New chemical method for selective generation of C_{70}^{n-} ($n = 1, 2, 3$) anions and formation and properties of an aqueous colloidal solution of C_{70}

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Facile methods for selective generation of C_{70}^{n-} ($n = 1, 2, 3$) and preparation and properties of an aqueous colloidal solution of C_{70} are reported. C_{70} was reduced by Al–Ni alloy (or Zn, Sn) to obtain C_{70}^{-}, C_{70}^{2-}, C_{70}^{3-} in aqueous caustic–THF or mixtures of DMSO and THF with varying compositions, without the need for stoichiometric control of the reducing agents. The reduction products were confirmed by ESI–MS, VIS–NIR and EPR spectra. C_{70}^{n-} ($n = 1, 2, 3$) are stable to water under our experimental conditions, but very sensitive to oxygen. The oxidation reaction of C_{70}^{-} with oxygen follows $C_{70}^{-}\rightarrow C_{70}$, but oxidation of C_{70}^{3-} follows $C_{70}^{3-}\rightarrow C_{70}^{2-}\rightarrow C_{70}(OH)_x$, which was monitored by NIR absorption spectra and species characterized by FT–IR and ^{1}H NMR. The C_{70} sol is formed while C_{70}^{-} is oxidized in aqueous solution. The sol is composed of C_{70} which was confirmed by UV, FTIR and APCI–MS. The aqueous colloidal solution of C_{70} possesses excellent optical limiting properties and biological activity, illustrated by strong cytotoxicity to U937 cells upon visible light irradiation.

Introduction

The success of synthesizing macroscopic amounts of fullerene C_{70} with a high purity has advanced the development of fullerene-C_{70} chemistry.[1] The reports published include a variety of C_{70} derivatives[1] and metal complexes,[1] and the electrochemistry[2] and photophysics[3] of C_{70}. C_{70}^{n-} ($n = 1$–6) anions have been generated by electrochemical methods,[2] γ-irradiation[4] and free electron capture,[5] but, to our knowledge, there are very few chemical methods for the generation of C_{70} anions,[6] especially for selective generation. It is important therefore to explore a facile and selective method for the aqueous solution phase generation of C_{70}^{n-}.

Over the past few years, many research groups have begun to investigate the biological uses of fullerenes. It was found that fullerene derivatives exhibit several types of biological activity, both *in vitro* and *in vivo*.[7] It was discovered that fullerene could convert oxygen from the triplet to the singlet state, which could kill cancer cells,[8] that substituted fullerenes are able to inhibit HIV-1 protease[9,7d] and exhibit photo induced DNA scission properties[7a] and that fullerene can be incorporated into artificial lipid bilayers.[10] However, the application of fullerenes in biology has been hampered by their poor water solubility. It remains a challenge to produce solutions of fullerenes in polar solvents, particularly in water. The solubilization of fullerene molecules in polar solvents, especially in water, has been recently reported using ultrasound,[11] γ-cyclodextrins,[12] calixarenes,[13] the detergent Tween-20,[14] phospholipids,[15] micelles, liposomes,[15,16] vesicles,[10] polyvinylpyrrolidone[17] and Triton X-100.[18] It is valuable to explore a new method for producing fullerenes in aqueous solution without any additional agents and to study the bioactivity of the fullerenes. Fullerenes as a promising nonlinear optical material have attracted much attention recently, particularly as intensity dependent absorption makes these materials suitable candidates for optical limiters[19] for the eye and for sensor protection from high intensity beams. They also show promise in the fabrication of optical switches and optical bistable devices.[20]

In the present work, we report a new chemical method for selective generation of C_{70}^{n-} ($n = 1, 2, 3$) and for preparation of an aqueous solution of C_{70} without additives with concentrations up to 0.45 mg ml^{-1}. The optical limiting responses and strong photo-induced cytotoxicity to U937 cells of the C_{70} sol are described.

Experimental

Materials and equipment

C_{70} was purchased with purity 99%. Tetrahydrofuran (THF) and toluene were distilled from sodium–benzophenone in a schlenk system. DMSO was distilled in a schlenk system, with argon bubbled through it under reflux for 10 min before use. Water was distilled and then had argon bubbled through it under reflux for 10 min before use. Zinc powder, Al–Ni alloy, tin and NaOH are chemically pure and were used as purchased.

