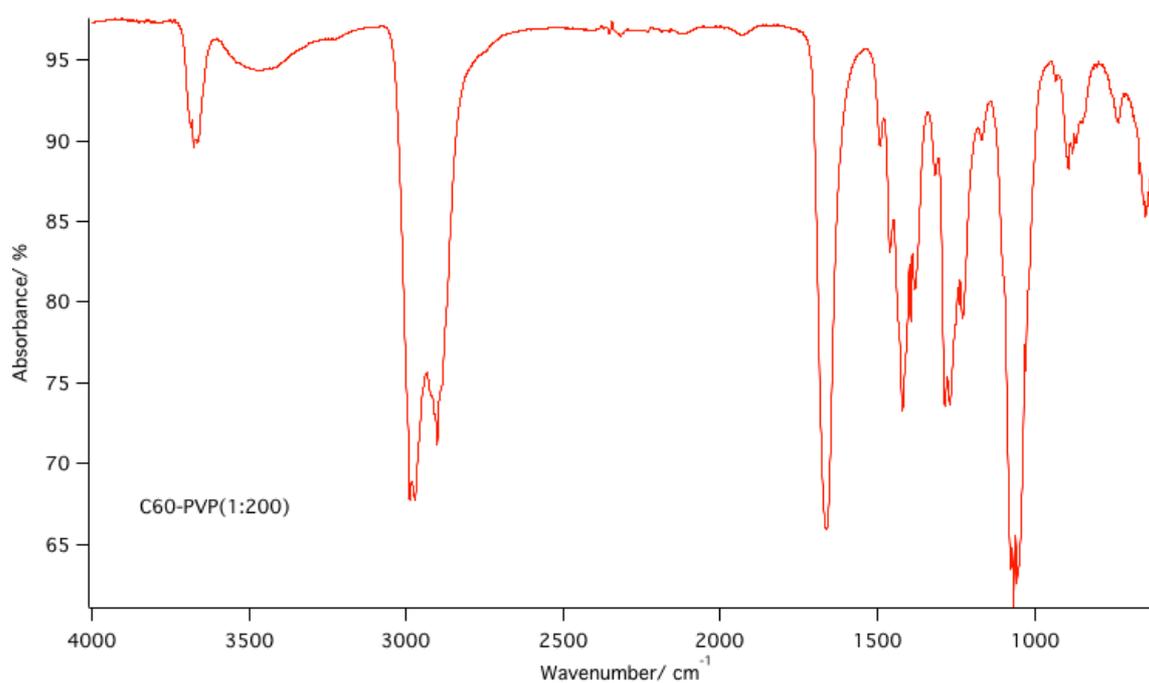


Figure S14. IR Spectrum of C₆₀-PVP copolymer **2a** (1:200) (neat).

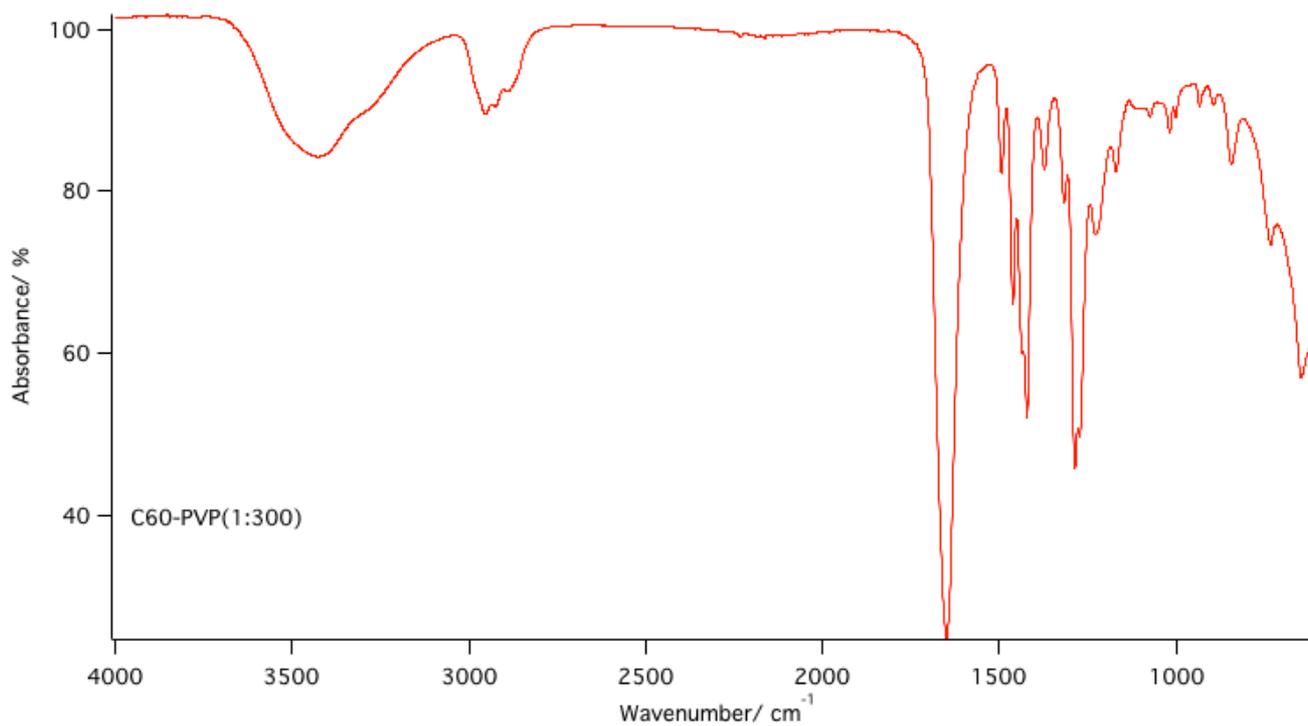


Figure S15. IR Spectrum of C₆₀-PVP copolymer **2a** (1:300) (neat).

