Polymer Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Journal Name

RSCPublishing

COMMUNICATION

RAFT synthesis of poly(vinylpyrrolidone) amine and preparation of a water-soluble C_{60}-PVP conjugate

Cite this: DOI: 10.1039/x0xx00000x

Safwan Aroua,[a] Elisha Gabrielle V. Tiu,[a] Maxime Ayer,[a] Takashi Ishikawa[b] and Yoko Yamakoshi[a]*

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

An amine-terminated poly(vinylpyrrolidone) (PVP) was prepared by a radical polymerization using a xanthate-type RAFT agent. The obtained PVP (*ca.* 20 kDa) was converted to PVP-NH_2, which was coupled with a C_{60} anhydride to successfully provide a well-defined C_{60}-PVP conjugate.

Poly(vinylpyrrolidone) (PVP) is a colorless, amorphous, amphiphilic, and biologically compatible polymer[1] and has been used as a non-toxic polymer since 1939, when Reppe and co-workers initially prepared it from *N*-vinylpyrrolidone (NVP). In addition to its original use as a plasma expander,[2] PVP has been widely used as a dispersion agent in many industries including in pharmaceutical products (*e.g.* povidone iodine), food additives (*e.g.* E1201), quasi-drugs, and cosmetics.[3] The amphiphilic property of PVP is based on the hydrophilic cyclic amide unit in the side chain and the hydrophobic methylene/methine moieties in the backbone (Fig. 1).

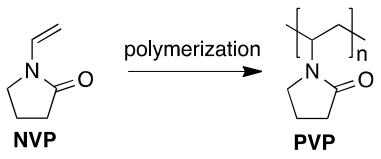


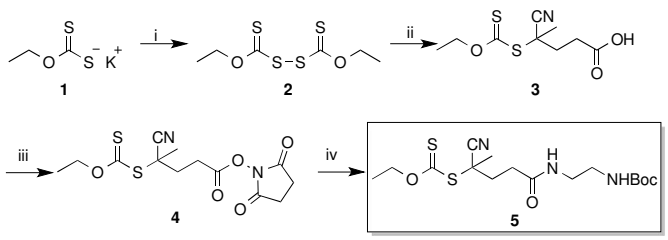
Fig. 1 Structures of *N*-vinylpyrrolidone (NVP) and poly(vinylpyrrolidone) (PVP).

One of the most commonly used biocompatible polymers in pharmaceutical research is polyethylene glycol (PEG).[4] The advantages of the use of PEG include improvement of drug solubility and stability *in vivo* (*i.e.* extended lifetime in blood), non-immunogenicity, and passive targeting of inflamed tissues and organs *via* enhanced permeation and retention (EPR) effect. In addition, monodispersed PEGs are commercially available which enables suitable design of the molecules with certain molecular weights on demand. However, *in vivo* safety of PEGs is not completely proven and preparation of alternative biocompatible polymers is currently in demand.

For the purpose of biological applications of extremely hydrophobic fullerenes (C_{60}, C_{70} and the higher C_{2n}), we have previously used PVP as a non-toxic detergent to prepare water-soluble complexes (C_{2n}/PVP)[5] and copolymers (C_{2n}-PVP).[6, 7] These fullerene materials provided sufficient water-solubility for *in vivo* biological assays such as photo DNA cleavage,[8, 9] enzyme inhibition,[10] and photo antimicrobial tests.[11] While the PVPs used in these previous studies were of wide polydispersity, it is important to develop polymers with well-defined structure for future clinical purposes.[12] In this study, we report highly-controlled preparation of PVP *via* a reversible addition-fragmentation chain transfer (RAFT) radical polymerization method and further conjugation (*PVPylation*) to a hydrophobic pharmaceutical core. Previous studies have shown the importance of reaction efficiency in PEGylation[4] (conjugation of PEG) protocols, therefore an amine-terminated PVP and a C_{60} anhydride derivative were chosen for PVPylation. In this study, we used a PVP with a terminal amine moiety, which was introduced by a RAFT reagent simultaneously controlling the polymerization, and C_{60} anhydride to carry out the efficient PVPylation.