Mass spectra were recorded on a Finnigan mat API SSQ-710 and LCQ Mass Spectrometer. UV and NIR spectra were recorded on a Shimadzu UV-3100. IR Spectra were recorded on a Bruker IFS66V FT–IR spectrometer. Analysis of metal in metal fulleride was completed on a Jarrell-Ash 1100+2000 Inductively Coupled Plasma Quantometer (ICP). NMR spectra were recorded at room temperature on a Bruker AM-500MHz spectrometer. EPR spectra were recorded on a Bruker ER 200-D-SRC 10/12 spectrometer. Electrochemical data were measured on EG&G M273 and CHI660 electrochemical analyzers.

The one-compartment electrochemical cell was of airtight design with high-vacuum glass stopcocks connecting to the schlenk system. The working electrode consisted of a platinum microdisk with an area 0.00196 cm^2 approximately sealed in glass. The counter electrode consisted of a platinum spiral, and the quasi-reference electrode was a silver spiral. The potentials are always referenced to SCE. $E_{1/2}$ values correspond to $(E_{pc} - E_{pa})/2$ from CV. In some experiments an SCE reference electrode was used, separated from the working electrode compartment by a sintered glass frit and a glass carbon electrode was used in DMSO solution.

A Q modulation Nd: YAG laser with pulse duration of 15 ns at 532 nm was employed as the light source. The laser beam was focused on a 10 mm thick glass cell, in which the sample was placed, by a lens with a 2 mm diameter beam spot. The input and output energies were measured by a two channel energy meter R938, while the incident beam intensity was varied with a Glam prism.

U937 cells, provided by Shanghai Institute of Cell Biology, were cultured in RPMI 1640 and 10% (v/v) Fetal Bovine Serum at 37 °C under 5% CO_2. The cells, in the logarithmic growth state, were suspended in the medium at a concentration of 5.0×10^5 cells ml^{-1}. Then, various amounts of C_{70} sol were added to each tube containing 2 ml of the above cell suspension respectively, followed by incubation for 1 h in a CO_2 incubator. After centrifugation the supernatant was discarded and the cells were dispersed in 2 ml of fresh culture medium. After an addition of 1 ml of the cell suspension solution to each well of 24-well tissue culture plates, the plate was exposed to visible light of Lux 4000 at 20 °C for 30 min, then 100 µl of the cell suspension was taken from each well of the above 24-well tissue plate and added to each well of the 96-well tissue plate. After further incubation for 24 h, 20 µl of the 5 mg ml^{-1} solution of MTT[21] were added to each well, followed by incubation for 4 h at 37 °C, then addition of 100 µl of the extraction buffer. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured by a DG5031 ELISA Scanner. The cells of each well were observed and photographed with a Nikon Inverted Microscope. The procedure was carried out under strictly bacteria-free conditions.

Solution of C_{70}^- in THF

In a typical experiment, 8.0 mg C_{70} powder (0.01 mmol), 100 mg Al–Ni alloy (2.5 mmol, excess) and 400 mg solid NaOH pellets (10 mmol) were put in a bottle, which was evacuated and filled with argon, then 10 ml THF were added with stirring. With the exception that C_{70} was slightly soluble in THF, the other starting materials did not dissolve. After adding 3 ml of deoxygenated water, NaOH solid pellets dissolved with accompanying effervescence. The reduction took place rapidly and the color of the THF layer turned from slightly yellow to red–orange. After 1 h the red–orange THF solution was separated from the colorless aqueous caustic NaOH and used directly for electrospray mass spectroscopy (ESI–MS), VIS–NIR and EPR measurements.

Solution of C_{70}^{2-} in DMSO–THF

To a suspension of 4.0 mg C_{70} powder (4.76×10^{-3} mmol), 50 mg Al–Ni alloy (1.16 mmol, excess) and 300 mg solid NaOH pellets (7.5 mmol) in a mixture of 5 ml DMSO and 5 ml THF in a schlenk system, 2 ml of degassed water were added. As the reduction reaction proceeded, the NaOH solid pellets dissolved in the water with accompanying effervescence, and the color of the solution turned to red–orange at first, and finally olive–green; the lower layer was colorless aqueous caustic with black Ni powder. After 4 h, the olive–green solution of C_{70}^{2-} was separated from the aqueous caustic lower layer and used as a sample for EPR, CV and NIR measurements.

