



Anti-Radiation Damage Performance of Fullerene Ethanolamine

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Water soluble fullerene ethanolamine derivative was synthesized by adding fullerene (C_{60}) into the ethanolamine under nitrogen atmosphere protection. Its molecular structure was characterized by FTIR and ESI-MS. Hydroxyl radicals were generated by adding ethanol solution and nitronyl nitroxide radicals were generated by adding 4-hydroxy-2,2,6,6-tetramethyl piperidine solution. With irradiation of γ -ray, scavenging radicals efficiency of fullerene ethanolamine derivative reached to 49.2 %; with irradiation of fast neutron, fullerene ethanolamine derivative showed excellent scavenging radicals efficiency compared with other materials in experiments, which was decreased first and then increased and attained 65 % at most. The results reveal that no matter irradiation by γ -rays or high-energy neutrons, fullerene ethanolamine derivative presents favorable scavenging radicals efficiency and may be used as a affective protectant for two irradiations.

Keywords: Fullerene ethanolamine derivative, γ -Ray, Neutron, Scavenging radicals.

INTRODUCTION

With the development of nuclear technology, although it benefits society, it produces severe nuclear radiation risk. Ionization radiation will destroy the balance of free radical in human, which play an important role on cell phospholipid¹ and DNA damage. Not only is free radical root of many diseases², but it is related intimately to decrepitude. So it is crucial to find out high capability free radical scavenging medicament.

Fullerene (C_{60}) was researched worldwide with its peculiar structure and character. Fullerene (C_{60}) as a scavenging radical sponge^{3,4} has the abilities of anti-oxidation and reaction with free radical^{5,6}. Fullerene (C_{60}) in fact, is not soluble in water or in polar organic solvents, which is limited to some extent in the application of biochemistry field. There have been several attempts to overcome the problem of solubility. The most widely used methodologies are: (1) encapsulation or micro-encapsulation in special carriers; (2) suspension with the help of co-solvents; (3) chemical fictionalization for the introduction of solubilizing appendages⁷. We have synthesized water soluble fullerene ethanolamine derivative through introducing solubilizing ethanolamine added to C_{60} .

So far it is limited in the study of water soluble fullerene derivatives radiation biology and mainly in γ -ray irradiation protection capability, while fullerene derivatives in neutron irradiation protection are less studied. Therefore our aim was to evaluate the radicals scavenging efficiency of fullerene

ethanolamine derivative. This paper mainly describes free radical scavenging activity based on the irradiation of neutron and γ -ray.

EXPERIMENTAL

Fullerene ethanolamine derivative was synthesized under N_2 protection by mixture of C_{60} and ethanolamine for 24 h^{8,9}. The product was purified through the following processes: (a) adding four times methanol to the solution after reaction; (b) static for 10 min and deposition appeared; (c) centrifugal separation. It was triple above steps and drying under vacuum for several hours. Its FTIR spectrum (Fig. 1), which showed a broad hydroxy absorption centered at 3338 cm^{-1} , a stronger C-O stretching absorption centered at 1071 cm^{-1} and C-N stretching absorption located at 1322 and 1232.71 cm^{-1} . Two

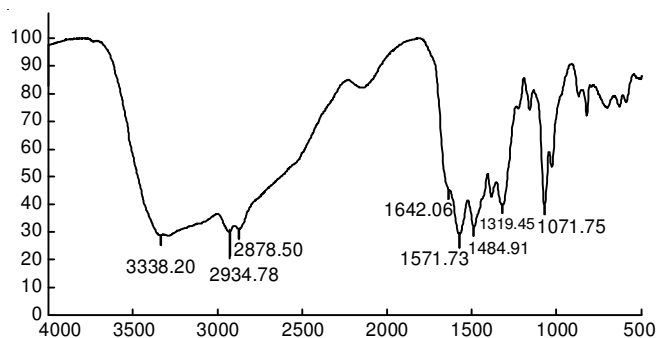


Fig. 1. FTIR spectrum of fullerene ethanolamine derivative

weak C-H absorption bands at 2935 and 2878 cm^{-1} were also observed. A weak alkene absorption at 1642.06 cm^{-1} , caused by electron deficiency of fullerene itself.

Fig. 2 is the electrospray ionization mass spectrometry (ESI-MS) of fullerene ethanolamine derivative. The main product was adducted three ethanolamine molecules and the chemical formula can be expressed as $\text{C}_{60}(\text{NH}_2\text{CH}_2\text{CH}_2\text{OH})_3$.