The controlled radical polymerization (CRP) of NVP was reported by several groups using Cu-catalysed atom transfer radical polymerization (ATRP),[13] RAFT,[14, 15] and organometal-mediated living radical polymerization (LRP such as SBRP[16, 17] and TERP[18]). The RAFT technique, initially reported in 1998,[19] uses a RAFT agent such as xanthate derivatives and an azo-initiator. The polymerization can be carried out in bulk or in solvents. The choice of the RAFT agent is dependent on the class of monomers; more activated monomers (MAMs, monomers with stabilized (conjugated) vinyl group; *e.g.* styrene, methyl acrylate, acrylonitrile, acrylamide) or less activated monomers (LAMs, monomers with unstabilized (non-conjugated) vinyl group; *e.g.* NVP, vinyl acetate, *N*-vinylcarbazole). The main advantages of the RAFT technique compared to the other LRP methods are (1) it is more suitable for the polymerization of LAMs including NVP and (2) direct introduction of end-group is possible by the suitable design of the RAFT agent. For example, an aldehyde-terminated PVP was prepared *via* a RAFT polymerization and applied to protein modification.[20] In this study, we therefore selected RAFT polymerization to prepare a well-defined PVP with an amine end group.[21] By referring a previously reported efficient RAFT

agent, (*S*)-(2-cyano-2-propyl) *O*-ethyl xanthate[14, 20] as an analogue, we designed a RAFT reagent **5** (Scheme 1), that holds a Boc-protected amine group. The synthesis of **5** was carried out using potassium *O*-ethyl xanthate **1** as a starting material as shown in Scheme 1.



Scheme 1 Synthesis of an amine-terminated RAFT agent **5**. *Reagents and conditions:* (i) KI, I_2, H_2O, rt, 12 h, 70%; (ii) ACVA, dioxane, 95 ºC for 4 h then rt for 12 h, 70%; (iii) NHS, DCC, CH_2Cl_2, –15 ºC then rt, 10 min, 85%, (iv) *N*-Boc-1,2-diaminoethane, TEA, CH_2Cl_2, –15 ºC, 30 min, 80%.

RAFT agent **5** was used for the polymerization of NVP in the presence of AIBN as a radical initiator (Fig. 2a). Upon increasing the reaction time, the M_n and M_w of the polymer products increased as shown in the table in Fig. 2b.

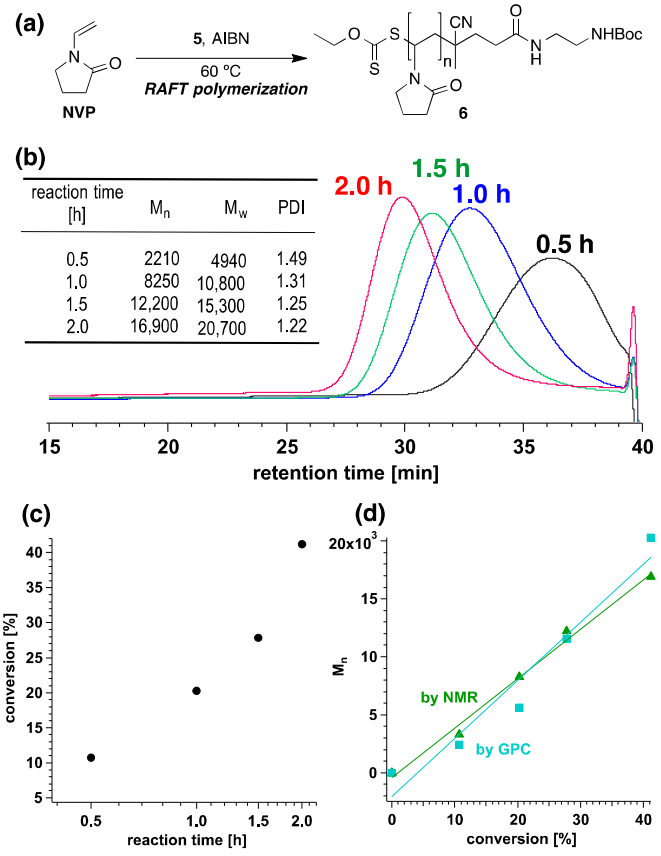


Fig. 2 (a) Scheme for RAFT polymerization of NVP in the presence of **5**. *Reagents and conditions:* NVP (120 mmol, 9.38 mol•L^{-1}), AIBN (48.1 µmol, 0.04 mol%), and **5** (217.7 µmol, 0.18 mol%). (b) GPC diagrams and table for the M_n and M_w values of the products at 0.5, 1.0, 1.5 and 2.0 hrs of reactions. (c, d) The kinetic study of polymerization based on (c) evolution of the conversion with the reaction time and (d) correlation of M_n with the conversion (%) estimated by NMR and GPC.

