Aqueous solutions of fluorescent ultrafine C_{60} nanoparticles have been successfully prepared by an electrochemical method, which involves reducing a C_{60} film to C_{60}^{-} anions in the presence of tetrabutylammonium (TBA^+) cations in acetonitrile solution and transferring to water. The nanoparticles are highly emissive in the visible region. The structure of the fluorescent C_{60} nanoparticles was deduced as a ~5 nm sphere with a solid core of aggregated C_{60} and an anionic shell of C_{60}^3−, which renders the nanoparticles water soluble. The photostable and nontoxic C_{60} nanoparticles can easily penetrate into live cells, and are thus primed for a host of biomedical applications.

Introduction

Fluorescent nanoparticles have generated much excitement for a wide variety of promising applications, especially in biomedical sensing, labeling and imaging. Very recently, carbon-based fluorescent nanomaterials have been developed as a benign alternative to high performance semiconductor quantum dots by avoiding the use of heavy metals, which have caused serious health and environmental concerns. Carbon nanoparticles and carbogenic dots, nanodiamonds, shortened carbon nanotubes (CNTs) and graphene quantum dots (GQDs) have shown bright and colorful tunable photoluminescence. However, as a new form of carbon with precisely controllable size and structure, fullerenes both in isolation and in aggregation fall short of strong luminescence.

Fullerenes[60] (C_{60}) has long been targeted for applications in optoelectronic devices, organic photovoltaics and biotechnology. However, the fluorescence of C_{60} and its mono-adduct is very weak, with reported quantum yields of the order of only 10^{-4} in the 620–900 nm region. In addition, the insolubility of fullerenes in aqueous solution further limits their application in biology.

Several approaches have been used to make fullerenes water soluble, which include chemical derivatization with highly polar moieties, transfer of genuine fullerenes to water by formation of supramolecular complexes, such as those with cyclodextrins, calixarenes, and formation of colloids either with or without the use of surfactants or lipids.

Herein, we report an unusual electrochemical generation of water soluble, strongly fluorescent C_{60} nanoparticles, overcoming many of the above problems. The nanoparticles are highly emissive in the visible region, and easily penetrate into live cells. Moreover, in bioimaging applications, the simplicity of our method should thwart the demanding functionalizations with organics/inorganics often needed for the practical use of C_{60}.

Experimental

Preparation of C_{60} nanoparticle solutions

C_{60} fullerene (99.9%) was purchased from PKU (Beijing, China). All chemical solvents and reagents were purchased from J&K. The electrochemical preparation of fullerene (C_{60}) nanoparticles was performed on CHI 705 by constant potential electrolysis of a C_{60} film in an acetonitrile solution of 0.01 M tetrabutylammonium perchlorate ((TBA)ClO_4) as the supporting electrolyte, under a high purity N_2 atmosphere. The C_{60} film was obtained by dripping a drop of a C_{60} m-toluene solution several times onto a Pt foil followed by drying in air. The C_{60}-covered Pt foil was placed in the electrochemical cell as the working electrode, with a Pt wire as the counter electrode, and an Ag/AgCl reference electrode. For electrolysis, the applied potential was ~2.0 V. The size of the fullerene nanoparticles was controlled by the electrical current of the cell. Only under low electrical current (~0.1 mA) could the ultra fine C_{60} nanoparticles (4–5 nm) be obtained. A resistor (1000 Ω) was connected between the working electrode and the electrochemical cell to control and limit the electrical current. The C_{60} nanoparticles could also be obtained by potential cycling between ~2.0 V and +2.0 V (see Fig. S1, ESI†).

The aqueous solution of the nanoparticles was obtained by transferring the acetonitrile solution to water by evaporation of the acetonitrile, dissolution of the remaining solid in water, and filtration of the resulting solution to remove the supporting electrolytes and the very small amount of insoluble C_{60} nanoparticles. The filtrate was centrifuged at 12 000 rpm for 10 min to obtain a translucent solution of the C_{60} nanoparticles.

