

# Water-soluble C<sub>60</sub>- and C<sub>70</sub>-PVP polymers for biomaterials with efficient <sup>1</sup>O<sub>2</sub> generation†

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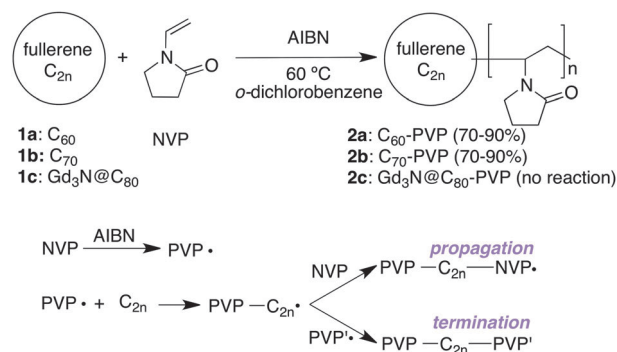
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Highly water-soluble fullerene polymers were successfully prepared by a simple direct free-radical copolymerization of *N*-vinylpyrrolidone and intact C<sub>60</sub> or C<sub>70</sub> as a radical-capping agent. Using AIBN as a radical initiator, the polymers (C<sub>60</sub>- or C<sub>70</sub>-PVP) with significantly high molecular weight (~30 kDa) and with efficient <sup>1</sup>O<sub>2</sub> 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<sub>60</sub> derivatives,<sup>1–5</sup> various water-soluble materials were reported as biocompatible fullerenes<sup>6–9</sup> addressing photodynamic therapy (PDT) and MRI contrast enhancement agents. Recent attention in this field has been directed towards polymeric fullerene materials.<sup>10</sup> For targeting inflammatory diseases such as cancers, where vascular leakage is often limited, polymeric materials (*M<sub>w</sub>* > 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<sup>11</sup> or copolymers.<sup>12</sup> In this study, we aimed to develop a versatile procedure for preparation of fullerene (C<sub>2n</sub>)-PVP copolymers using radical polymerization of *N*-vinylpyrrolidone (NVP) in the presence of intact fullerenes that react with <sup>•</sup>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<sub>2n</sub>-vinyl derivatives) and will allow easy access to the



**Fig. 1** Preparation of fullerene-PVP copolymers by the direct free-radical copolymerization of NVP and C<sub>60</sub>, C<sub>70</sub>, or Gd<sub>3</sub>N@C<sub>80</sub>. AIBN (0.075–0.25 equiv.) was used as a radical initiator. The initial ratio of C<sub>2n</sub> and NVP considered in the reaction was 1 : 100, 200, 300, 400, or 500.

preparation of water-soluble higher fullerenes such as C<sub>70</sub> and Gd<sub>3</sub>N@C<sub>80</sub> with biologically attractive properties including a longer lifetime of the triplet excited state and paramagnetism, respectively. There are only few studies on water-soluble materials of such higher fullerenes. And particularly with C<sub>70</sub>, similar reactions are expected to occur as radical addition reactions are known.<sup>13</sup>

Studies on such direct free-radical copolymerization reactions of fullerenes involving intact C<sub>60</sub> were initially reported as C<sub>60</sub>-poly(styrene) copolymers.<sup>14–18</sup> 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<sub>60</sub> forming covalent bonds. A similar procedure was used to prepare other polymers such as C<sub>60</sub>-poly(carbonate)<sup>19</sup> and C<sub>60</sub>-poly(methylmethacrylate).<sup>20,21</sup> Conjugation with controlled polymers generated by nitroxide-mediated radical polymerization was also reported.<sup>22–24</sup> However, direct radical copolymerization of intact C<sub>60</sub> 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.<sup>25,26</sup>

In the present study, we tested several different ratios of NVP (100–500 equiv.) to C<sub>2n</sub> to optimize the conditions for the preparation of C<sub>2n</sub>-PVP polymers with a sufficiently large molecular

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weight and high water-solubility. C<sub>60</sub>, C<sub>70</sub>, and Gd<sub>3</sub>N@C<sub>80</sub> 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,<sup>27,28</sup> a relatively large amount of AIBN (0.075–0.25 equiv. of NVP, 40 equiv. of C<sub>2n</sub>) was added to each reaction.<sup>29</sup> The reactions were carried out in *o*-dichlorobenzene, a good solvent for fullerenes.