This polymerization step was evaluated by a kinetic study based on (1) conversion from monomer to polymer at different reaction time scale (0.5, 1.0, 1.5 and 2.0 h) (Fig. 2c) and (2) the molecular weight M_n versus conversion yield (Fig. 2d). Polymerization was carried out with NVP (120 mmol, 9.38 mol•L^{-1}), AIBN (48.1 µmol), and **5** (217.7 µmol). The conversion to the polymer was monitored by ^{1}H-NMR and the relative amount of the polymer to the unreacted monomer in the whole reaction mixture was estimated at each reaction time point. The mesurements were based on the integration values of vinyl protons (4.33 ppm, 2H for NVP) of unreacted monomer and the pyrrolidone + methine + methylene protons (1.0 – 4.0 ppm, 9H per unit for PVP plus 6H for NVP) (Figures S1-4). The M_n of polymer fraction (PVP precipitated in Et_2O for each reaction time point) was determined by both GPC (solvent: DMF with 0.1% LiBr, calibration standard: PMMA) and ^{1}H-NMR integration of PVP (3.0 – 4.1 ppm, 3H per unit) relative to the integration of CH_2 group in xanthate end group (4.60 ppm, 2H) (Figure S5). The M_n values determined by GPC had very good agreement to the ones calculated by ^{1}H-NMR integration and were highly correlated with conversion (Fig. 2d), confirming the evidence of controlled polymerization using **5**.

The polymer **6** with a molecular weight of M_n *ca.* 20 kDa (PDI = 1.18, prepared by polymerization for 3 hours), was subjected to the subsequent radical induced reduction using Bu_3SnH to remove the xanthate end-group,[22] resulting in an H-terminated polymer **7** (Fig. 3a). Evidence of the xanthate reduction was clearly shown by UV-vis spectrum of **6** as the absorption at 280 nm, corresponding to the xanthate moiety, completely disappeared after the reaction (Figure S26). Subsequent deprotection of the Boc group was carried out by treating the polymer with TFA, and the production of the amine-terminated PVP polymer **8** (Fig. 3a) was confirmed by ^{13}C-NMR (Figure S27). The molecular size distribution of PVP **6** and **8** were briefly compared with commercially available undefined PVP (PVP K-30 from TCI) to show their well-defined size (Fig. 3b).

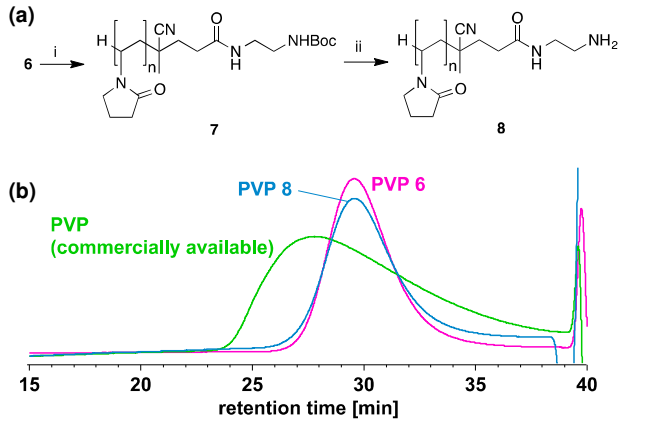


Fig. 3 (a) Scheme for the conversion of polymer **6** (M_n *ca.* 20 kDa) to the amine-terminated PVP **8**. *Reagents and conditions:* (i) AIBN, Bu_3SnH, *o*-dichlorobenzene/DMF, 70 ºC, 4 h, 87%; (ii) TFA, rt, 12 h, 95%. (b) GPC traces of PVP **6** and **8** in comparison to the commercially available uncontrolled PVP K-30 (**6**: Mn = 19,500, Mw = 22,900, PDI = 1.18; **8**: Mn = 19,900, Mw = 25,800, PDI = 1.29; PVP K-30: Mn = 19,200, Mw = 42,600, PDI = 2.22).