Sample characterization

A JEOL 2011 transmission electron microscope (TEM) and a Hitachi S-4800 scanning electron microscope (SEM) were used to investigate the morphologies of the fluorescent C_{60} nanoparticles. A UVF300 confocal fluorescence microscope equipped with a C60 nanoparticles. See DOI: 10.1039/c0jm02492a

† Electronic supplementary information (ESI) available: Further characterization of C_{60} nanoparticles. See DOI: 10.1039/c0jm02492a
with an argon ion laser and neon-helium laser was used to obtain fluorescence microscopy images. Excitation wavelengths of 405, 488, and 543 nm were used. X-ray diffraction measurements (XRD) were carried out with a Rigaku D/max-2500 using filtered Cu Kα radiation. The FT-IR spectra were measured using an AVATAR 360 spectrophotometer. The vis-NIR spectrum was obtained with a JASCO V-570 spectrometer at room temperature. The fluorescence lifetime decay of the fullerene (C60) nanoparticles was measured in a cuvette using a time resolved fluorimeter (Horiba TemPro system) with LED for excitation (370 nm) and a monochromator for emission.

Results and discussion

The electrochemical preparation was performed in a conventional three-electrode cell, which consisted of a C60 film-covered Pt foil as the working electrode, a Pt wire counter electrode and an Ag/AgCl reference electrode in an acetonitrile solution of 0.01 M (TBA)ClO4 as the supporting electrolyte. The C60 electrode was electroreduced at a constant potential of −2.0 V vs. Ag/AgCl. A series resistance was introduced in the working electrode to control and limit the electrical current in order to produce uniform, ultrafine C60 nanoparticles. During the reduction process, the solution was found to change from colorless to yellow and finally dark brown, which emitted intense blue light upon irradiation with an UV lamp (Fig. 1a, b, c). A luminescent aqueous solution of the nanoparticles was obtained by transferring the acetonitrile solution to water: i) evaporation of the acetonitrile, ii) dissolution of the remaining solid in water, and iii) filtration of the resulting solution to remove the supporting electrolytes and the very small amount of insoluble C60 nanoparticles. The filtrate was then centrifuged at 12 000 rpm for 10 min, affording a translucent solution of the C60 nanoparticles.

The C60 nanoparticles are strongly fluorescent both in solution and in the solid state, with emissions spanning the visible wavelength range from 400 to 600 nm (Fig. 1d and Fig. S2, ESI†) when excited by light with wavelengths from 390 to 490 nm. The brightness of the photoluminescence is reflected by the high emission quantum yields; with 350 nm excitation, the quantum yields were measured to be about 5–6%. The UV-vis absorption spectrum reveals that the first absorption band is at 270 nm, corresponding to the small size of the nanoparticles.25,26

When a small aliquot of the aqueous solution was carefully dropped onto a cover glass followed by the evaporation of water, the deposited C60 nanoparticles are strongly emissive in the visible region when excited at 405, 488 and 543 nm, as shown in the confocal fluorescence microscopy images in Fig. 2. The observed broad and colorful fluorescence, dependent on the excitation wavelengths, could be due to the finite nanoparticle size distribution (see results below on the nanoparticle size distribution).

Transmission electron microscopy (TEM) was used to determine the size and morphology of the C60 nanoparticles by simply dropping a solution onto a copper grid for direct observation after drying. It can be seen that the C60 nanoparticles are uniformly spherical with a narrow size distribution around 5 nm (Fig. 3). From XRD patterns of dried C60 nanoparticles, a hexagonal close-packed (hcp) structure is evident but the major peaks such as (110), (112) and (300) are substantially broadened (Fig. S3, ESI†), implying that the diameter of the C60 nanoparticles is ultrasmall.27 The FT-IR spectrum (Fig. S4a, ESI†) is dominated by absorption peaks at 1429, 1182, 577, and 528 cm⁻¹, which are clearly ascribable to the typical four dipole-allowed, IR-active vibrational modes of the C60 skeleton (Fig. S4b, ESI,† 1429, 1181, 576 and 527 cm⁻¹) but with slight shifts.28 These pronounced vibrational features evince that the nanoparticles are indeed composed of C60 and its ions.

