Synthesis, Characterization and Biological Activity of C_{60} Derivative

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ABSTRACT: A novel fullerene-maleic anhydride derivative was synthesized via radical polymerization. It is soluble in polar solvents such as water, dimethyl sulfoxide, and tetrahydrofuran etc. The product was characterized by FTIR, UV-Vis, GPC, and Transmission electron microscope (TEM). TEM analysis shows that the average particle diameter is about 38 nm. The *in vitro* antitumor activity of the fullerene-maleic anhydride derivative has been tested and the result shows that the derivative exhibits better antitumor activity against HeLa cells and bone tumor cells. To prove water-soluble fullerene derivatives photodynamic effect on tumor cell, i.e., the photodynamic effect on mouse bone tumor *in vivo* we injected the fullerene -maleic anhydride derivatives into the mouse bone tumor body. We found that with I-W lamp (500 W) illumination the mouse bone tumor body was strongly damaged. The antitumor mechanism of water-soluble fullerene-maleic anhydride derivative was investigated for the first time. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 3120–3123, 2007

Key words: fullerene derivative; water solubility; antitumor activity; nanoball

INTRODUCTION

Since C_{60} was detected in 1985,[1] the increasing interest and significance of the work in the fullerene field led to the award of the 1996 Nobel prize in the chemistry field.[2] To make a water-soluble fullerene was one of the great challenges in fullerene chemistry and scientific interest in the potential biochemical, biophysical, and biological applications of these fascinating molecules.[3] There were some attempts to make fullerene (C_{60}) derivatives that were soluble in an aqueous environment.[4–10] As a number of fullerene (C_{60}) derivatives have been prepared for such a purpose, most of them have been of only poor aqueous solubility, these results have limited application in biological field.

For the investigation of such biological properties, it is necessary that the C_{60} derivatives should be soluble in polar solvents, particularly water. It is therefore quite important to synthesize fullerene derivatives with a versatile anchor group that can be used for conversion to polar functional addends.[11] Considering that maleic anhydride is a compound with high significant biological activities, one can foresee that maleic anhydride derivative of C_{60} might have important medicinal applications. In this article, a novel water-soluble maleic anhydride derivative of fullerene C_{60} was synthesized, and activity of biology of water-soluble maleic anhydride derivative of fullerene C_{60} was studied for the first time.

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EXPERIMENTAL

Synthesis

The fullerene-maleic anhydride derivative was synthesized by typical free radical polymerization. A solution of 80 mg of C_{60} in 70 mL of toluene was mixed with 3 g of maleic anhydride. The mixture was deoxygenated by bubbling dry nitrogen gas for $\sim$ 30 min, followed by the addition of 100 mg of AIBN (azobisisobutyronitrile). The polymerization reaction was carried out in a glass bottle at 65–75°C for 10 h. The products were precipitated from the toluene solution into petroleum ether. The precipitation procedure was repeated several times, yielding a solid sample with almost black color. The solid sample was further purified by washing with acetone/petroleum ether and THF/hexane. After drying under vacuum, 1.2 g of the final copolymer sample was obtained. Polymer of neat maleic anhydride was prepared under the same conditions.

Cytotoxicity

The *in vitro* cytotoxicity against HeLa cells and bone tumor cells were evaluated by the study of inhibition

of growth rate. To examine the effect of light, incubation was carried out with or without irradiation of light for 30 min each day during the 3-day period of incubation. The fullerene maleic anhydride derivative was dissolved in a small amount of DMSO, and these solutions were further diluted with 100 μL of 1640 medium to afford solutions suitable for the fullerene-maleic anhydride derivative concentration range studied. Each solution was placed into a 96-well to be used as a reference microplate (100 μL/well). HeLa cells were suspended in 1640 medium containing 10% of fetal calf serum at a concentration of 5×10^4 cells/mL, and 100 μL of the solution were added to the 96-well plate. After 3 day incubation at 37°C, MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)solution was added to each well and the plate was incubated at 37°C for 4 h. The mitochondrial dehydrogenase of viable cells reduces MTT to a blue formazane product that can be measured spectrometrically.