DMPO (5,5-dimethyl-1-pyrroline-N-oxide) was purchased from Sigma-Aldrich and HTMP (4-hydroxy-2,2,6,6-tetramethyl piperidine) was purchased from Nantong Huikang International Ltd.

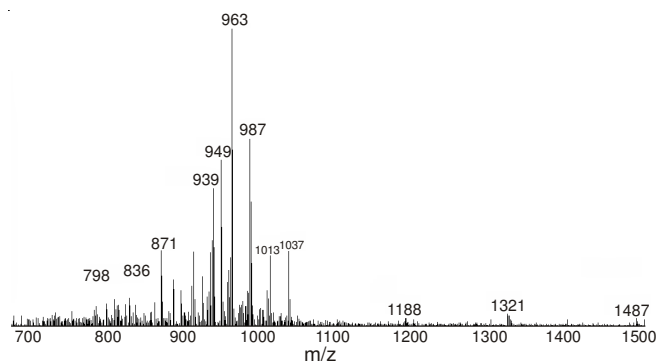


Fig. 2. ESI-MS of fullerene ethanolamine derivative

Free radical generation: Samples were divided into experimental samples, which contain free radical generation material, fullerene ethanolamine derivative, DMPO and water and blank antitheses samples, which didn't contain fullerene ethanolamine derivative.

Hydroxyl radicals were generated by adding 500 μL of 1 M ethanol solution with ^{60}Co γ -ray radiation for 10 min, which the dose rate was 145 Gy/min and the total dose was 1450 Gy. Nitronyl nitroxide radicals were generated by adding 500 μL of 0.2 M HTMP solution with 14 MeV fast neutron radiation for 430 min, which the neutron flux rate was $2.57 \times 10^{10} \text{ s}^{-1}$ and the total dose was 30 Gy. The generation of radical species was monitored by electron spin resonance (ESR) spectroscopy (ESP300, Bluker) using the spin-trapping technique with DMPO as trapping agent. Free radical can be identified by characteristic spectral line of ESR signal. Height of the secondary peak was defined as the intensity of free radical signal and the scavenging efficiency can be calculated according to the following formula:

Scavenging efficiency =

$$\left(1 - \frac{\text{peak height of the experimental samples}}{\text{peak height of the blank antitheses samples}} \right) \times 100 \%$$

RESULTS AND DISCUSSION

Hydroxyl radicals scavenging generated by γ -ray radiation: The ESR spectra of hydroxyl radicals captured by DMPO in different concentrations of fullerene ethanolamine derivative is shown in Fig. 3 and its hydroxyl radicals scavenging activity is presented in Fig. 4.

It indicates that with the concentration of fullerene ethanolamine derivative increasing between 0.07 and 0.2 mg/mL, intensity of hydroxyl radicals is reduced gradually, while

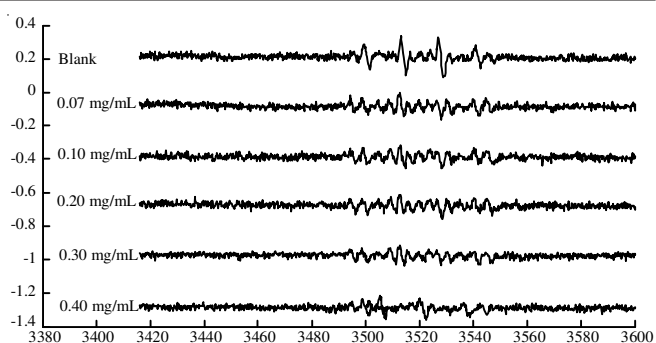


Fig. 3. ESR spectra of hydroxyl radicals in fullerene ethanolamine derivative concentrations

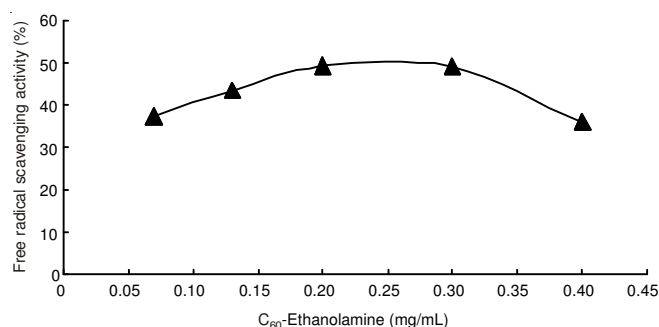


Fig. 4. Scavenging efficiency curve of fullerene ethanolamine derivative to hydroxyl radicals created by γ -ray radiation

efficiency of free radical scavenging enhances by degrees. When concentration of fullerene ethanolamine derivative is 0.2 mg/mL, the efficiency of free radical scavenging reaches 49.2 %. When concentration was greater than 0.2 mg/mL, fullerene ethanolamine derivative did not offer enough scavenging radical activity and it was dropped for scavenging efficiency, which is similar to vitamin C^{10} .