C60 is insoluble in acetonitrile, but its anions, especially C60⁻, were reported to be soluble in acetonitrile.29–31 Similarly, the hydrophobic C60 could be made water soluble by appropriate substitutions, transformation into stabilized anions, and aggregation to form bilayer vesicles.29,32 Significantly, in our case, a characteristic peak around 1400 nm in the vis-NIR absorption spectrum of the C60 nanoparticles solution is clear evidence for the existence of C60⁻ in the nanoparticles (Fig. 4).33 This is consistent with the finding that the electrolysis potential is crucial for the production of the C60 nanoparticles. When the potential was more positive (e.g., −1.8 V) than −2.0 V, no changes in the solution were observed by the electrolysis. On the other hand, when the supporting electrolyte was changed from (TBA)ClO4 to KPF6 or KClO4, the electrolysis failed to generate a fluorescent C60 nanoparticle solution. However, when (TBA)BF4 or (TBA)PF6 was used as the supporting electrolyte, a strongly
fluorescent C₆₀ nanoparticle solution could be obtained again. This accentuates the role played by the TBA⁺ cation in the formation of the C₆₀ nanoparticles. This is not surprising given that the effect of supporting electrolyte on the reduction of C₆₀ films has already been reported previously; here with TBA⁺, the C₆₀ film was less stable for the potential scans beyond the third reduction waves. With hindsight, we consider that this is associated with the formation of small size C₆₀ nanoparticles in the solution. During the reduction process, TBA⁺ ions would diffuse into the C₆₀ film to balance the negative charges acquired therein. Because TBA⁺ has a size close to that of the C₆₀ molecule, the insertion of TBA⁺ would drive structural reorganizations in the C₆₀ film. As illustrated in Fig. 1a, after C₆₀ was reduced to C₆₀⁻ ions in sufficient quantity, the adjoining TBA⁺ species would be able to wrap around the C₆₀⁻ ions to form ~5 nm nanoparticles, which are soluble in and thus would enter the acetonitrile solution thereafter. Probably, this process would engulf some neutral C₆₀ molecules into the core of the nanoparticles. With regard to the smaller cations such as K⁺, however, the much easier intercalation into the C₆₀ film would not trigger the structural reorganization process necessary for overcoming the activation energy involved in forming the C₆₀ nanoparticles. Additionally, this nanoparticle forming mechanism by reduction–intercalation–wrapping–dissolution can also explain our observation that controlling the electrolysis current is another important factor in generating the uniform, ultrafine C₆₀ nanoparticle solution: when the series resistance was not used to limit the electrolysis current, only an insoluble black precipitate (consisting of ~20 nm nanoparticles) was obtained (Fig. S5, ESI†) because the overly rapid charging of the C₆₀ film precluded a smooth intercalation, wrapping and dissolution process.

On the basis of the experimental results presented above and the relevant properties of C₆₀ established previously, we can deduce the structure of the fluorescent C₆₀ nanoparticles as a ~5 nm sphere with a solid core of aggregated C₆₀ and an anionic shell of C₆₀⁻ (Fig. 1b), which renders the nanoparticles water soluble.

Strong luminescence has been reported in various surface-functionalized carbon nanoparticles and CNTs. Surface passivants such as PEG (poly-(ethylene glycol)) have been considered to be the origin of the fluorescence. To test this possibility in our system, PEG2000N was added to a TBA⁺ supporting electrolyte solution to generate the C₆₀ nanoparticle solution. Although a dilute C₆₀ nanoparticles solution could still be obtained, no fluorescence was observed, implying that the strong luminescence observed in our system arises from a novel

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**Fig. 2** Confocal fluorescence microscopy images (the scale bars are all 5 μm) of the C₆₀ nanoparticles at the excitation wavelengths of 405 (a), 488 (b) and 543 nm (c).

**Fig. 3** TEM image of the fluorescent C₆₀ nanoparticles (a). Size distribution of the C₆₀ nanoparticles determined by TEM (b) and by DLS (dynamic light scattering) (c). It can be seen that the size distributions determined by TEM and DLS agree well with each other.

**Fig. 4** Vis-NIR spectrum of the C₆₀ nanoparticles in an acetonitrile solution.
structure of the C60 nanoparticles rather than surface passivation. Such a structure may involve an electronic symmetry breaking of the C60 molecules engendered by the special charge distribution in the neutral core/anionic shell C60 nanoparticles, which would partially allow the intrinsically forbidden transitions (see Fig. 1b). One would expect that such an electronic perturbation mechanism should be most effective in an ultrafine size regime in much the same way as our photoluminescence data have revealed. We notice that hexapyrrolidine derivatives of C60 were shown to emit bright visible light and this was also interpreted as a result of an electronic perturbation of the π-system.\textsuperscript{37} Further studies are required to better understand the origin of the strong fluorescence from the C60 nanoparticles.

