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Study of the fluorescence quenching of 1-hydroxypyrene-3,6,8-trisulfonic acid by single-walled carbon nanotubes

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Abstract

This paper presents a study of the fluorescence quenching of 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS) in the presence of single-walled carbon nanotubes (SWCNT) using a fluorescence method. To investigate the quenching mechanism (dynamic or static) of HPTS, Stern-Volmer plots of single walled carbon nanotubes at different temperatures were used. The positive deviation from linearity in Stern-Volmer plots suggests that single walled carbon nanotubes follow a static quenching mechanism evidenced by the formation of a stable ground state complex. The results presented here help us to clarify the quenching mechanism in the interaction of a pyrene derived dye and carbon nanotubes. This study will open new possibilities in the use of the conjugate formed by SWCNTs and HPTS in the fabrication of a biosensor based on intracellular fluorescent probes.

Keywords: Single-walled carbon nanotubes; Static quenching; Fluorescence; 1-hydroxypyrene-3,6,8-trisulfonic acid.

Introduction

The 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS) is a fluorescent dye which contains three sulfate groups (**Fig. 1**) and has been used as an apoplastic marker (Wang *et al.* 1994, Wright *et al.* 1996). It is well known that its physiological pH HPTS would be highly charged thus preventing the entry to the cell and decreasing its range of applications. The pH detection at the intracellular level has become a challenge for the scientific community because it is a significant variable in understanding the action mechanism of different types of drugs within a specific cell. The study of the interactions between intracellular dyes and nanostructures is of crucial interest to understand the nature of the interaction and thus develop new types of optical biosensors. Recently, nanotechnology has provided different nanostructures to functionalize many biomolecules to facilitate the entry into the cell (Baties *et al.* 2013).

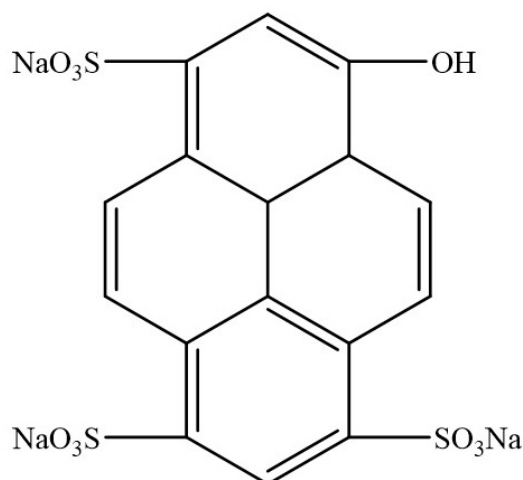


Figure 1. Chemical structure of HPTS.

Carbon nanotubes (CNTs) are unique tubular structures with diameters in the range of nanometers and large diameter/length ratio which have attracted the attention of researches due to the numerous applications in the biomedical field (Liu *et al.* 2009, Wang *et al.* 2009; Xing *et al.* 2010). One of the advantages possessed by the CNTs is the possibility of effectively crossing biological membranes. In an elegant study, it was shown that the ability of different types of CNTs to penetrate the cell and how this ability can be exploited for the use in a variety of biological systems and devices as biosensors (Xing *et al.* 2011). Chemically functionalized CNTs has been used to evaluate cellular internalization in the presence of different cellular uptake inhibitors (Kostarelos *et al.* 2007). The ability of CNTs have allowed advancements in the gene therapy research area.

In an interesting work, (Zhao *et al.*) developed a pH sensor system using single-walled carbon nanotubes (SWCNTs) functionalized with HPTS and coated with a layer of zinc phthalocyanine (Zhao *et al.* 2009). CNTs are promising candidates to facilitate the entry of HPTS to the cells and to be used as intracellular probes. Therefore, understanding the mechanism of interactions between SWCNTs and HPTS is of prime importance. CNTs have been used in the determination of fluorescence quenching mechanism of different dyes due to its ability as a quencher molecule. In a similar work, the effect of structural defect of SWCNTs and multiwall carbon nanotubes (MWCNTs) on their quenching fluorescence properties was studied (Singh *et al.* 2012). Ahmad *et al.* studied the static quenching mechanism of Rhodamine B by SWCNTs (Ahmad *et al.* 2009). Chiu *et al.* studied the quenching effect of different dyes by SWCNTs (Chiu *et al.* 2011).