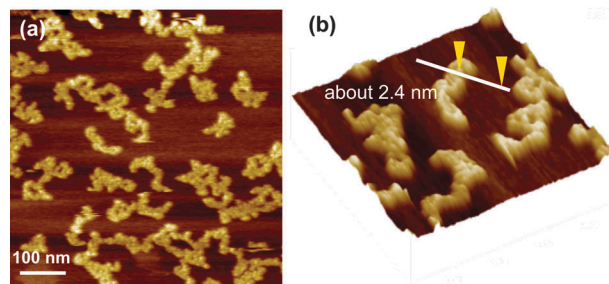
All co-polymerization reactions of C<sub>60</sub> or C<sub>70</sub> with NVP provided fullerene–PVP materials in sufficient yields (>70%, Table 1, column 3). However, the reaction of Gd<sub>3</sub>N@C<sub>80</sub> and NVP did not provide any polymeric product. We speculated that the radical quenching activity, which is common to many fullerenes, was too high in Gd<sub>3</sub>N@C<sub>80</sub> to provide any polymers (further details need to be clarified). C<sub>60</sub>–PVP (1 : 100–500) and C<sub>70</sub>–PVP (1 : 200–500) were thoroughly soluble in water. Only the C<sub>70</sub>–PVP (1 : 100) polymer was not soluble in water presumably due to the larger hydrophobic surface area of C<sub>70</sub>. The molecular weight of each copolymer (C<sub>60</sub>– or C<sub>70</sub>–PVP) was analyzed by GPC (solvent: DMF, calibration standard: poly(methylmethacrylate) (PMMA)). Interestingly, the *M*<sub>w</sub> of C<sub>2n</sub>–PVP polymers were highly correlated with the ratios of C<sub>2n</sub> and NVP. While the reaction with a higher ratio of NVP (lower ratio of C<sub>2n</sub>) provided polymers with higher *M*<sub>w</sub>, the reaction with the lower ratio of NVP provided polymers with lower *M*<sub>w</sub> (Table 1, columns 2 and 4). This result suggests that C<sub>60</sub> or C<sub>70</sub> are acting as an end-capping reagent of PVP (Fig. 1) and upon increasing the ratio of NVP, the PVP–C<sub>2n</sub> radical would have a higher possibility of reacting with NVP, leading to the higher *M*<sub>w</sub> 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<sub>2n</sub> 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

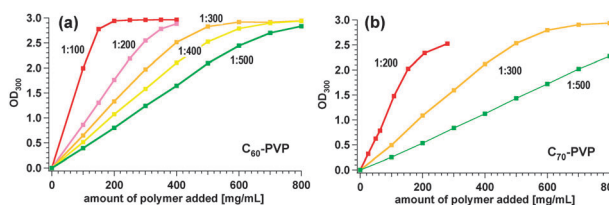
**Table 1** Characterization of C<sub>2n</sub>–PVP polymers

Reagents considered <sup>a</sup>		Properties of the obtained polymer				
C <sub>2n</sub>	Ratio of NVP to C <sub>2n</sub>	Yield [%]	<i>M</i> <sub>w</sub> <sup>b</sup> [kDa]	PDI	Particle size <sup>c</sup> [nm]	
					Fresh	1-day old
C <sub>60</sub>	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 <sub>70</sub>	100 <sup>d</sup>	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

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



**Fig. 2** AFM image of the C<sub>60</sub>–PVP (1 : 200) polymer on mica substrate.



**Fig. 3** Solubility curves of C<sub>60</sub>– (a) and C<sub>70</sub>–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.