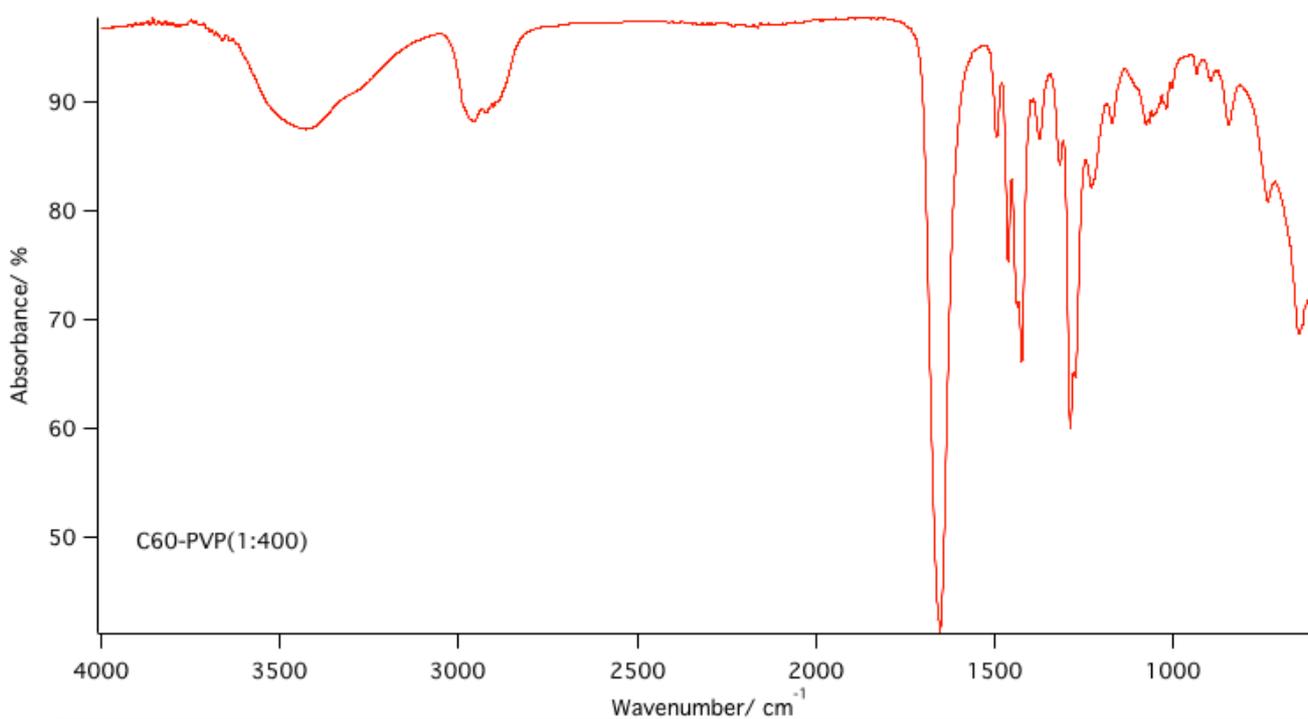


Figure S16. IR Spectrum of C₆₀-PVP copolymer **2a** (1:400) (neat).

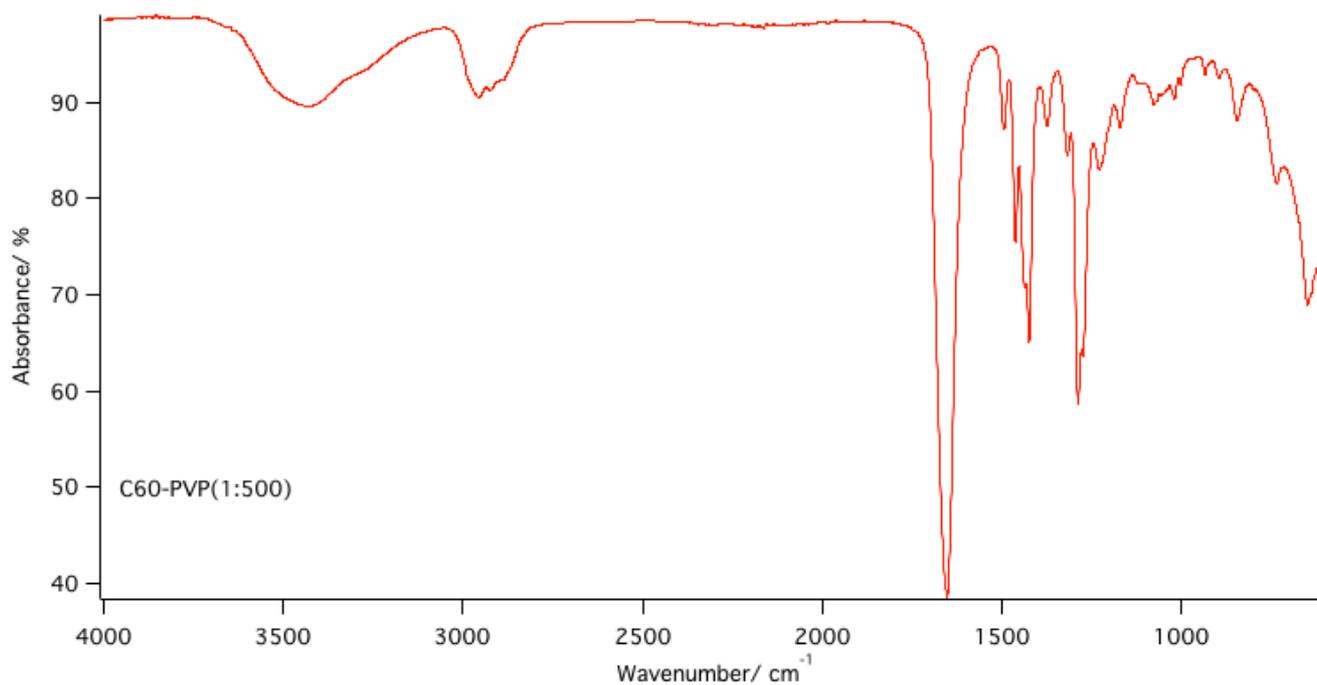


Figure S17. IR Spectrum of C₆₀-PVP copolymer **2a** (1:500) (neat).

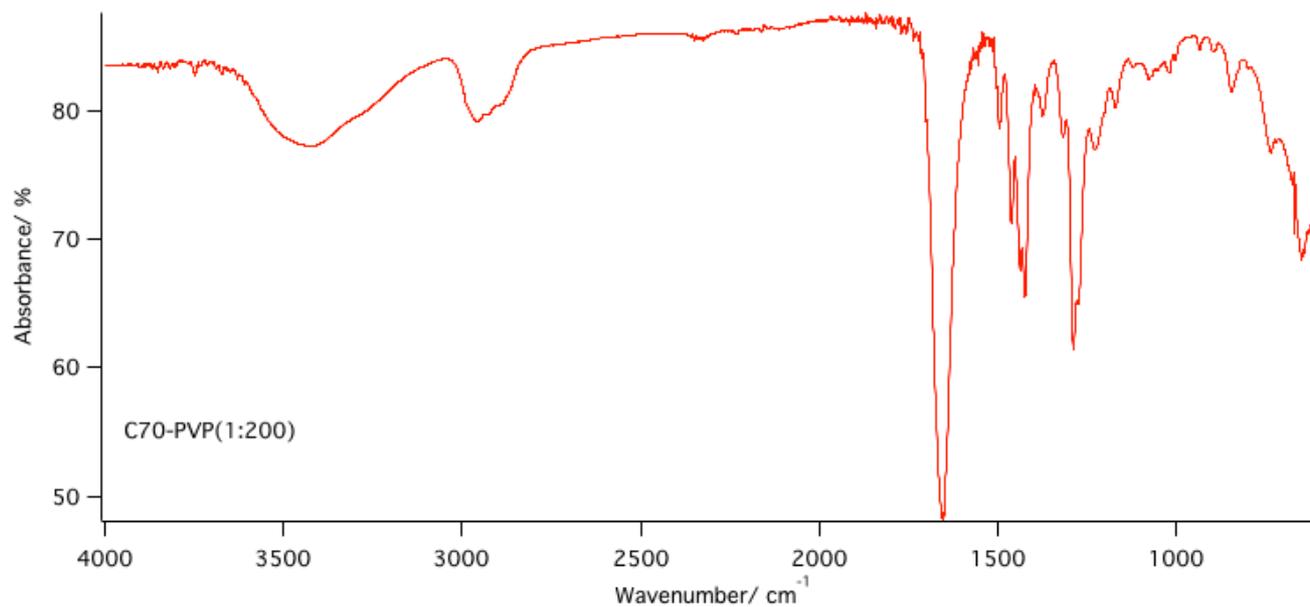


Figure S18. IR Spectrum of C₇₀-PVP copolymer **2b** (1:200) (neat).