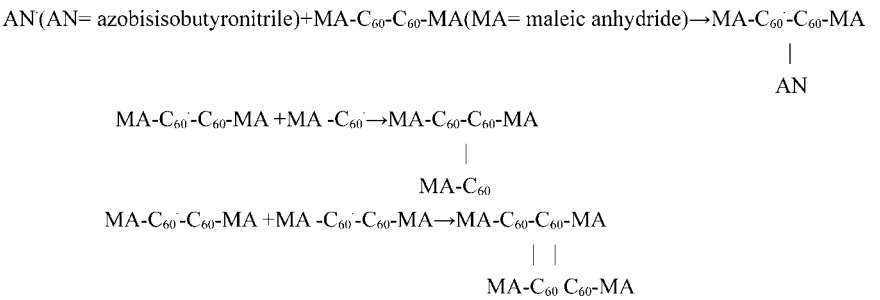
RESULTS AND DISCUSSION

Characterization of the fullerene derivative nanoball

The fullerene-maleic anhydride derivative was characterized by FTIR, UV-Vis, GPC, Transmission electron microscope (TEM), and SEM. FTIR spectra were measured in KBr matrices, and the results were compared with those of neat poly(maleic anhydride) samples prepared under the same experimental conditions. As shown in Figure 1, FTIR spectrum of the copolymers with C_{60} are nearly the same as those of neat poly(maleic anhydride). However, for the copolymer, the FTIR spectrum shows extra absorption in the 530 cm^{-1} region, which is typical with respect to substituted C_{60}.[12] UV absorption spectra were measured in THF, and the results were compared with those of poly(maleic anhydride), As shown in Figure 2, the UV absorption spectra of copolymer with C_{60} are different from those of poly(maleic anhydride). The somewhat structured absorption band of free C_{60} has been replaced by a steadily decreasing curve, typical for substituted C_{60}.[12] UV absorption wavelength maximum of copolymer with C_{60} contents is 256 nm, different from that of free C_{60} and poly(maleic anhydride), which is typical with respect to substituted C_{60}.[13–14] The emission wavelength can be attributed to the C_{60}-containing sites in the poly(maleic anhydride) structure.

The average molecular weight M_w obtained by GPC (Voria 5060 model). M_w of copolymer with C_{60} contents is 8560. The molecular structure of fullerene-maleic anhydride derivative the may be star polymer with fullerene as core and a grafting of one or several poly(maleic anhydride) chain segments.

The results could be used to support a proposed copolymer mechanism as follows.



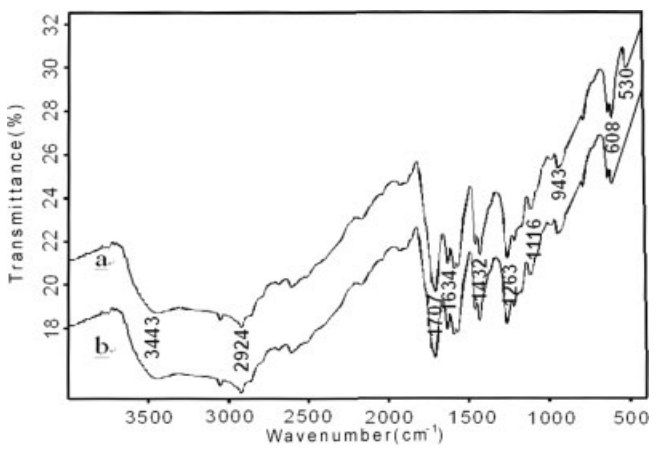


Figure 1 FTIR spectra of (a) C_{60}-maleic anhydride copolymer and (b) poly(maleic anhydride).

The results and the mechanism of radical-initiation could be used to support a proposed copolymer structure as follows.[14]

Morphology and size of the fullerene derivative in water were measured using a JEM 100CXII model TEM with a voltage of 200 kV. The synthesized fullerene derivative is soluble in water, giving a clear brown solution. TEM analysis indicates that the derivative possesses an ideal sphere in water with an average size of particle about 10–70 nm in diameter, as shown in Figure 3. For comparison, the appearance of the poly(maleic anhydride) in water takes lamellar shape. The physical structure of the fullerene derivative nanometer tiny balls may be described as follows: the core is hard fullerene, and the shell is poly(maleic anhydride), which may be relatively soft but elastic.

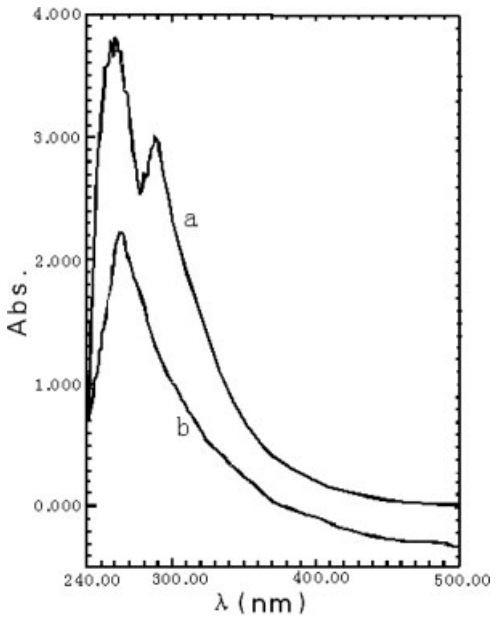


Figure 2 A comparison of (a) the C_{60}-maleic anhydride copolymer UV spectrum with (b) that of poly(maleic anhydride). All in THF.