Scavenging nitronyl nitroxide radicals genetated by neutron radiation: Scavenging efficiency of nitronyl nitroxide radical created by neutron radiation is decreased first then increased.

With radiation by 14 MeV fast neutron, as the concentration increased, the radicals scavenging efficiency of fullerene ethanolamine derivative presented fall first and then enhanced (Fig. 5b), while the radicals scavenging efficiency of fullerene ethylenediamine derivative and vanillin descended (Fig. 5a,c). When concentration of fullerene ethanolamine derivative is 0.5 mg/mL, the efficiency of free radical scavenging reaches 65 %.

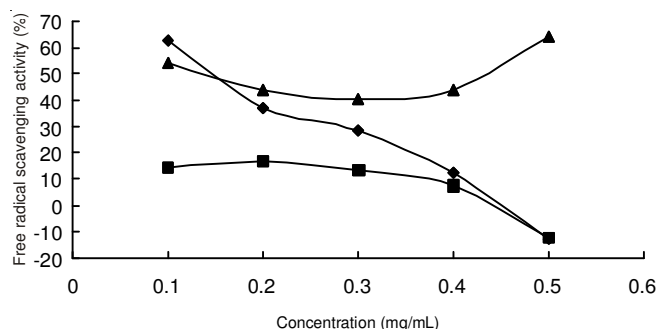


Fig. 5. Scavenging efficiency curve of fullerene ethanolamine derivative compared other materials to nitronyl nitroxide radicals created by neutron radiation, (a) fullerene ethanolamine derivative; (b) fullerene ethylenediamine derivative; (c) vanillin

Fast neutron belonged to high liner energy transfer (LET) and has great ionization density. The molecule structure of fullerene derivatives and vanillin were destroyed and came into being free radicals, which induced ESR signal intensity increased as the concentration enhanced. Correspondingly radicals scavenging efficiency decreased, even disappeared by degrees. Radicals scavenging efficiency of fullerene ethanolamine derivative was exceeded more than 40 % in the whole experimental concentration. These results prompted us that fullerene ethanolamine derivative has the idiosyncratic character with neutron radiation and has excellent scavenging radical activity.

Conclusion

Present results show that fullerene ethanolamine derivative has excellent scavenging radical activity. Scavenging radical efficiency of fullerene ethanolamine derivative is confirmed on radiation of γ ray and fast neutron, which reaches max 49 % and 65 %, respectively. Further work is required to decipher the mechanisms of fullerene ethanolamine derivative scavenging radical on radiation of fast neutron. The results have extraordinary reference value for searching effective high LET

protective medication. The results reveal that fullerene ethanolamine derivative presents favorable scavenging radicals efficiency and may be used as an affective protectant for both γ -rays and high-energy neutrons.

REFERENCES

1. A.C. Cheng, J.L. Hogan and M.A. Caffrey, *J. Mol. Biol.*, **229**, 291 (1993).
2. D. Harman, in ed.: W. A. Pryor, Free radical in Biology, Academic Press, New York, vol. 5, p. 255 (1982).
3. C.N. McEwen, R.G. McKay and B.S. Larsen, *J. Am. Chem. Soc.*, **114**, 4412 (1992).
4. M. Brettreich and A. Hirsch, *Tetrahedron Lett.*, **39**, 2731 (1998).
5. R. Taylor and D.R.M. Walton, *Nature*, **363**, 685 (1993).
6. I.S. Ayene, C.J. Koch and R.E. Krisch, *Radiat. Res.*, **146**, 501 (1996).
7. T. Da Ros and M. Prato, *Chem. Commun.*, 663 (1999).
8. F. Wudl, A. Hirsch, K.C. Khemani, T. Suzuki, P.-M. Allemand, A. Koch, H. Eckert, G. Srdanov and H.M. Webb, Survey of Chemical Reactivity of C_{60} , Electrophile and Dieno-Polarophile Par Excellence, ACS Symp. Series, pp. 161-175 (1992).
9. R. Seshadri, A. Govindaraj, R. Nagarajan, T. Pradeep and C.N.R. Rao, *Tetrahedron Lett.*, **33**, 2069 (1992).
10. M.H.L. Green, J.E. Lowe, A.P.W. Waugh, K.E. Aldridge, J. Cole and C.F. Arlett, *Mutat. Res.*, **316**, 91 (1994).