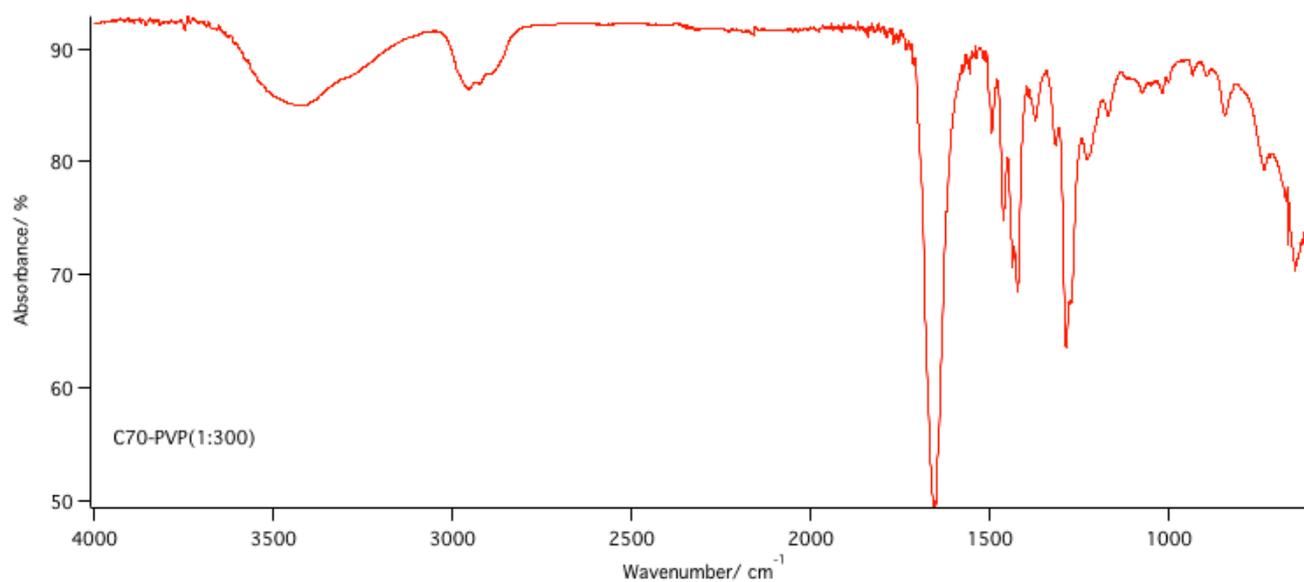


Figure S19. IR Spectrum of C₇₀-PVP copolymer **2b** (1:300) (neat).

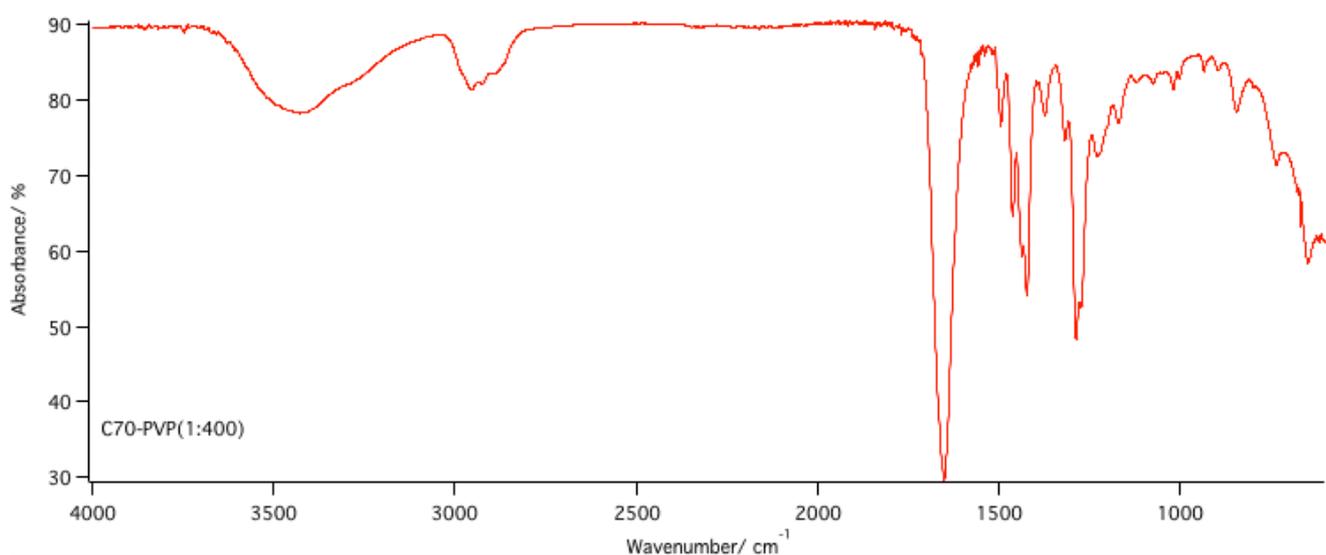


Figure S20. IR Spectrum of C₇₀-PVP copolymer **2b** (1:400) (neat).

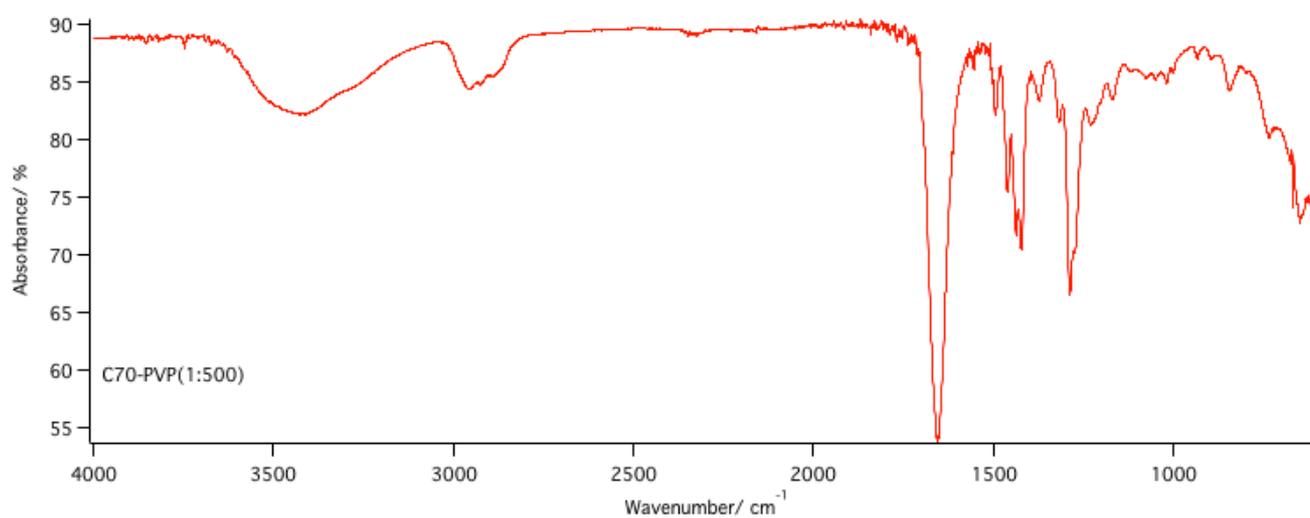


Figure S21. IR Spectrum of C₇₀-PVP copolymer **2b** (1:500) (neat).

3.3 Solubility in water. An aliquot of the each polymer and was dissolved in Milli-Q water followed by filtration through 0.45 μm RC syringe filters (Whatman) in order to prepare the samples of concentration range (100-800 mg/mL). Each filtrate was then diluted with Milli-Q (100 times) and subjected to the UV-Vis measurement on JASCO V570 UV-Vis spectrometer.