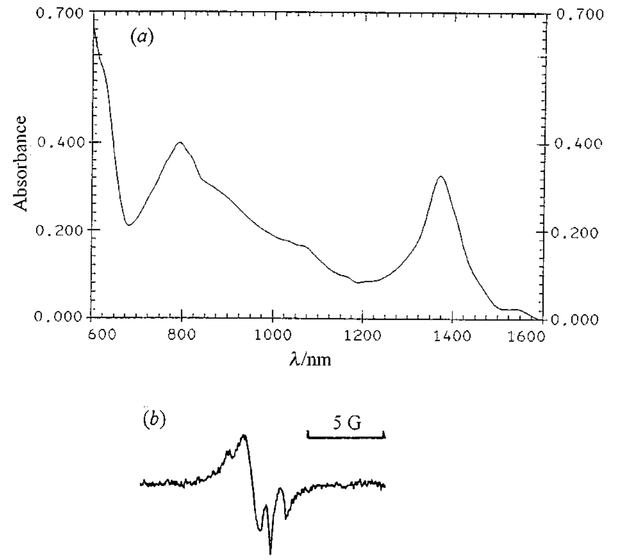

Fig. 1 C_{70} anions generated by reaction of C_{70} and Al–Ni alloy in aqueous NaOH–THF. (*a*) NIR spectrum of C_{70}^- in THF (1.9×10^{-4} mmol ml^{-1}, THF as blank, slit: 5 nm); (*b*) EPR spectrum ($T = 295$ K).

Solution of C_{70}^{3-} in DMSO

4.0 mg C_{70} powder (4.76×10^{-3} mmol), 50 mg Al–Ni alloy (1.16 mmol, excess) and 300 mg solid NaOH pellets (7.5 mmol) were put in a bottle, which was evacuated, then filled with argon. 10 ml DMSO were added to the mixture with stirring. After adding 2 ml of deoxygenated water, effervescence was observed and the color of the solution turned to brown–green then deep red–yellow. After 4 h the deep red–yellow DMSO solution was separated from the aqueous caustic lower layer and used as a sample for EPR, CV and NIR measurements.

Aqueous colloidal solution of C_{70}

A solution of C_{70}^- (16 ml), containing 7.2 mg C_{70} in THF, was prepared by the method described above. This was added dropwise to 50 ml of undegassed distilled water. The THF was removed under reduced pressure, to give an aqueous colloidal solution of C_{70} containing 0.45 mg ml^{-1} C_{70}.

Results and discussion

Selective generation and properties of C_{70}^{n-} (n = 1, 2, 3)

C_{70} is slightly soluble in THF, forming a pale yellow solution. The near-IR region for C_{70} is featureless, but there are present two absorption bands at 791, 1370 nm [Fig. 1(*a*)], characteristic of the C_{70}^- anion,[2g,6e] in the near-IR (NIR) absorption spectrum of the red–orange THF solution formed by reducing C_{70} with Al–Ni–NaOH–H_2O. The ESI–MS spectrum shows a very strong peak at $m/e = 840$ with anion mode; the minor peaks at $m/e = 856$ and $m/e = 872$ were assigned to $C_{70}O$ and $C_{70}O_2$ from the reaction of C_{70}^- with trace amounts of oxygen in the flow phase THF. The EPR spectrum of the red–orange solution has four peaks ($T = 295$ K) with $g = 2.0019$, 2.0004, 2.0011, 1.9997 [Fig. 1(*b*)], which is characteristic of C_{70}^-.[2b] The separate EPR peaks seem reasonable because of C_{70}^- anisotropy, in view of the fact that the symmetry of C_{70} is only D_{5h}. The intensities of the peaks at $g = 2.0019$, 2.0004, 1.9997, decrease but that at $g = 2.0011$ does not, as the microwave power decreases; the reason for this is not known to us at present. Radiofrequency Inductively Coupled Plasma (ICP) analysis data shows that the counterpart cation is Na^+, forming an NaC_{70} compound in THF solution.

C_{70} was reduced in DMSO–NaOH–H_2O by Al–Ni alloy to generate a deep red–orange solution of DMSO and its NIR