To the best of our knowledge, there are no results regarding the quenching effect of SWCNTs on the fluorescence properties of HPTS. In this study, we have prepared and characterized a conjugate between SWCNT and HPTS dye to investigate the quenching effect of the SWCNTs using a fluorescence method based on Stern-Volmer plots.

Materials and methods

Materials

SWCNTs with diameters ranging from 0.8 nm to 2 nm and lengths between 500 nm and 2000 nm were purchased from Unydim, California. Folic acid and 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS) were obtained from Sigma-Aldrich Corp. All chemicals used in this work were of analytical grade.

Synthesis

Solubilization of single-walled carbon nanotubes

5 mg of SWCNTs were sonicated for 20 min in 5 mL of a 5 mM aqueous solution of folic acid (FA). FA solution was prepared as described by Castillo and coworkers (Castillo *et al.* 2013). FA molecules were dissolved in water with an addition of 50 μL of NaOH (1 M) due to the poor solubility of FA. The FA powder (0.029 g) was mixed with H_2O (25 mL) until a final pH of 10 was reached and magnetically stirred until a yellow-to-clear transition was observed. The solution was centrifuged at 2800 rpm for 20 min; the supernatant was collected and carefully separated from the solid. After aggregation, a large bundle of nanotubes was found. The conjugate was washed several times with MilliQ water and centrifuged to eliminate excess free FA. Finally, the SWCNT solution was stored at 4 °C. To estimate the concentration of SWCNT/FA suspension, a similar methodology used by Regev (Attal *et al.* 2006) was employed.

Synthesis of the single-walled carbon nanotube-HPTS conjugate

4 mg of HPTS was dissolved in 10 mL distilled water to a final concentration of 1 mM. Then aliquots of 10 μL of HPTS were added to different concentrations of SWCNTs/FA suspensions (10 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$). The solutions were magnetically stirred for 30 min and mixing before fluorescent measurements.

Measurements and tool techniques

Scanning electron microscopy images To characterize the functionalization of SWCNT with FA, Scanning Electron Microscopy (SEM) images were recorded using a Quanta FEG SEM microscope (Oregon, USA) with a maximal spatial resolution of 1.0 nm.

Atomic force microscopy images

All atomic force microscopy (AFM) images were recorded using an XE-150 Premier cross-functional AFM (Park Systems, Korea) with a PPPNVHR AFM probe (Nanosensors, Switzerland).

Ultraviolet-visible (UV-Vis) absorption spectrum

UV-Vis absorption spectrum was collected at room temperature in a quartz cell with a 1.0 cm path length with an Agilent-HP 8453 spectrophotometer.

Raman scattering spectroscopy

Raman measurements were performed using a Thermo Scientific DXR Raman microscope. An optical microscope was coupled to a single grating spectrometer with 5 cm^{-1} FWHM spectral resolution and ± 2 wavenumber accuracy. A frequency-stabilized single diode laser was operated at 532 nm. Raman spectra were recorded using a 10x long working distance objective, 0.1 mW laser power and 15 s signal accumulation times. The power density was kept low ($\sim 12\text{ kW/cm}^2$) of the sample to minimize photo induced and thermal effects.

Fluorescence methods

Fluorescence measurements were made on standard luminescence spectrophotometer Perkin Elmer LS-55 using quartz cuvettes (10 mm path length). The fluorescence emission spectra were recorded in the range of 400-600 nm following an excitation wavelength at 458 nm. Both excitation and emission bandwidths were adjusted at 2.5 nm. The HPTS concentration was fixed at $10\text{ }\mu\text{M}$, and the SWCNT/FA concentrations were varied from 10 to $100\text{ }\mu\text{g/mL}$. To evaluate the effect of the temperature on the HPTS-SWCNT interaction, fluorescence emission was recorded at three different temperatures ($5\text{ }^{\circ}\text{C}$, $25\text{ }^{\circ}\text{C}$ and $45\text{ }^{\circ}\text{C}$).

Results and Discussion

The conjugate was prepared by sonication of a solution of FA (3.5 mM) with 5 mg of SWCNT. **Fig. 2a** and **Fig. 2b** show the dispersion SWCNT and FA before and after the ultrasonication step. It can be appreciated the debundled of SWCNT by the action of FA solutions. To obtain a clearer image of the debundling of SWCNT by AF, **Fig. 2c** shows an AFM image of several nanotubes covered by FA. On the other hand, the **Fig. 2c** is showed a picture of the solutions of SWCNT-FA, free HPTS, and HPTS conjugated to the nanotubes. **Fig. 2d** shows UV-vis absorbance spectra to SWCNT/FA of the free HPTS, SWCNT/FA, and HPTS conjugated. The HPTS conjugated to the nanotubes showed an absorption peak at 240 nm which corresponds to the Plasmon absorption of tubular graphite (Attal *et al.* 2006). An electronic transition around 375 nm absorbance band was observed, and probably this corresponds to the characteristic peaks of FA (See UV-vis spectrum of FA, supporting information SI). Additionally, at 402 nm a band belonging to HPTS is observed (**Fig. 2b**, white line).