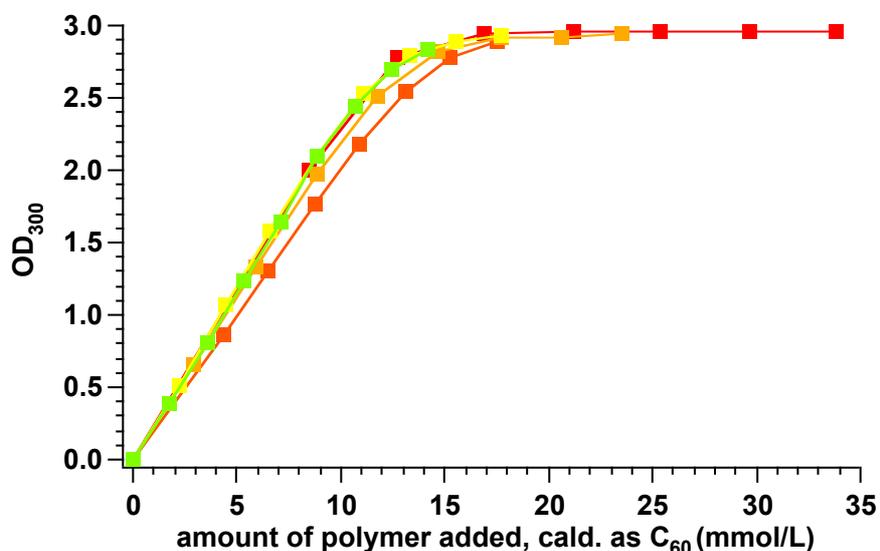


Figure S22. Solubility curves of C_{60} -PVP copolymer **2a** (1:100-500). Unit for the X-axis is amount of C_{60} -PVP polymer added estimated in mmol/L of C_{60} (ratios of subjected C_{60} -NVP: 1: 100 (red), 1:200 (dark orange), 1:300 (orange), 1:400 (yellow), and 1:500 (green)).

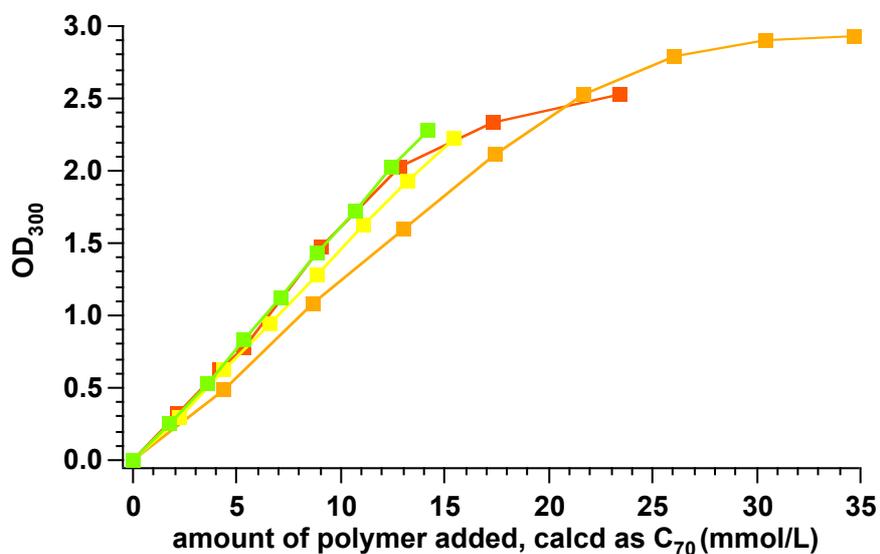


Figure S23. Solubility curves of C_{70} -PVP copolymer **2b** (1:200-500). Unit for the X-axis is amount of C_{70} -PVP polymer added estimated in mmol/L of C_{70} (ratios of subjected C_{70} -NVP: 1:200 (dark orange), 1:300 (orange), 1:400 (yellow), and 1:500 (green)).

3.2 GPC analysis. Samples were prepared with 3-5 mg of each polymer in 0.4 mL of dimethyl formamide (DMF) with 0.1% LiBr (GPC grade, Fischer Chem.) and filtered prior to the analysis with 0.22 micron PTFE hydrophilic syringe filters (Simplepure). Aliquot of each solution (250 μ L) was subjected to GPC analysis on JASCO PU-2080 Plus HPLC pump, JASCO MD-2018 Plus UV detector, JASCO RI02031 Plus RI detector, ChromNAV Chromatography Data System (JASCO Co., Tokyo, JPN), and columns in linear combination: linear (2), 10^4 Å, 10^3 Å, 500 Å columns (5 μ m, 300 mm x 7.8 mm, Phenomenex, Torrance CA, USA) using PMMA for callibration and DMF with 0.1% LiBr as mobile phase at 60°C inside JASCO CO-2065 Plus column oven. Results are summarized in Tables S3 and S4.

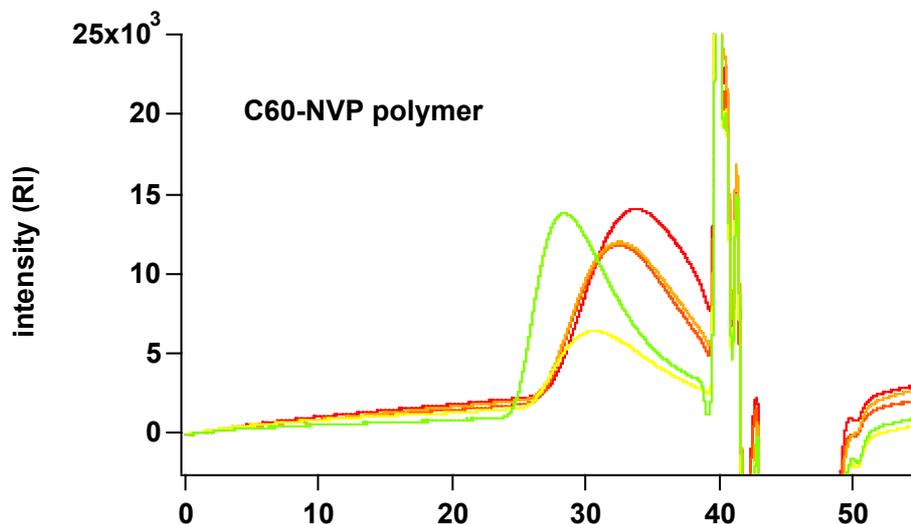


Figure S24. GPC traces of C₆₀-PVP **2a** (ratios of subjected C₆₀-NVP: 1: 100 (red), 1:200 (dark orange), 1:300 (orange), 1:400 (yellow), and 1:500 (green)).

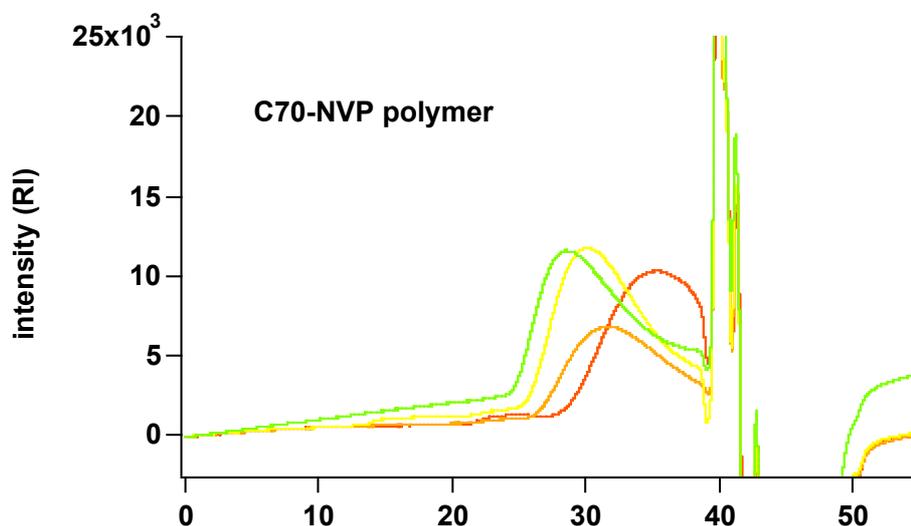


Figure S25. GPC traces of C₇₀-PVP **2b** (ratios of subjected C₇₀-NVP: 1:300 (orange), 1:400 (yellow), and 1:500 (green)).