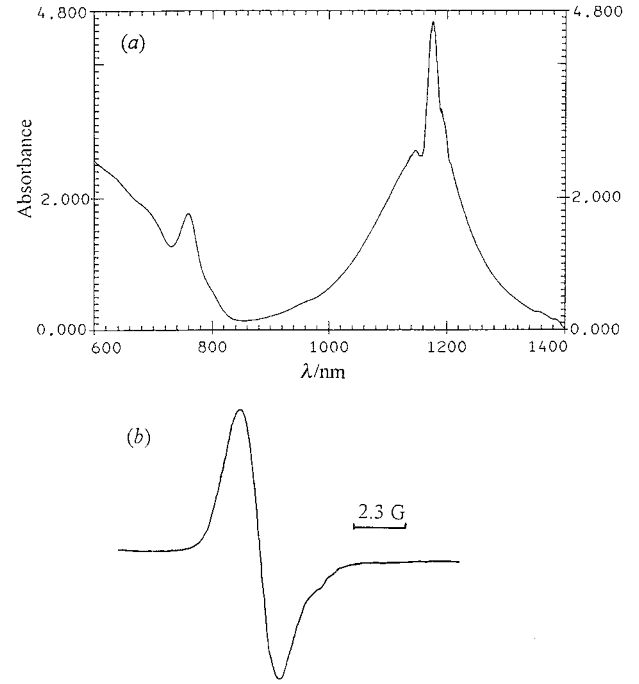

Fig. 2 C_{70}^{3-} anions generated by C_{70} with Al–Ni alloy in aqueous NaOH–DMSO. (*a*) NIR spectrum of C_{70}^{3-} in DMSO (3.5×10^{-4} mmol ml^{-1}, DMSO as blank, slit: 5 nm); (*b*) EPR spectrum ($T = 298$ K).

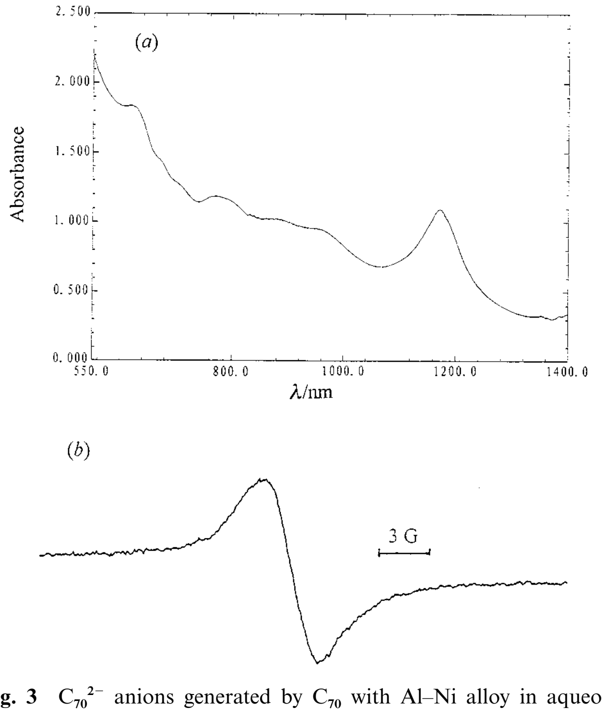

Fig. 3 C_{70}^{2-} anions generated by C_{70} with Al–Ni alloy in aqueous NaOH–THF–DMSO (1 : 1). (*a*) NIR spectrum of C_{70}^{2-} in THF–DMSO (3.6×10^{-4} mmol ml^{-1}, THF–DMSO 1 : 1 as blank, slit: 5 nm); (*b*) EPR spectrum ($T = 298$ K).

spectrum presents two absorption bands at 1178 nm and 759 nm [Fig. 2(*a*)], which accords with that of C_{70}^{3-}.[2g] The EPR spectrum of C_{70}^{3-} in DMSO has a main peak with $g = 2.0029$, $\Delta H_{pp} = 2.3$ G ($T = 298$ K) [Fig. 2(*b*)] and two minor shoulder peaks at room temperature. These results indicate that C_{70}^{3-} was indeed formed under our experimental conditions. The reaction goes through an intermediate stage of C_{70}^{2-}, and stops giving C_{70}^{3-} as the final product.

When using a mixture of DMSO and THF instead of DMSO, but otherwise the same experimental conditions as above, similar olive–green solutions were obtained, and the NIR spectrum presents an absorption band at 1170 nm [Fig. 3(*a*)] which is characteristic of C_{70}^{2-}.[2g,6e] The EPR spectrum has a main peak at $g = 2.0020$, $\Delta H_{pp} = 3$ G ($T = 298$ K) [Fig. 3(*b*)] which is the same as that cited for C_{70}^{2-} in CH_2Cl_2.[2b]

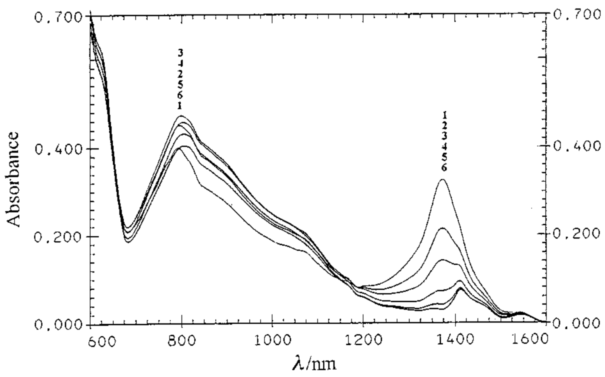

Fig. 4 NIR spectra of the oxidation process of C_{70}^{-} (1.9×10^{-4} mmol ml^{-1}) in THF. Scans were recorded at 5 min intervals.