Raman scattering spectroscopy (RSS) is a technique mainly used to observe vibrational, rotational and other low-frequency modes in a system (Rindzevicius *et al.* 2017). In this study, we employed RSS to evaluate the effect SEM image of the conjugate SWCNT before (a) and after (b) the modification with FA; (c) topographic image on the silicon substrate of SWCNT covered by FA using non-contact mode image; (d) images of solutions of SWCNT/FA, HPTS and SWCNT/FA-HPTS and (e) UV-vis absorbance spectra of the SWCNT/FA-HPTS (black line), SWCNT/FA (blue line) and free HPTS (red line) (ultra-purified water was used as a reference solution). of the HPTS on the structure of CNT and thus show its functionalization. **Fig. 3** shows the presence of two Raman peaks located at 1537 cm^{-1} (D band) and 1591 cm^{-1} (G band).

These vibrations modes have been used to evaluate the presence of surface defects on the walls of CNTs. In a recent report, Koh *et al.* 2011 analyzed the presence of (i) the disorder-induced D band, and (ii) the G band to investigate dispersion states and covalent functionalization of SWCNTs and to map COOH groups on oxidized SWCNTs. Hence, inelastic scattering spectra can provide a more detailed evidence of the vibrational Raman modes induced by the coating of HPTS around CNTs.