The water soluble, strongly fluorescent fullerene nanoparticles are an ideal candidate for biomedical applications, as has been proposed for quite some time.\textsuperscript{38–40} To demonstrate the prospects of the new C60 nanoparticles as a bioimaging agent, human lung cancer cells (A549) and breast cancer cells (MCF-7) were assayed for evaluating cell imaging performance, permeability and cytotoxicity. The cells were cultured in a 6-well microscopy chamber with a density of $3 \times 10^5$ cells per well, and were then incubated in a fluorescent C60 nanoparticle aqueous solution (160 $\mu$g mL$^{-1}$) for 24 h. After incubation, the cells were washed three times and then fixed with 4% paraformaldehyde for 20 min at room temperature. As shown in Fig. 5 and Fig. S6, ESI,\textsuperscript{†} the cells became brightly blue, green and red when illuminated at excitation wavelengths of 405, 488 and 543 nm, respectively. The bright spots were mostly found in the cytoplasmic area of the cells, and the light intensity in the central region corresponding to the nucleus was weak (see the arrowheads). Moreover, the confocal section image (Fig. S6, ESI\textsuperscript{†}) shows that the vision is dimmer at both upper and lower sides than that at the center of the cells, connoting an efficient internalization of the C60 nanoparticles into the A549 and MCF-7 live cells; they have been incorporated into the cytoplasmic areas, not just adsorbed on the outer membrane surfaces. In addition, the fluorescence images in Fig. 5 at different excitation wavelengths match very well spatially in the same scanning area. After repeated excitation and generation of the images in Fig. 5 for at least 1000 runs, no changes in emission intensities were observed, signifying the high photostability of the C60 nanoparticles in the cells (Fig. S7, ESI\textsuperscript{†}).

Cytotoxicity of the fluorescent C60 nanoparticles was also tested with the A549 and MCF-7 cells. Cell death or viability reduction was not observed for greater than 80% of cells after addition of the C60 nanoparticles to the cell culture solution with a concentration up to 100 $\mu$g mL$^{-1}$ for 3 days (Fig. 6). This means that the fluorescent C60 nanoparticles can image live cells for a long period of time without causing cell damage. Notably, the C60 nanoparticles induced different levels of cytotoxic effects on these two kinds of cells when the concentration exceeded 200 $\mu$g mL$^{-1}$. For example, when the concentration of C60 nanoparticles was increased from 200 to 1000 $\mu$g mL$^{-1}$, the amount of live A549 cells decreased by 27%, whereas that of live MFC-7 cells deceased...

![Image](image_url)

**Fig. 5** Confocal fluorescence microscopy images of A549 (a–c) and MCF-7 (d–f) cells with the fluorescent C60 nanoparticles incorporated at the excitation wavelengths of 405, 488 and 543 nm, respectively. The arrowheads point to the nucleus of the cells.

![Image](image_url)

**Fig. 6** Cell viability (cytotoxicity assay) for A549 (black) and MFC-7 (grey) cells as a function of the added C60 nanoparticle concentration.
by 73%. Fluorescent C$_{60}$ nanoparticles are certainly unique for the bioimaging of live cells, offering high brightness, prolonged photostability and very low cytotoxicity.

Conclusions

In conclusion, we have successfully prepared fluorescent C$_{60}$ nanoparticle solutions by electrolyzing C$_{60}$ films to C$_{60}^{-}$ anions in the presence of TBA$^+$ cations. These C$_{60}$ nanoparticles, easily solubilized in water, have shown intense photoluminescence, excellent photostability, are non-cytotoxic and can easily penetrate into live cells. To the best of our knowledge, this is the first time that pure C$_{60}$ is used as a fluorescent labelling agent in live-cell imaging. Compared with other carbon-based fluorescent nanomaterials, such C$_{60}$ nanoparticles are expected to be more practicable in biomedical applications, such as drug delivery, due to the extensive functionalization schemes of C$_{60}$ that have been well developed over the years. Detailed follow-up work is underway in our laboratory and will be reported in due course.

Acknowledgements

This work is supported by NSFC (20773015, 30672491, 21073018), Beijing Municipal Commission of Education, the Fundamental Research Funds for the Central University and Hong Kong RGC GRF No. 604608.

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