One of the most notable features of the reduction reaction of C_{70} with Al–Ni alloy is that C_{70}^{n-} ($n = 1$, 2 and 3) can be selectively generated depending upon the polarity of the solvent irrespective of the amount of the reducing agents. Although the Al–Ni alloy used in our experiments is in excess, only the monoanion C_{70}^{-} is generated in aqueous caustic–THF, C_{70}^{2-} in DMSO–THF and C_{70}^{3-} in DMSO. The second point of note is that in aqueous caustic conditions, only C_{70} anions were formed without any $C_{70}H_n$, which was formed under acid conditions.[22]

The chemical generation of C_{70}^{n-} ($n = 1, 2, 3$) under these experimental conditions could be rationalized by considering the relevant redox potentials. The $E_{1/2}$ (which is solvent polarity dependent) of the $C_{70}^{0/1-}$, $C_{70}^{1-/2-}$ and $C_{70}^{2-/3-}$ couples in various solvents are listed in Table 1. With the increase of polarity of the solvent from THF, THF–DMSO and DMSO, the $E_{1/2}$ of $C_{70}^{n-}/C_{70}^{(n+1)-}$ couple shifted anodically by 20–60 mV. So, the selective reduction reaction could be reached in the solvent or mixed solvent with suitable polarity.

Oxidation of C_{70}^{-}

C_{70}^{-} is sensitive to air and is oxidized easily by O_2. The color of the THF solution turns from red–orange to black–red and then a black powder deposits on exposure to air. The precipitate is soluble in toluene and its UV and IR spectra are the same as those of C_{70}. The ESI–MS spectrum showed a main peak at $m/e = 840$. These results indicate that C_{70}^{-} is oxidised by air to form C_{70} in THF. The oxidation process, monitored by NIR (Fig. 4), showed the intensity of the band of 1370 nm decreases with time. The oxidation reaction in THF is as follows: $C_{70}^{-} \rightarrow C_{70}$.

Oxidation of C_{70}^{2-}

The oxidation reaction of C_{70}^{2-} in DMSO–THF, monitored by NIR (Fig. 5), showed the intensity of the band of 1178 nm decreases with time; no 1370 nm band appeared and the olive–green solution turned to brown–red. A brown precipitate was obtained by evaporating the solvent. The precipitate is $C_{70}(OH)_x$, which was confirmed by FT–IR. The IR spectrum has absorptions at 3420 (br, s, OH), 1651 (s), 1371 (vs), 1020 (m), 838 (m), 688 (m), 629 (m) cm^{-1}, which indicates that it is composed of $C_{70}(OH)_x$.[24] The process is different to the oxidation of C_{60}^{2-}, which follows $C_{60}^{2-} \rightarrow C_{60}^{-} \rightarrow C_{60}$.[25] The oxidation product $C_{70}(OH)_x$ was soluble in water or water–methanol, but did not dissolve in methanol, ethanol, *etc.*

Oxidation of C_{70}^{3-}

The oxidation of C_{70}^{3-} in DMSO monitored by NIR (Fig. 6)

Table 1 $E_{1/2}$ (V *vs.* SCE) for C_{70} in solvents containing 0.1 M (TBA)ClO$_4$

			Reductions			
Species	*T*/K	Solvent	1st	2nd	3rd	References
C_{70}	298	CH_2Cl_2[a]	−0.41	−0.80	−1.2	2(*b*)
	223	Toluene–DMF (9 : 1)	−0.33	−0.76	−1.25	23
	298	THF	−0.27	−0.83	−1.37	2(*a*)
	293	THF	−0.27	−0.82	−1.41	This work[b]
	298	DMSO–THF (1 : 1)	−0.18	−0.64	−1.08	This work[c]
	293	DMSO	−0.14	−0.41	−0.82	This work[c]

[a] 0.1 M (TBA)BF$_4$. [b] Working electrode = Pt, sweep rate = 0.05 V s^{-1}. [c] Working electrode = glassy carbon, sweep rate = 0.05 V s^{-1}.