Cytotoxicity against tumor cells

The fullerene-maleic anhydride derivative has shown cytotoxicity against HeLa cell upon irradiation of light. No inhibition was observed in total darkness at fullerene-maleic anhydride derivative concentration >100 μg/mL. In the present studies, the *in vitro* cytotoxicity was compared for fullerene-maleic anhydride derivative at different concentration by the investigation on inhibition of growth rate, as shown in Table I.

To examine the effect of light, incubation was carried out with or without irradiation of light with 500 W at a 42 cm distance, for 30 min in every 24 h during a 72 h period of incubation at 37°C.

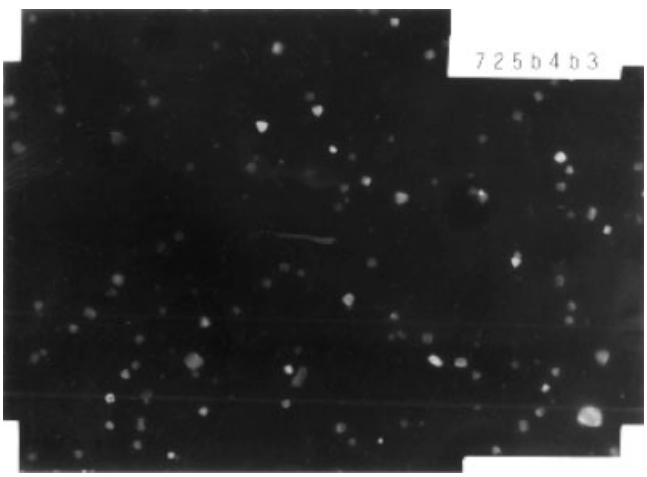


Figure 3 TEM of maleic anhydride with C_{60}. Magnification: 72,000×.

TABLE I
Inhibition of Growth Rate of Fullerene Derivative at Different Concentration

Concentration (μg/mL)	Inhibition of growth rate (%)
0	9.02
25	36.26
50	86.07
75	90.88
100	92.98

To examine the effect of light, incubation was carried out with or without irradiation of light with 500 W at a 42 cm distance, for 30 min in every 24 h during a 72 h period of incubation at 37°C.

The result shows that the new fullerene-maleic anhydride derivative exhibits better antitumor activity at low concentration of fullerene derivative being 50 μg/mL, and the inhibition of growth rate is dose-dependent ($P < 0.05$).

To prove water-soluble fullerene derivatives photodynamic effect on tumor cell, i.e., the photodynamic effect on mouse bone tumor *in vivo* we injected the fullerene -maleic anhydride derivative into the mouse bone tumor body. We found that with I-W lamp (500 W) illumination the mouse bone tumor body was strongly damaged. Under the intensity of 500 W and the concentration of C_{60} (50 μg/mL), mouse average survival time extended 2 days and the tumor size reduced 0.1 cm and tumor weight decreased 0.1 g. In the other way bone tumor cells was cocultured *in vitro* with fullerene -maleic anhydride derivative for three days. The result of the killing was examined by MTT method after illuminating the fullerene -maleic anhydride derivative with I-W lamp. Bone tumor cell *in vitro* were obviously damaged. Fullerene -maleic anhydride derivative is a kind of strong photosensitize. It can kill mouse bone tumor cells both *in vivo* and *in vitro*.

Based on the above experimental results, the antitumor mechanism of water-soluble fullerene-maleic anhydride derivative were investigated for the first time. It was believed that cell death is mainly because of phospholipid and protein damage within the cell membrane. When irradiated with light, the fullerene-maleic anhydride derivative became excited to a singlet excited state and through intersystem crossing passed to an excited triplet state. Subsequently, they could be quenched by O_2 to produce singlet oxygen, a species that reacts readily with amino acids, nucleic acids, and membrane phospholipids, leading to cell damage and death.[15]

CONCLUSIONS

In summary, a new water-soluble fullerene-maleic anhydride derivative nanoball has been synthesized and

characterized by FTIR, UV-Vis, GPC, and TEM. It is soluble in polar solvents such as water, dimethyl sulfoxide, and tetrahydrofuran etc. The antitumor activity of the fullerene-maleic anhydride derivative has been tested and the result shows that the fullerene-maleic anhydride derivative exhibits better antitumor activity both *in vivo* and *in vitro*. The antitumor mechanism of water-soluble fullerene-maleic anhydride derivative was investigated for the first time. The fullerene-maleic anhydride derivative may become a potential medicine for anticancer uses.

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