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Water-soluble C_{60}– and C_{70}–PVP polymers for biomaterials with efficient $^1O_2$ generation†

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Highly water-soluble fullerene polymers were successfully prepared by a simple direct free-radical copolymerization of *N*-vinylpyrrolidone and intact C_{60} or C_{70} as a radical-capping agent. Using AIBN as a radical initiator, the polymers (C_{60}– or C_{70}–PVP) with significantly high molecular weight (∼30 kDa) and with efficient $^1O_2$ generation were obtained.

The bioapplications of fullerenes have been discussed for more than a decade due to their high photosensitivity and metal encapsulation ability. The main obstacle to developing fullerene biomaterials is their extremely low solubility in water or water-miscible solvents. Following the initial studies on water-soluble C_{60} derivatives,[1–5] various water-soluble materials were reported as biocompatible fullerenes[6–9] addressing photodynamic therapy (PDT) and MRI contrast enhancement agents. Recent attention in this field has been directed towards polymeric fullerene materials.[10] For targeting inflammatory diseases such as cancers, where vascular leakage is often limited, polymeric materials (M_w > 20 kDa) are selectively accumulated due to the enhanced permeation and retention (EPR) effect. Previously, we have reported that water-soluble fullerene biomaterials in combination with biocompatible poly(vinylpyrrolidone) (PVP) can be used for preparing complexes[11] or copolymers.[12] In this study, we aimed to develop a versatile procedure for preparation of fullerene (C_{2n})–PVP copolymers using radical polymerization of *N*-vinylpyrrolidone (NVP) in the presence of intact fullerenes that react with •PVP on their double bonds to form a covalent bond (Fig. 1). This procedure does not require the preparation of fullerene monomer derivatives (*e.g.* C_{2n}-vinyl derivatives) and will allow easy access to the preparation of water-soluble higher fullerenes such as C_{70} and $Gd_3N@C_{80}$ with biologically attractive properties including a longer lifetime of the triplet excited state and paramagneticity, respectively. There are only few studies on water-soluble materials of such higher fullerenes. And particularly with C_{70}, similar reactions are expected to occur as radical addition reactions are known.[13]

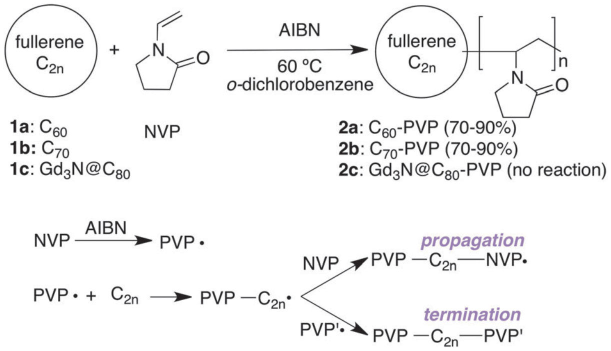

Fig. 1 Preparation of fullerene–PVP copolymers by the direct free-radical copolymerization of NVP and C_{60}, C_{70}, or $Gd_3N@C_{80}$. AIBN (0.075–0.25 equiv.) was used as a radical initiator. The initial ratio of C_{2n} and NVP considered in the reaction was 1 : 100, 200, 300, 400, or 500.

Studies on such direct free-radical copolymerization reactions of fullerenes involving intact C_{60} were initially reported as C_{60}–poly(styrene) copolymers.[14–18] In those studies, AIBN was used as a radical initiator and the poly(styrene) radical generated *in situ* was speculated to attack the double bonds of C_{60} forming covalent bonds. A similar procedure was used to prepare other polymers such as C_{60}–poly(carbonate)[19] and C_{60}–poly(methylmethacrylate).[20,21] Conjugation with controlled polymers generated by nitroxide-mediated radical polymerization was also reported.[22–24] However, direct radical copolymerization of intact C_{60} and NVP was not investigated except in a few studies using lauroyl peroxide or benzoyl peroxide as the radical initiators. However, these results provided low yields and insufficient water-solubility.[25,26]

In the present study, we tested several different ratios of NVP (100–500 equiv.) to C_{2n} to optimize the conditions for the preparation of C_{2n}–PVP polymers with a sufficiently large molecular

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weight and high water-solubility. C_{60}, C_{70}, and $Gd_3N@C_{80}$ were used as fullerene cores for polymerization with AIBN as a radical initiator (Fig. 1). Considering the radical quenching activities of fullerenes, which often disturb radical polymerization,[27,28] a relatively large amount of AIBN (0.075–0.25 equiv. of NVP, 40 equiv. of C_{2n}) was added to each reaction.[29] The reactions were carried out in *o*-dichlorobenzene, a good solvent for fullerenes.