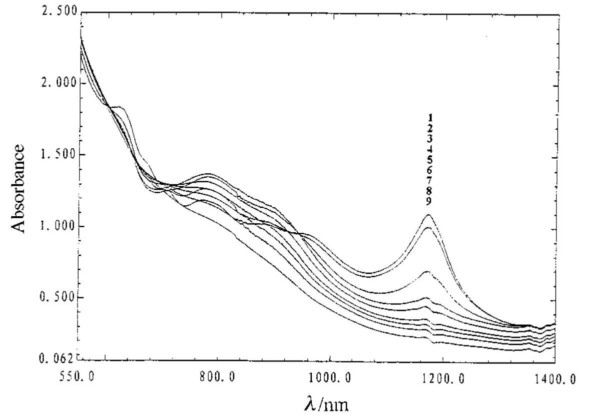

Fig. 5 NIR spectra of the oxidation process of C_{70}^{2-} (3.6×10^{-4} mmol ml^{-1}) in THF–DMSO. Scans were recorded at 3 min intervals.

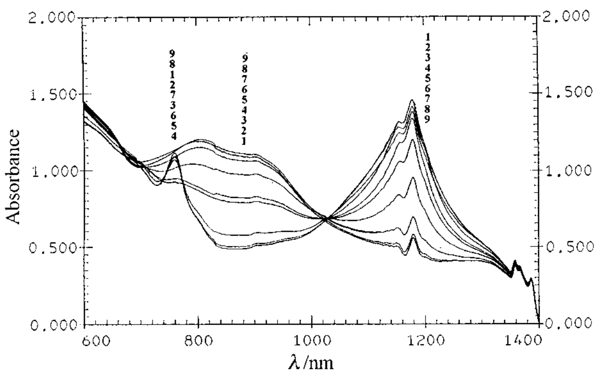

Fig. 6 NIR spectra of the oxidation process of C_{70}^{3-} (3.0×10^{-4} mmol ml^{-1}) in DMSO. Scans were recorded at 5 min intervals.

showed the intensities of the bands at 759 and 1178 nm, which are characteristic bands of C_{70}^{3-} and C_{70}^{2-}, decrease gradually, with broad bands at 800–1000 nm increasing with time. It is notable that the 1370 nm band, which is assigned to C_{70}^{-}, was not observed during the oxidation of C_{70}^{3-}. When C_{70}^{3-} in DMSO is completely oxidized, the solution changes from deep red–yellow to brown–yellow, then becomes colorless and a brown precipitate is observed. The precipitate was centrifugated and washed with ethanol then dissolved in water or water–methanol. The ^{1}H NMR shows a broad peak at 3.59 ppm. The IR (KBr pellets) shows the absorptions at 3401–3420 (br, s, OH), 1648 (s), 1371 (vs), 1080 (m), 1021 (m), 958 (w), 893 (w), 838 (w), 688 (w), 629 (w), 564 (w), 498 (w) cm^{-1}. These indicate that the precipitate is composed of $C_{70}(OH)_x$. The oxidation process in DMSO follows $C_{70}^{3-} \rightarrow C_{70}^{2-} \rightarrow C_{70}(OH)_x$, with no intermediate C_{70}^{-} product found.

We notice that the absorption bands at 750–800 nm for C_{70}^{n-} (n = 1, 2, 3) have similar behavior during the oxidation process, in that they all increase first then decrease later for reasons we do not understand at present.

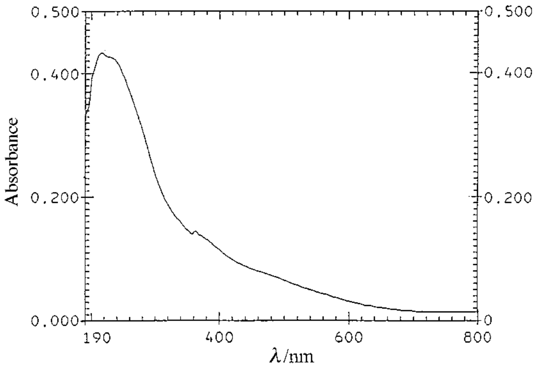

Fig. 7 UV spectra of the C_{70} aqueous sol (0.01 mg ml^{-1}, T = 298 K, H_2O as blank).