The previously reported C_{60}/PVP complex or C_{60}–PVP copolymer with uncontrolled sizes had efficient photoinduced reactive oxygen species (ROS) generation ability showing their

potential as photodynamic therapy (PDT) agents for tumor treatment.[6-9] Importantly, such polymeric materials can target tumors *in vivo* due to their EPR effect, where the larger molecules (>15 kDa) tend to be accumulated much more than in normal tissues.[23, 24] Therefore, it is very important to have a well-defined size of polymer in such PDT agents. The prepared well-defined PVP-NH_2 **8** (M_n = 19,900, M_w = 25,800, PDI = 1.29) was subjected to a reaction with C_{60} anhydride **9**[25] in excess (7.5 equiv, due to the difficulty in separation of PVP-NH_2 and C_{60}-PVP) for the amide conjugation (Fig. 4a). The reaction was carried out in a mixture of 1-methylnaphthalene and DMF, good solvents for both C_{60} anhydride **9** and PVP-NH_2 **8**, respectively. Based on the calibrated UV-Vis measurement of C_{60}-Prato adduct (due to the poor solubility of **9**, its precursor, the bis-*tert*-butyl ester was used), the C_{60}-PVP conjugate **10** was obtained quantitatively. The GPC trace of **10** was almost identical to the one of **8** (Figure S35) with well-defined molecular size. The obtained polymer **10** was highly soluble in water (clear solution, >10 mM, inset in Fig. 4a).

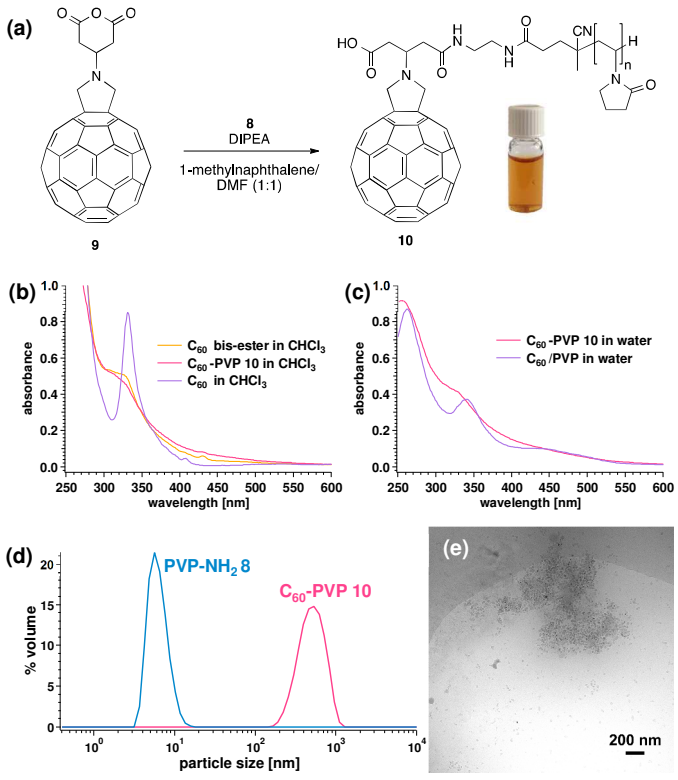


Fig. 4 (a) Synthesis of a C_{60}–PVP conjugate **10** and its aqueous solution (inset). (b) UV-Vis spectra of Prato bis-ester derivative, C_{60}-PVP **10** and pristine C_{60} (15.0 μM in $CHCl_3$, PVP-NH_2 solution in $CHCl_3$ was used as a blank solution for the measurements of **10**). (c) UV-Vis spectra of C_{60}-PVP **10** and C_{60}/PVP complex (15.0 μM in water, aqueous solution of PVP-NH_2 and PVP were used as blank solutions respectively). (d) DLS data of freshly prepared aqueous solutions (1.0 mM,) of PVP-NH_2 **8** (mean: 6.0 nm, width: 2.2 nm) and C_{60}-PVP **10** (mean: 560 nm, width: 270 nm). (e) CryoTEM image of C_{60}-PVP **10** in water (1 mM).