All co-polymerization reactions of C_{60} or C_{70} with NVP provided fullerene–PVP materials in sufficient yields (>70%, Table 1, column 3). However, the reaction of $Gd_3N@C_{80}$ and NVP did not provide any polymeric product. We speculated that the radical quenching activity, which is common to many fullerenes, was too high in $Gd_3N@C_{80}$ to provide any polymers (further details need to be clarified). C_{60}–PVP (1 : 100–500) and C_{70}–PVP (1 : 200–500) were thoroughly soluble in water. Only the C_{70}–PVP (1 : 100) polymer was not soluble in water presumably due to the larger hydrophobic surface area of C_{70}. The molecular weight of each copolymer (C_{60}– or C_{70}–PVP) was analyzed by GPC (solvent: DMF, calibration standard: poly(methylmethacrylate) (PMMA)). Interestingly, the M_w of C_{2n}–PVP polymers were highly correlated with the ratios of C_{2n} and NVP. While the reaction with a higher ratio of NVP (lower ratio of C_{2n}) provided polymers with higher M_w, the reaction with the lower ratio of NVP provided polymers with lower M_w (Table 1, columns 2 and 4). This result suggests that C_{60} or C_{70} are acting as an end-capping reagent of $^{\bullet}$PVP (Fig. 1) and upon increasing the ratio of NVP, the PVP–C_{2n} radical would have a higher possibility of reacting with NVP, leading to the higher M_w value. To ensure that the fullerene cores were covalently bound to the polymer (not by complexation), aqueous polymer solutions were extracted with toluene in the presence of oversaturated NaCl. No fullerene was detected in the organic layer indicating that C_{2n} materials were water-soluble due to covalent functionalization.

The particle size was analyzed by dynamic laser scattering (DLS) using both freshly prepared and 1-day-old solutions. The distribution of particle size of polymers generally became uniform after keeping the solutions in water at room temperature for 1-day with the shift to the smaller particle size ranges (Fig. S26, ESI†). Despite the difference in M_w values, the sizes of the polymer particle measured by DLS were not significantly different, presumably because the polymers are not globular in water. The AFM image of the C_{60}–PVP polymer supports this speculation and showed that the polymers formed a linear bundled shape rather than the micellar form (Fig. 2). The distribution of particle size was narrower in the polymers with higher ratios of NVP (1 : 400–500) presumably due to the longer hydrophilic PVP part that helps in the formation of uniform C_{2n}–PVP particles in the aqueous solution.

Table 1 Characterization of C_{2n}–PVP polymers

Reagents considered[a]		Properties of the obtained polymer				
					Particle size[c] [nm]	
C_{2n}	Ratio of NVP to C_{2n}	Yield [%]	M_w[b] [kDa]	PDI	Fresh	1-day old
C_{60}	100	71	10.9	1.75	6.3/27.6	6.2/23.2
	200	93	13.3	1.75	8.02	4.94
	300	74	13.3	1.76	5.9/15.8	5.1
	400	72	18.6	1.86	5.3	5.7
	500	73	32.5	1.96	6.6	6.5
C_{70}	100[d]	85	—	—	—	—
	200	73	7.9	1.76	5.3/24.9	6.6/21.7
	300	87	15.3	1.86	5.0	4.8
	400	71	19.4	2.38	5.5	5.8
	500	70	29.7	2.47	6.9	7.0

[a] AIBN with 0.25–0.075 equiv. to NVP was used. [b] Analyzed by GPC. The chromatogram is shown in Fig. S24 and S25 (ESI). [c] Analyzed by DLS (mean). Shown in detail in Fig. S26 (ESI). [d] Not soluble in water.

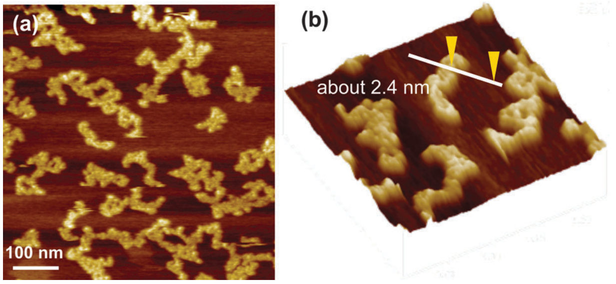


Fig. 2 AFM image of the C_{60}–PVP (1 : 200) polymer on mica substrate.

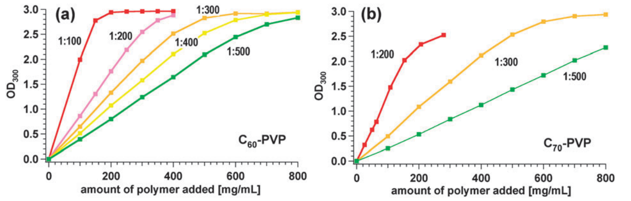


Fig. 3 Solubility curves of C_{60}– (a) and C_{70}–PVP (b) in water. To an aliquot of each polymer, Milli-Q water was added and subsequently filtered. The filtrates were diluted with Milli-Q water before the absorption measurements.

Solubility curves of the C_{60}– and C_{70}–PVP polymers are shown in Fig. 3a and b. As calculated based on the molar concentration of C_{2n}, the solubility of all of the polymers were in the same range ($\sim$10 to 15 mM for both C_{60}– and C_{70}–PVP, Fig. S22 and S23, ESI†) and were sufficient for many bio-applications.