Aqueous colloidal solution of C_{70}

THF solution of C_{70}^{-} is added dropwise to deionized water under air to give a dark red aqueous sol. Various concentrations of the sol are prepared conveniently by this method. The sol, even at concentrations as high as 0.45 mg ml^{-1}, is very stable and may be stored for three months at least. The aqueous sol with proper dilution was used as a sample for atmospheric chemical ionization mass spectrometry. The spectrum obtained shows only one main peak at *m*/*e* 840 which indicates that the sol is composed of C_{70} and the species detected are C_{70}^{-} or $(C_{70})_n^{n-}$. The peak *m*/*e* = 884 might be assigned to $C_{70}CO_2^{-}$, which was formed by reaction with CO_2 in the flow phase. There is no indication of other modified fullerenes being formed. As usual with an aqueous sol, the C_{70} sol will be precipitated when an electrolyte such as NaCl, BaCl$_2$, NaOH or HCl is added. The IR spectrum (KBr pellets) of the resulting precipitate shows the characteristic bands of C_{70}[26] at 1430, 1074, 1027, 796, 673, 578, 535 and 458 cm^{-1} and the UV spectrum of the precipitate in toluene or in THF is the same as C_{70}. The precipitates do not dissolve in H_2O again, but dissolve in toluene and THF. These may be well understood by considering the role of the charged bilayer of the C_{70} sol particles. The oxidation reaction may follow eqn. (1).

$$4\,C_{70}^{-} + O_2 + 2H_2O \longrightarrow 4\,C_{70}(\text{sol}) + 4\,OH^{-} \qquad (1)$$

The C_{70} sol is stable, and toluene cannot extract C_{70} from the sol when it is added to the sol and shaken. The UV spectrum of the C_{70} sol is shown in Fig. 7. There is one broad absorption band at 242 nm which is similar to that of C_{70} in water with polyvinylpyrrolidone.[17] It indicates that the aggregation state of C_{70} is present in C_{70} sol.

Optical limiting properties of C_{70} sol

Optical limiting properties refers to an increase in absorption with increased input light intensity. This behavior is attributed to a reverse saturation mechanism which results from the population of an excited state that has a greater absorption cross

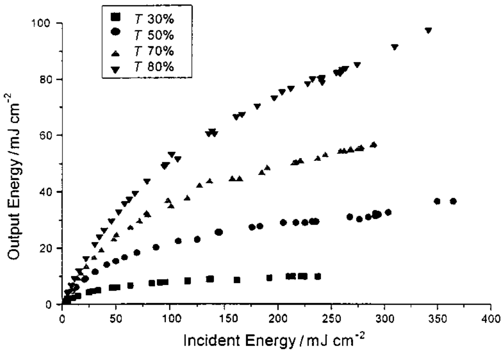

Fig. 8 Optical limiting responses of various transmitting C_{70} aqueous sol to 10 ns, 532 nm optical pulses.

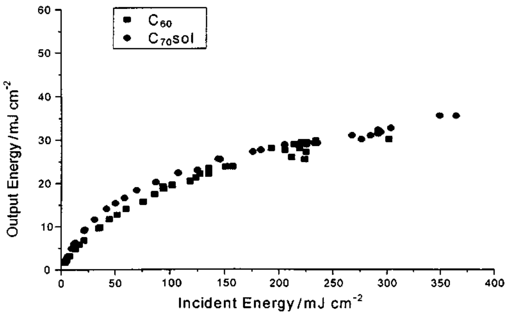

Fig. 9 Comparison of the optical limiting response of solution of C_{70} aqueous sol to C_{60} benzene solution with 50% transmitting.

section than that of the ground state. Recently, reverse saturatable absorption has been observed in C_{60} and C_{70}. In the continuing search for practical applications of the fullerenes, the optical limiting properties occupy a leading position.

The optical limiting responses of various transmitting C_{70} sol to 532 nm optical pulses are shown in Fig. 8. At very low fluences the optical response of the C_{70} sol obeys Beer's law. With the incident intensity increasing, the transmittance energy decreases obviously. At an incident intensity of *ca.* 100 mJ cm^{-2} for 30% transmission, the transmittance of C_{70} sol begins to level out. The transmitted fluence is effectively held at *ca.* 8 mJ cm^{-2}. The 80, 70, 50% transmitting samples behaved similarly. The output limiting fluence is about 55 mJ cm^{-2} for a C_{70} sol with 70% transmission, which is much smaller than *ca.* 350 mJ cm^{-2}, the value for C_{70} solution in toluene.[19a] Fig. 9 shows that the optical limiting behavior of a C_{70} sol with 50% transmission is comparable with a similar transmitting sample of C_{60} benzene solution. Because at 50% transmission the C_{70} sol concentration (0.0244 mg ml^{-1} C_{70}) is much lower than that of the C_{60} benzene solution (0.66 mg ml^{-1}), the C_{70} aqueous sol solutions must have a higher absorption at 532 nm than the C_{60} benzene solutions. Therefore, the higher ground-state absorption of C_{70} results in a smaller ratio of excited-state to ground-state absorption and a higher threshold for optical limiting. Our experiment shows that the optical limiting performance of the C_{70} sol is better than that of C_{60} and C_{70} benzene solutions.