3.3 DLS analysis. An aliquot (12.5 mg) of each polymer sample was dissolved in Milli-Q water (1 mL) and filtered through a 0.22 micron PTFE hydrophilic syringe filters (Simplepure). Samples were subjected to the analysis immediately (within 30 min) and after sitting overnight at room temperature. Dynamic light scattering was recorded on Zetasized Nano S (Malvern) using a fresh disposable cuvette (half-mikro PS, 1.6 mL, VWR Int.).

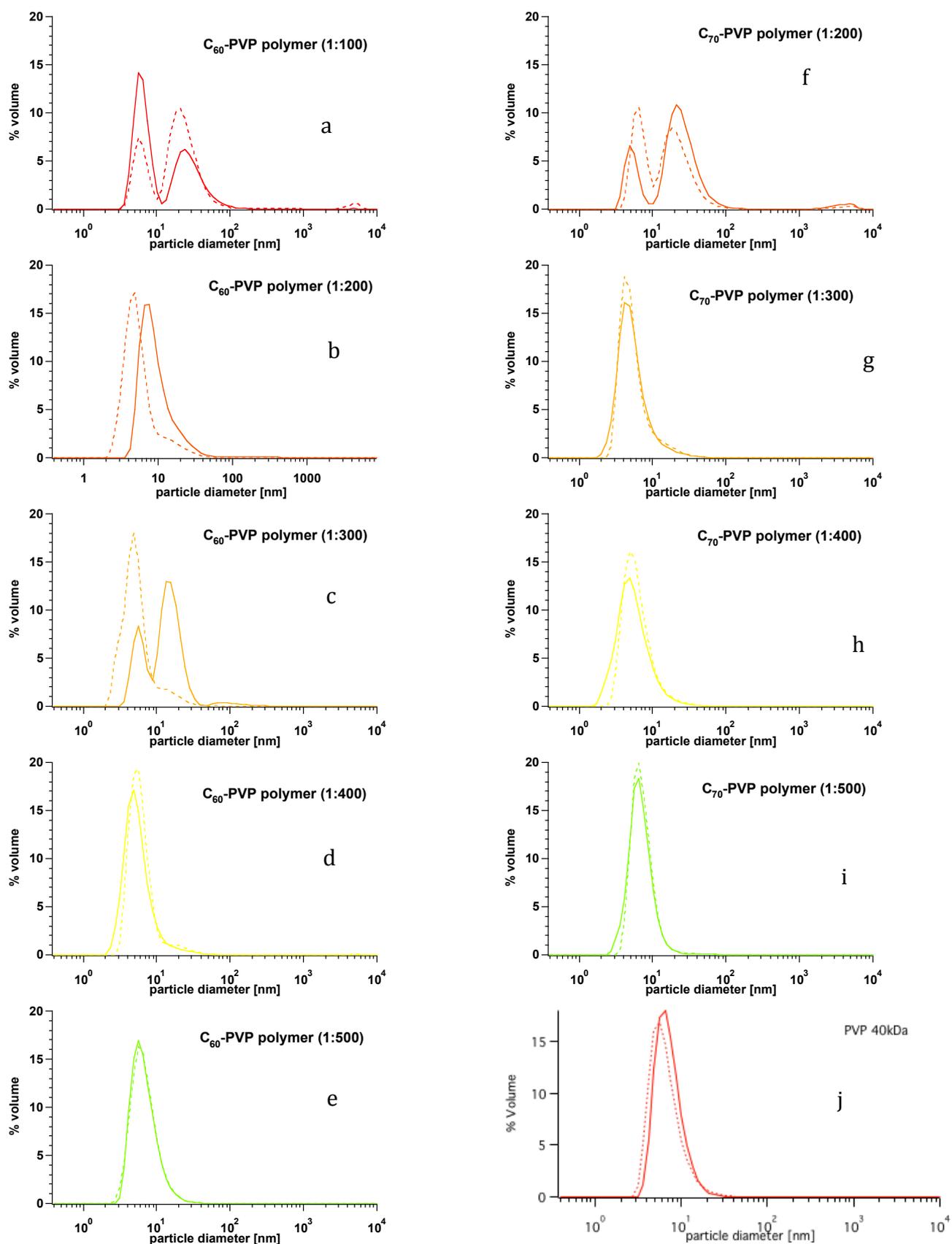


Figure S26. Distribution curves of the polymer particle size in aqueous solution by DLS. (a)-(e) C_{60} -PVP, (f)-(i) C_{70} -PVP with ratios of subjected fullerene-NVP of 1:100 (a), 1:200 (b and f), 1:300 (c and g), 1:400 (d and h), and 1:500 (e and i), PVP 40kDa (TCI) (j) Solid lines: fresh solution, dotted lines: solution kept for one day. Data are the average of three-times-measurements.

3.4 AFM imaging. An aliquot (30 μL) of polymer solution (concentration about $0.3 \times 10^{-5} \text{M}$ in water) was deposited onto the freshly cleaved HOPG surface, air-dried for 5 min, and finally dried with N_2 gas. All of the AFM images were recorded using Nanoscope IIIa SPM (Bruker) under ambient condition. AFM characterizations were operated at tapping mode using a typical Si cantilever with resonant frequency around 340 kHz.

3.5 $\text{O}_2^{\cdot -}$ generation measured by EPR method with DEPMPO as a spin trapping agent.

Superoxide was detected by an EPR method using 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) as a spin-trapping agent. To a 0.5 mM fullerene aqueous solution (40 μL), 5 mM DETAPAC in 250 mM phosphate buffer (20 μL), water (8 μL), 625 mM DEPMPO in DMSO (22 μL), 100 mM NADH or distilled water (10 μL) were added and mixed well under an aerobic condition. The mixed solution was collected in a capillary, irradiated with a 200-W photoreflexor lamp at a distance of 10 cm, and subjected immediately to EPR measurement. The generation of $\text{O}_2^{\cdot -}$ was detected as signals due to DEPMPO-OOH formed by the reaction of $\text{O}_2^{\cdot -}$ with DEPMPO.