As one of the evaluations of C_{2n}–PVP polymers as PDT agents, reactive oxygen species (ROS) generated under visible light irradiation were estimated using ESR spin-trapping methods (Fig. 4). In general, C_{60} and C_{70} can be excited by visible light irradiation to generate either singlet oxygen ($^1O_2$) or superoxide radical anions ($O_2^{\bullet -}$) *via* an energy transfer type II pathway or *via* an electron transfer type I pathway, respectively (Scheme 1).

According to the previous studies,[30,31] including ours,[32] $^1O_2$ generation from photoexcited C_{60} or C_{70} was generally observed in less polar solvents (*e.g.* benzene) and not in the aqueous solution. Instead, $O_2^{\bullet -}$ is often detected in aqueous solution in the presence of electron donors with a physiological concentration. This is shown in the control experiments using C_{60}– or C_{70}–PVP complexes as a DEPMPO–OOH signal in Fig. 4a (3rd and 4th lines). However in our present study, no $O_2^{\bullet -}$ generation from aqueous solutions of C_{60}– and C_{70}–PVP copolymers was detected in the presence of an electron donor. Alternatively, $^1O_2$ generation was clearly observed as a signal of 4-oxo-TEMPO,

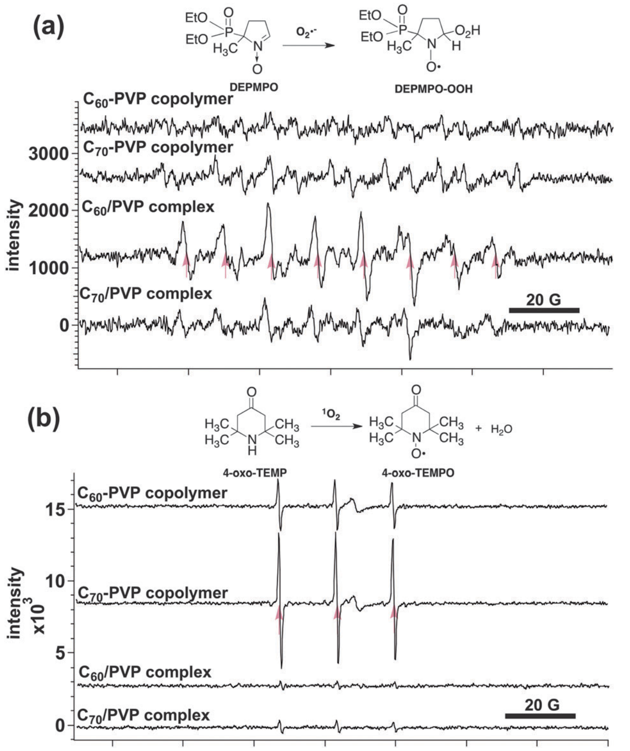
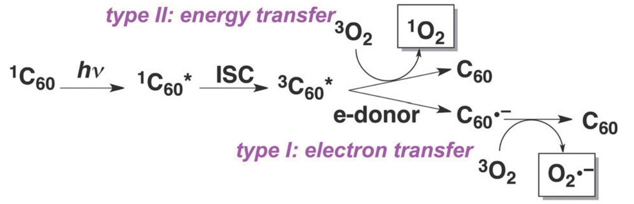


Fig. 4 ROS generation of C_{60}– and C_{70}–PVP copolymers under visible light irradiation and in the aqueous solution. (a) $O_2^{\bullet-}$ generation trapped with DEPMPO (200 W photoreflector lamp, 3 min, in the presence of NADH as an electron donor). (b) $^1O_2$ generation trapped with 4-oxo-TEMP (200 W photoreflector lamp, 5 min). Arrows correspond to the signals of the adducts of ROS and spin-trapping agents. C_{60}– and C_{70}–PVP complexes were used in the control experiment.



Scheme 1 Types I and II pathways of ROS generation from photoexcited C_{60}.

a $^1O_2$ adduct of 4-oxo-TEMP (Fig. 4b, the C_{60}– or C_{70}–PVP copolymer). This result clearly shows that type II energy transfer reaction of $^3C_{60}^*$ and $^3C_{70}^*$ is favoured in C_{2n}–PVP copolymer solution even in polar media (Scheme 1). Interestingly, the $^1O_2$ generation from C_{70}–PVP was higher than that from C_{60}–PVP, which may be related to the longer lifetime of the triplet excited state of C_{70} (130 μs)[31] compared to C_{60} (40 μs).[30] Although the detailed mechanism of ROS generation should be investigated further, such efficient generation of ROS from C_{60}– and C_{70}–PVP shows their high potential as biocompatible polymer PDT agents.

In summary, the C_{60}– and C_{70}–PVP copolymers were successfully prepared *via* an easy-to-handle polymerization method of NVP using C_{60} or C_{70} as a terminal group. The ratio of C_{2n} and NVP can efficiently affect the size of the polymers. The obtained polymers showed high water-solubility and efficient $^1O_2$ generation showing high potential as a PDT agent. A detailed mechanistic study of $^1O_2$ generation is in progress.

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