Biological properties of C_{70} sol

The cytotoxicity of C_{70} sol under Lux 4000 visible light irradiation is given in Table 2. It shows obvious biological effects of the C_{70} sol on U937 cells.

Fig. 10 shows the photomicrographs of the growth state of the U937 cells cultured for the third day after irradiation of light: (*a*) containing 20 µg ml^{-1} C_{70} aqueous sol; (*b*) the blank contrast without C_{70} aqueous sol; (*c*) containing 40 µg ml^{-1} C_{70} aqueous sol; (*d*) containing 40 µg ml^{-1} C_{70} aqueous sol in the dark. From Fig. 10 we can see that the U937 cell density is much less in the sample containing C_{70} aqueous sol than in that of the blank contrast with light irradiation [Fig. 10(*a*) and (*b*)], and decreases with increasing concentration of the C_{70} aqueous sol [Fig. 10(*a*) and (*c*)]. Comparing the samples containing the same concentration of C_{70} sol under different light conditions [Fig. 10(*c*) and (*d*)], the cell density of the sample under light irradiation is markedly less. These results show clearly the biological effect of the C_{70} sol on U937 cells under irradiation; the C_{70} kills or inhibits the growth of the U937 cells. This may be caused by superoxide production or the production of singlet oxygen.[7k] Further investigations are in progress.

Table 2 Cytotoxicity of C_{70} aqueous sol to U937 cells under Lux 4000 visible light irradiation (MTT Method)

C_{70} sol concentration/ µg ml^{-1}	Absorption (570 nm)	
	Light	Dark
40	0.749 ± 0.105[a]	1.554 ± 0.106
20	1.053 ± 0.085[a]	1.422 ± 0.077
0	1.693 ± 0.091	1.740 ± 0.048

[a] $P < 0.01$ (*t* test) sample containing C_{70} aqueous sol under visible light irradiation; comparison with blank without C_{70} aqueous sol, and with the same concentration of C_{70} aqueous sol in the dark. Data = mean ± S.D. ($n = 8$).

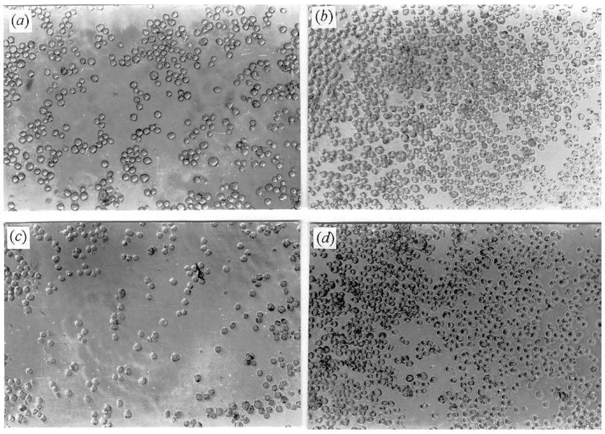

Fig. 10 Photomicrographs of the growth state of the U937 cells cultured for the third day after irradiation with light: (*a*) containing 20 µg ml^{-1} C_{70} aqueous sol, (*b*) the blank contrast without C_{70} aqueous sol, (*c*) containing 40 µg ml^{-1} C_{70} aqueous sol, (*d*) containing 40 µg ml^{-1} C_{70} aqueous sol in the dark.

Conclusions

In conclusion, $C_{70}{}^{n-}$ ($n = 1, 2, 3$) anions can be generated by reaction of C_{70} with Al–Ni alloy (or Zn, Sn) in aqueous caustic–THF or DMSO–THF with varying composition, without the need for stoichiometric control. To our knowledge, it is the first selective production of $C_{70}{}^{n-}$ ($n = 1, 2, 3$) by chemical reaction in an aqueous system. $C_{70}{}^{n-}$ is very sensitive to oxygen and the oxidation of $C_{70}{}^{3-}$ follows $C_{70}{}^{3-}\rightarrow C_{70}{}^{2-}\rightarrow C_{70}(OH)_x$. However, $C_{70}{}^{-}$ is oxidized to form $C_{70}{}^{0}$ as a black powder in organic solvent, but generates C_{70} sol in aqueous solution, which is very stable for a long period. The C_{70} sol possesses optical limiting properties and has strong photo-induced biological activity. Efforts are now under way to synthesize and characterize various new fulleride salts and organo-fullerenes with a variety of electrophiles, to elucidate the response of fullerene films to optical pulses, and to use C_{70} sol in clinical applications.

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