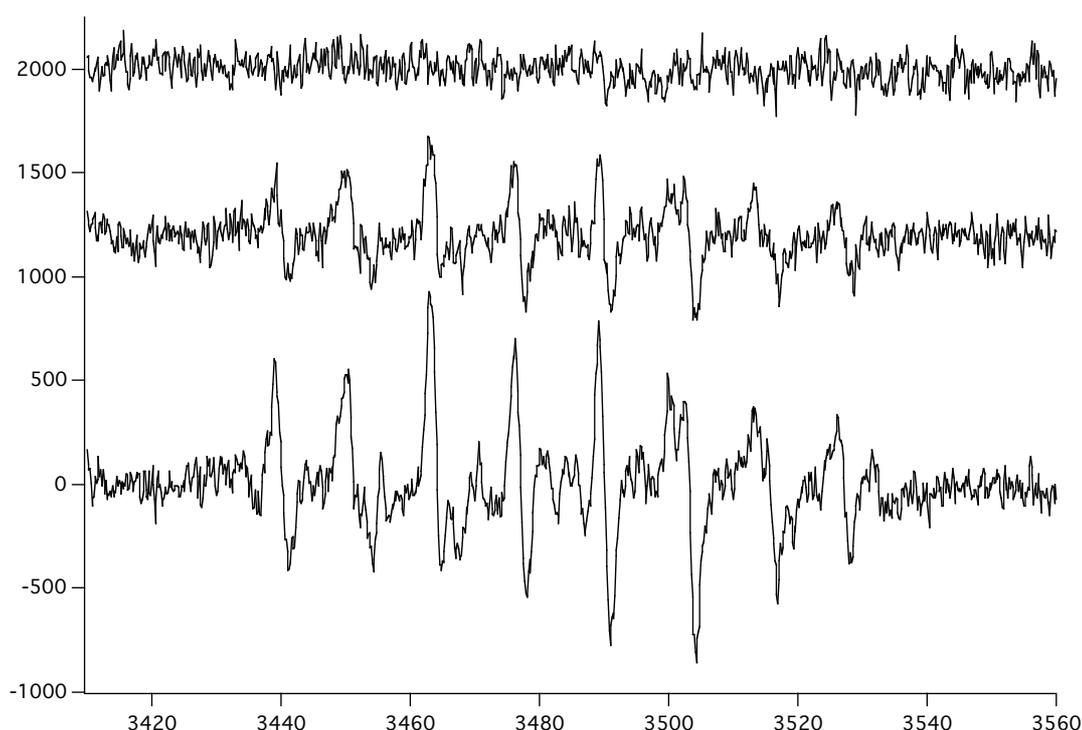


Fig. S27. X-band ESR spectrum of DEPMPO adduct with superoxide generated in C_{60} /PVP complex aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time: 0 min (top), 1 min (middle), 3 min (bottom). Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

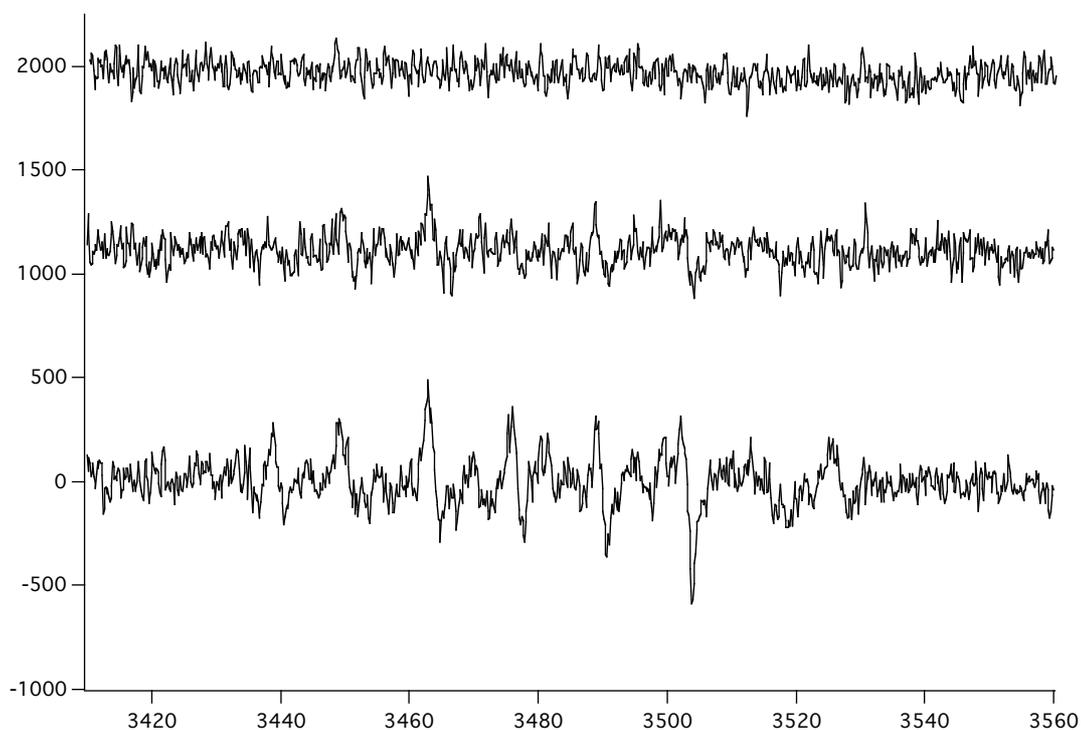


Fig. S28. X-band ESR spectrum of DEPMPO adduct with superoxide generated in C_{70} /PVP complex aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time: 0 min (top), 1 min (middle), 3 min (bottom). Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

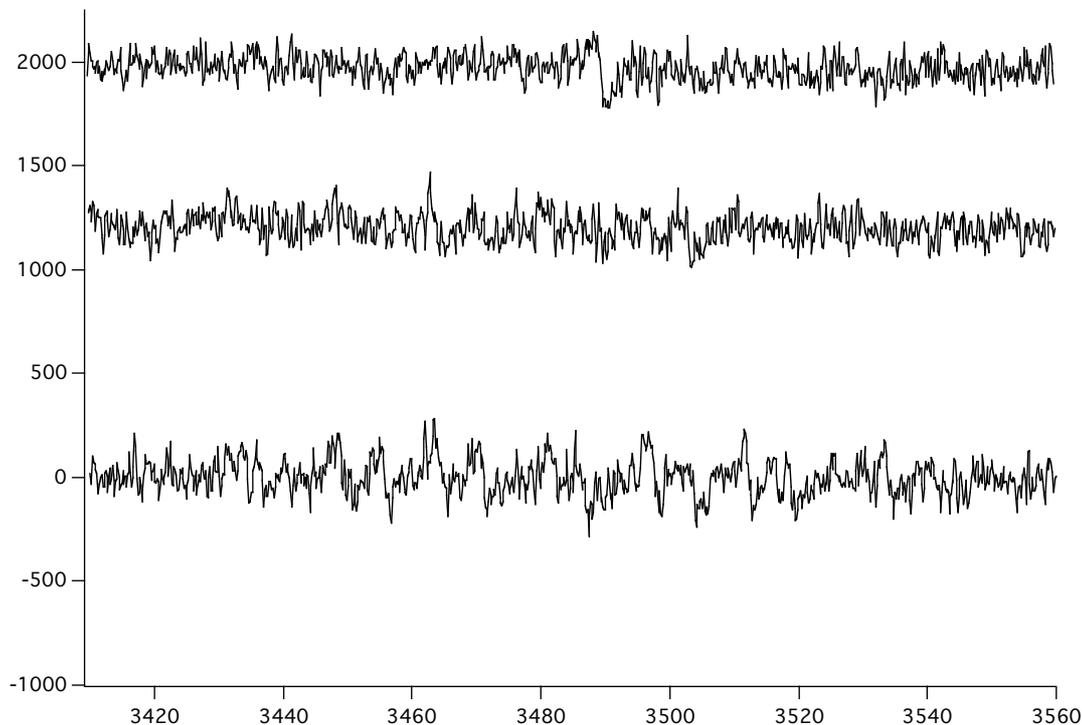


Fig. S29. X-band ESR spectrum of DEPMPO adduct with superoxide generated in C_{60} -PVP 1:500 polymer aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time: 0 min (top), 1 min (middle), 3 min (bottom). Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