The C_{60}-PVP conjugate **10** was characterized by UV-vis spectroscopy (Fig. 4b, c) and dynamic light scattering (DLS) (Fig. 4d). Compared to the UV-vis spectra of pristine C_{60} (in $CHCl_3$), the spectra of both Prato bis-ester derivative (precursor of **9**)[26] and C_{60}-PVP **10** showed significant broadening of the band at 330 nm presumably due to the breaking of symmetry of the C_{60} cage (Fig. 4b). Similar situation was observed in the aqueous solution of C_{60}-PVP **10** in comparison to pristine C_{60} complexed with PVP (Fig. 4c). In the $CHCl_3$ solution of C_{60}-PVP **10**, increase of the intensity around 400-500 was observed compared to the small molecule Prato derivative indicating the aggregation of the molecules.[27]

Although the GPC diagrams of PVP-NH_2 **8** and C_{60}-PVP **10** were almost identical indicating that they are in the same range of the molecular sizes (Figs. S31 and S35), DLS data indicated that their particle size in water were very different (Fig. 4d). In comparison to the size distribution of PVP-NH_2 **8** (with a peak top of <10 nm), C_{60}-PVP **10** showed larger particle size (transparent solution, DLS peak top at *ca.* 600 nm). These DLS results were consistent under storage for 6 days in aqueous solution showing the stability of the sizes of **8** and **10** (Figs. S36-37). In addition, previously reported undefined C_{60}-PVP copolymer[7] with similar molecular weight range had smaller range of particle size (peak top <10 nm by DLS). CryoTEM measurement was used to investigate the morphology of the larger size of C_{60}-PVP. As shown in Fig. 4e, C_{60}-PVP **10** formed small particles with 10-20 nm that further create larger aggregates with several hundreds of nm (cryoTEM images of **10** in other positions are shown in Figure S37). In contrast, there was no significant object observed in the cryoTEM images of PVP-NH_2 **8**, indicating that **8** did not form any lager observable morphology in aqueous solution.

In summary, controlled polymerization of NVP was succesfully achieved using a RAFT agent. By subsequent end-group modification, an amine-terminated PVP was obtained. The PVP-NH_2 with ca. 20 kDa was succesfully conjugated to C_{60} fullerene to provide a water-soluble C_{60}-PVP with well-defined molecular weight. In addition, remaining carboxylic acid group in **10** can potentially be useful for further conjugation of ligands to target tumors for PDT application.

Authors thank Prof. Dr. M. Morbidelli (ETH Zürich) for his help in DLS measurements. Authors thank Prof. Dr. J.-C. Leroux and Ms. K. Fuhrmann (ETH Zürich) for their help in GPC calibration. This research was supported in part by the ETH Research Grant (ETH-25 11-1), Swiss National Foundation (200021-140451, 200021-156097), PRESTO program from JST, and American Heart Association Researcher's Developing Grant (0930140N).

Notes and references

[a]Laboratorium für Organische Chemie, ETH-Zürich, Vladimir-Prelog-Weg 3, CH-8093 Zürich, Switzerland.
E-mail: yamakoshi@org.chem.ethz.ch; Fax: +41 44 633 1235; Tel: +41 44 633 6420
[b]Paul-Scherrer Institute, CH-5232, Villigen, Switlzerland.