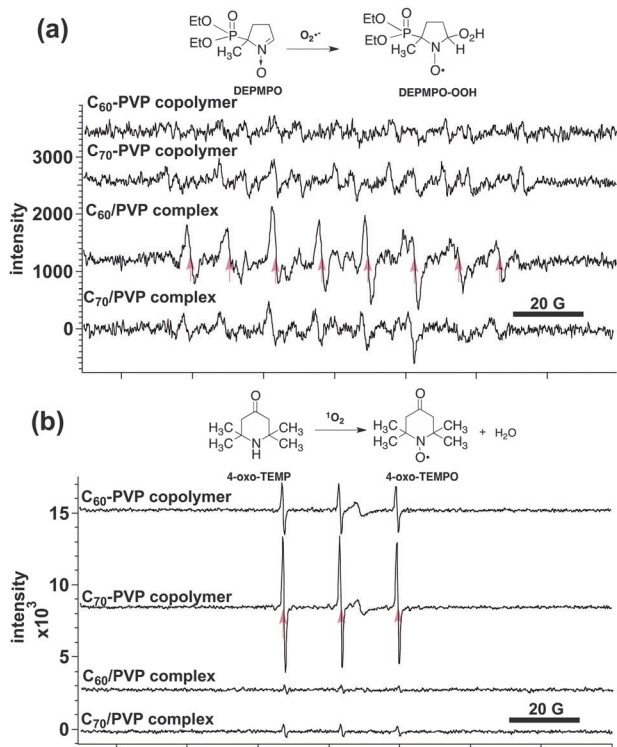
(Fig. S26, ESI<sup>†</sup>). Despite the difference in *M*<sub>w</sub> values, the sizes of the polymer particle measured by DLS were not significantly different, presumably because the polymers are not globular in water. The AFM image of the C<sub>60</sub>–PVP polymer supports this speculation and showed 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<sub>2n</sub>–PVP particles in the aqueous solution.

Solubility curves of the C<sub>60</sub>– and C<sub>70</sub>–PVP polymers are shown in Fig. 3a and b. As calculated based on the molar concentration of C<sub>2n</sub>, the solubility of all of the polymers were in the same range (~10 to 15 mM for both C<sub>60</sub>– and C<sub>70</sub>–PVP, Fig. S22 and S23, ESI<sup>†</sup>) and were sufficient for many bio-applications.

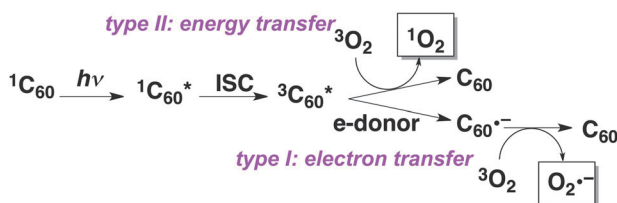
As one of the evaluations of C<sub>2n</sub>–PVP polymers as PDT agents, reactive oxygen species (ROS) generated under visible light irradiation were estimated using ESR spin-trapping methods (Fig. 4). In general, C<sub>60</sub> and C<sub>70</sub> can be excited by visible light irradiation to generate either singlet oxygen (<sup>1</sup>O<sub>2</sub>) or superoxide radical anions (O<sub>2</sub><sup>•−</sup>) via an energy transfer type II pathway or via an electron transfer type I pathway, respectively (Scheme 1).

According to the previous studies,<sup>30,31</sup> including ours,<sup>32</sup> <sup>1</sup>O<sub>2</sub> generation from photoexcited C<sub>60</sub> or C<sub>70</sub> was generally observed in less polar solvents (*e.g.* benzene) and not in the aqueous solution. Instead, O<sub>2</sub><sup>•−</sup> 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<sub>60</sub>– or C<sub>70</sub>–PVP complexes as a DEPMPO–OOH signal in Fig. 4a (3rd and 4th lines). However in our present study, no O<sub>2</sub><sup>•−</sup> generation from aqueous solutions of C<sub>60</sub>– and C<sub>70</sub>–PVP copolymers was detected in the presence of an electron donor. Alternatively, <sup>1</sup>O<sub>2</sub> 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 DEPMPPO (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  $\mu$ s)<sup>31</sup> compared to  $C_{60}$  (40  $\mu$ s).<sup>30</sup> 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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