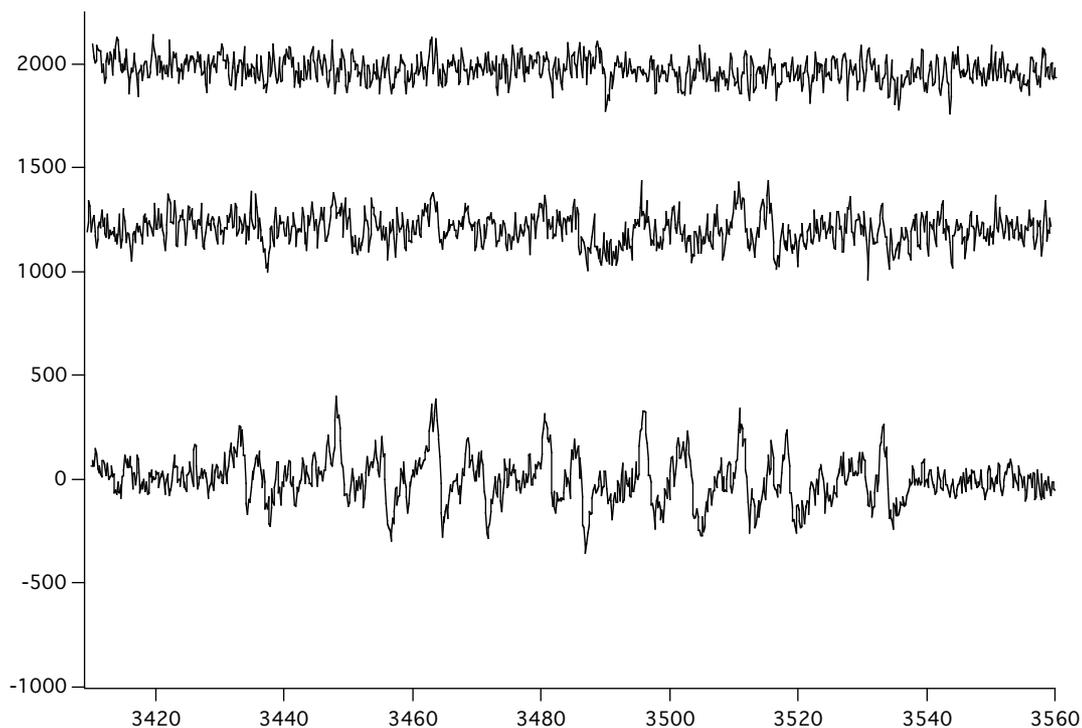


Fig. S30. X-band ESR spectrum of DEPMPPO adduct with superoxide generated in C₇₀-PVP 1:500 polymer aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time: 0 min (top), 1 min (middle), 3 min (bottom). Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

3.6 ¹O₂ generation measured by EPR method with TEMP as a spin trapping agent. Singlet oxygen was detected by an EPR method using 2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMP) as a spin-trapping reagent. As a standard, ¹O₂-generating compound Rose Bengal was employed. To a 0.5 mM fullerene or a Rose Bengal aqueous solution (40 μL), 250 mM phosphate buffer (20 μL), distilled water (32 μL), and 1 M 4-oxo-TEMP (8 μL) were added and mixed well under an aerobic condition. The mixed solution was introduced into a capillary, then placed into the EPR tube and irradiated with a 200-W photoreflexor lamp at a distance of 10 cm, and immediately subjected to EPR measurement. The generation of singlet oxygen was detected as an EPR signal due to 4-oxo-TEMPO formed by the reaction of ¹O₂ with 4-oxo-TEMP.

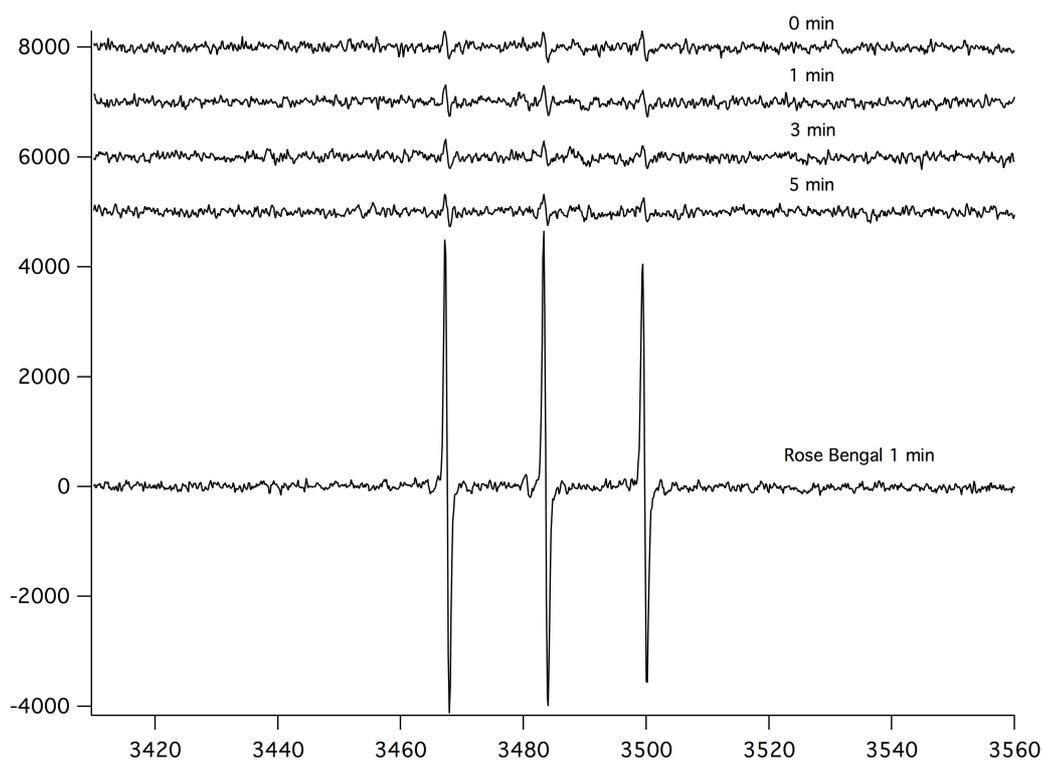


Fig. S31. X-band ESR spectrum of TEMP adduct with singlet oxygen generated in C₆₀/PVP complex aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time 0 min, 1 min, 3 min, 5 min. Rose bengal 1 min irradiation was used as a reference. Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