†Electronic Supplementary Information (ESI) available: experimental section of synthesis and characterization of RAFT agent, polymers, and C_{60}-polymer conjugates. See DOI: 10.1039/c000000x/

1. W. F. Bergfeld, D. V. Belsito, W. W. Carlton, C. D. Klaassen, A. L. Schroeter, R. C. Shank, T. J. Slaga and C. I. R. C. E. Panel, *Int. J. Toxicol.*, 1998, **17**, 95-130.
2. A. L. Gropper, L. G. Raisz and W. H. Amspacher, *Surg. Gynecol. Obstet.*, 1952, **95**, S521-S542.
3. F. Fischer and S. Bauer, *Chem. Unserer Zeit*, 2009, 43, 376-383.
4. S. N. S. Alconcel, A. S. Baas and H. D. Maynard, *Polym. Chem.*, 2011, **2**, 1442-1448.
5. Y. N. Yamakoshi, T. Yagami, K. Fukuhara, S. Sueyoshi and N. Miyata, *J. Chem. Soc. Chem. Commun.*, 1994, 517-518.
6. Y. Iwamoto and Y. Yamakoshi, *Chem. Commun.*, 2006, 2006, 4805-4807.

7. S. Oriana, S. Aroua, J. O. B. Sollner, X. J. Ma, Y. Iwamoto and Y. Yamakoshi, *Chem. Commun.*, 2013, **49**, 9302-9304.
8. Y. Yamakoshi, S. Sueyoshi, K. Fukuhara and N. Miyata, *J. Am. Chem. Soc.*, 1998, **120**, 12363-12364.
9. Y. Yamakoshi, N. Umezawa, A. Ryu, K. Arakane, N. Miyata, Y. Goda, T. Masumizu and T. Nagano, *J. Am. Chem. Soc.*, 2003, **125**, 12803-12809.
10. N. Iwata, T. Mukai, Y. N. Yamakoshi, S. Hara, T. Yanase, M. Shoji, T. Endo and N. Miyata, *Fullerene Sci.. Technol.*, 1998, **6**, 213-226.
11. Y. Kai, Y. Komazawa, A. Miyajima, N. Miyata and Y. Yamakoshi, *Fuller. Nanotub. Car. N.*, 2003, **11**, 79-87.
12. L. Nebhani and C. Barner-Kowollik, *Macromol. rapid commun.*, 2010, **31**, 1298-1305.
13. X. J. Lu, S. L. Gong, L. Z. Meng, C. Li, S. Yang and L. F. Zhang, *Polymer*, 2007, **48**, 2835-2842.
14. G. Pound-Lana and B. Klumperman, *Acs Symp. Ser.*, 2009, **1024**, 167-179.
15. R. Devasia, R. L. Bindu, R. Borsali, N. Mougin and Y. Gnanou, *Macromol. Symp.*, 2005, **229**, 8-17.
16. S. Yamago, B. Ray, K. Iida, J. Yoshida, T. Tada, K. Yoshizawa, Y. Kwak, A. Goto and T. Fukuda, *J. Am. Chem. Soc.*, 2004, **126**, 13908-13909.
17. B. Ray, M. Kotani and S. Yamago, *Macromolecules*, 2006, **39**, 5259-5265.
18. S. Yamago, Y. Ukai, A. Matsumoto and Y. Nakamura, *J. Am. Chem. Soc.*, 2009, **131**, 2100-2101.
19. J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559-5562.
20. G. Pound, J. M. McKenzie, R. F. M. Lange and B. Klumperman, *Chem. Commun.*, 2008, 3193-3195.
21. PVP-NH_2 was previously prepared using a chain transfer agent but in an uncontrolled manner. H. Y. Lee, S. A. Yu, K. H. Jeong, Y. J. Kim, *Macromol. Res.* 2007, **15**, 574-552.
22. Y. K. Chong, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 2007, **40**, 4446-4455.
23. Y. Matsumura and H. Maeda, *Cancer Res.*, 1986, **46**, 6387-6392.
24. R. Duncan and Y. N. Sat, *Ann. Oncol.*, 1998, **9**, 39-39.
25. S. Aroua, W. B. Schweizer and Y. Yamakoshi, *Org. Lett.*, 2014, **16**, 1688-1691.
26. Since C_{60} anhydride **9** was not throughly soluble in $CHCl_3$, we used similar Prato derivative with *tert*-butyl ester groups, we reported previously as a precursor in the preparation of **9** (ref. 25).
27. J. Lee, Y. Yamakoshi, J. B. Hughes and J. H. Kim, *Environ. Sci. Technol.*, 2008, **42**, 3459-3464.