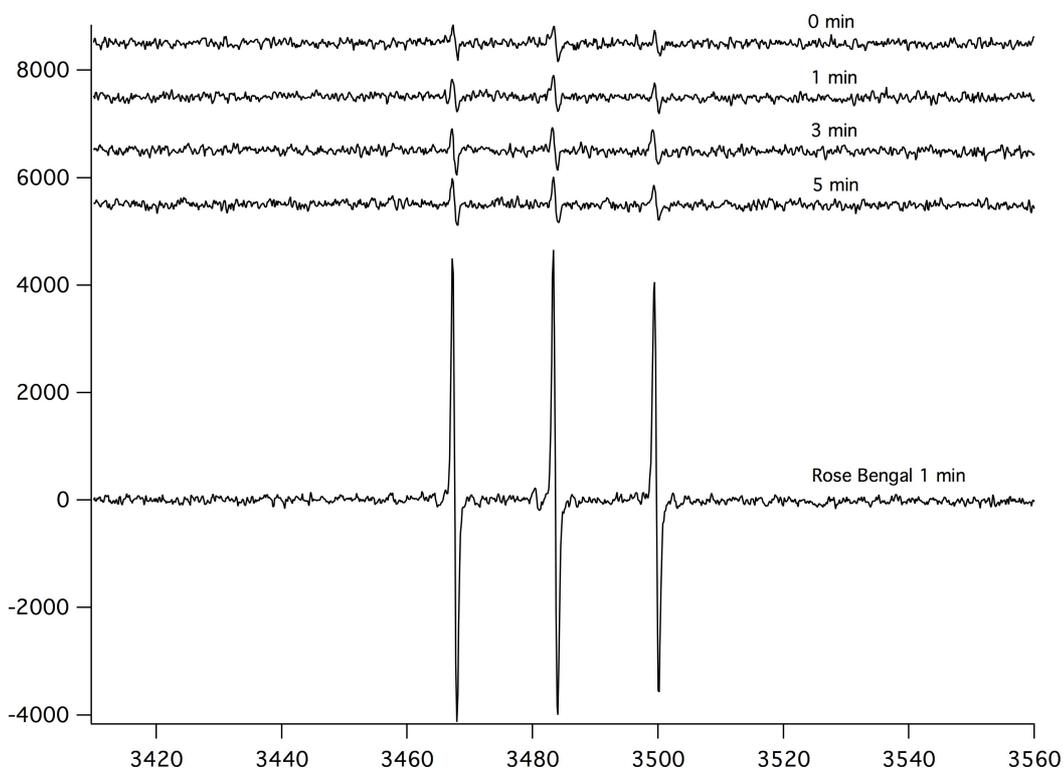


Fig. S32. X-band ESR spectrum of TEMP adduct with singlet oxygen generated in C₇₀/PVP complex aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time 0 min, 1 min, 3 min, 5 min. Rose bengal 1 min irradiation was used as a reference. Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

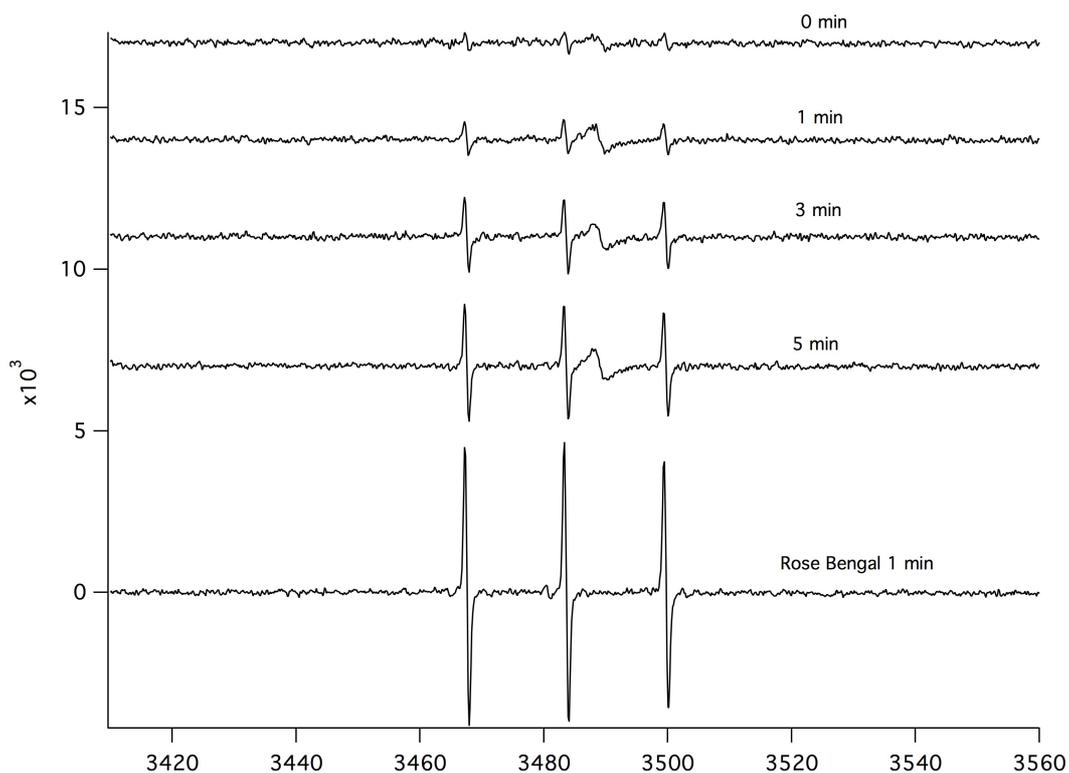


Fig. S33. X-band ESR spectrum of TEMP adduct with singlet oxygen generated in C_{60} -PVP 1:500 polymer aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time 0 min, 1 min, 3 min, 5 min. Rose bengal 1 min irradiation was used as a reference. Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.

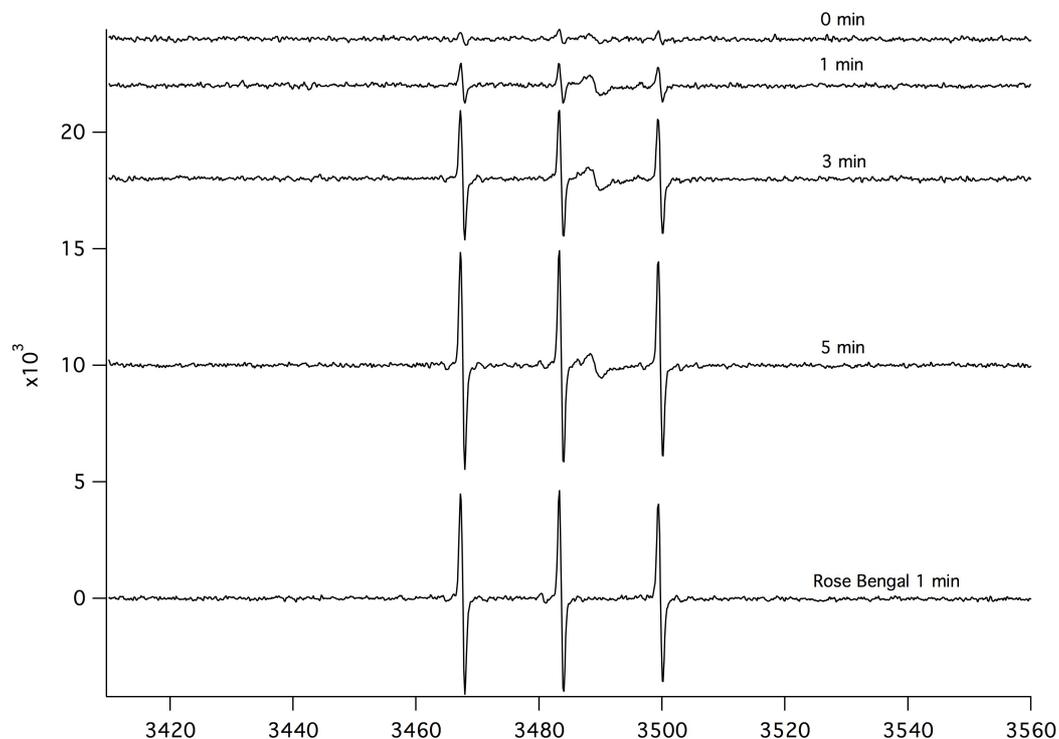


Fig. S34. X-band ESR spectrum of TEMP adduct with singlet oxygen generated in C_{70} -PVP 1:500 polymer aqueous solution under irradiation of 200-W reflector lamp. Light irradiation time 0 min, 1 min, 3 min, 5 min. Rose bengal 1 min irradiation was used as a reference. Experimental conditions: temperature 296 K, microwave frequency 9.790 GHz, microwave power 10 mW, receiver gain 5.0×10^4 , modulation amplitude 1.00 G, modulation frequency 100 kHz, sweep time 84 sec.