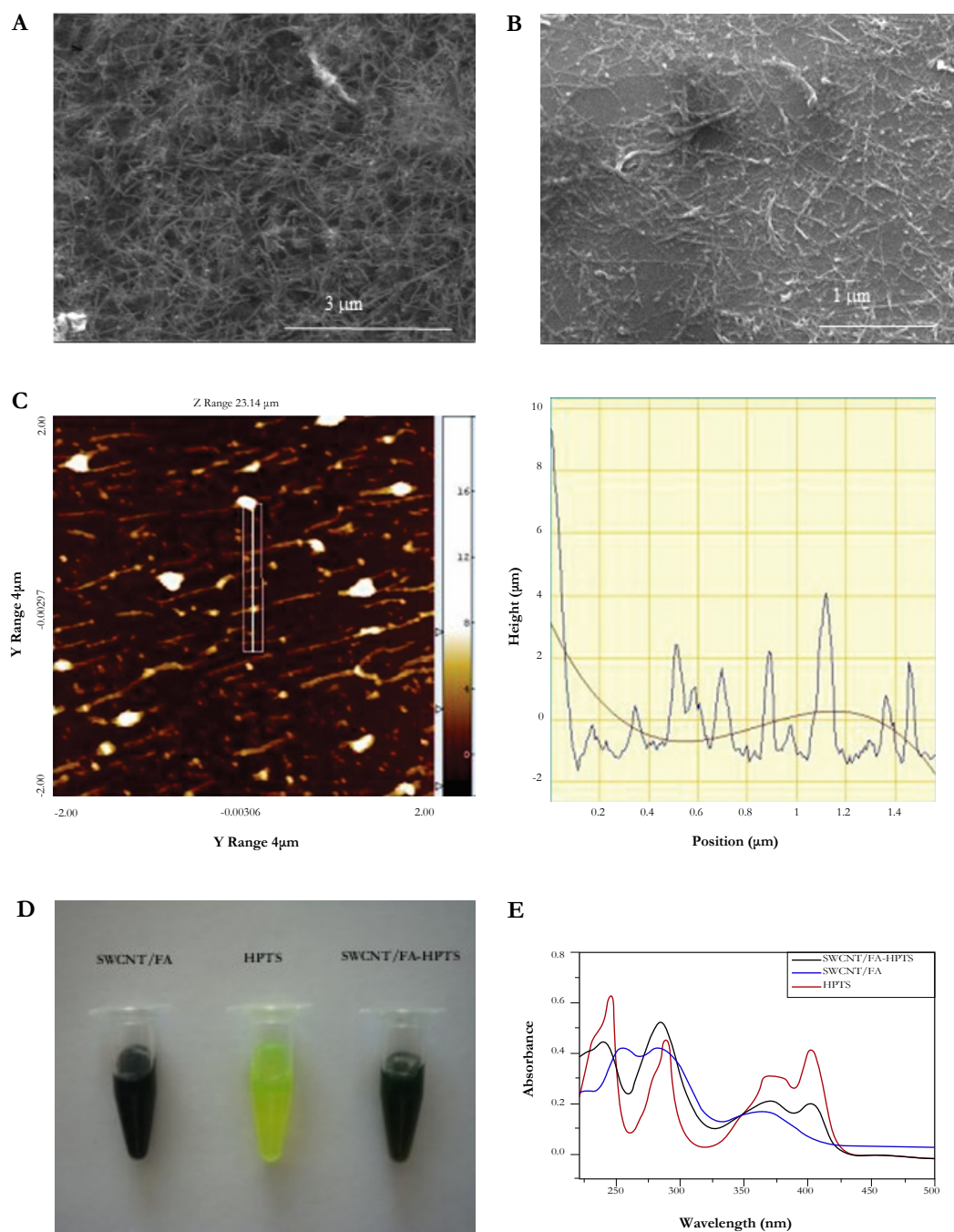


Figure 2. SEM image of the conjugate SWCNT before (a) and after (b) the modification with FA; (c) topographic image on the silicon substrate of SWCNT covered by FA using non-contact mode image; (d) images of solutions of SWCNT/FA, HPTS and SWCNT/FA-HPTS and (e) UV-vis absorbance spectra of the SWCNT/FA-HPTS (black line), SWCNT/FA (blue line) and free HPTS (red line) (ultra-purified water was used as a reference solution).

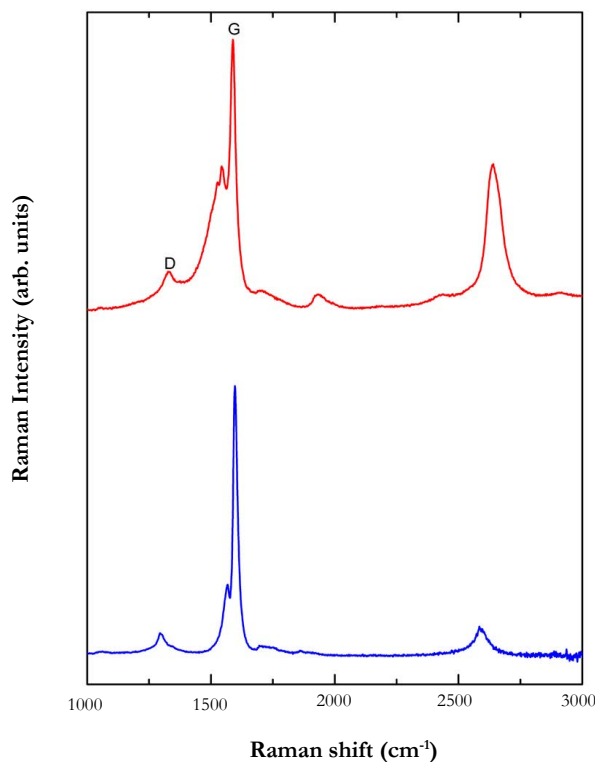


Figure 3. Raman spectra of SWCNT-FA (blue line) and SWCNT-FA in the presence of HPTS (laser 532 nm 0.1 mW).

The interaction of SWCNT-FA with HPTS was evaluated by measuring the intensity ratio between the D and G-bands. The I_D/I_G intensity ratios for the SWCNTs-FA and the HPTS functionalized nanotubes were found to be 0.098 and 0.21, respectively. These relatively small variations of the I_D/I_G ratios can be interpreted as an indication of a noncovalent interaction character between HPTS and carbon nanotubes functionalized with FA that inhibits the formation of significant structural defects.

The fluorescence spectrum of HPTS in the presence of different concentration of SWCNT at different temperatures is presented in **Fig. 4**. At a fixed concentration of HPTS, the addition of SWCNT produced a decrease in the fluorescence intensity of HPTS at 510 nm, SWCNTs have been considered as an efficient quencher (Zhiu *et al.* 2010) of a variety of dyes decreasing their fluorescence by a variety of electron transfer mechanisms (Ahmad *et al.* 2009).

Fig. 4. shows the fluorescence intensity decreases gradually with the increase of SWCNT concentration. This probably indicates some interaction between HPTS and the nanotubes.

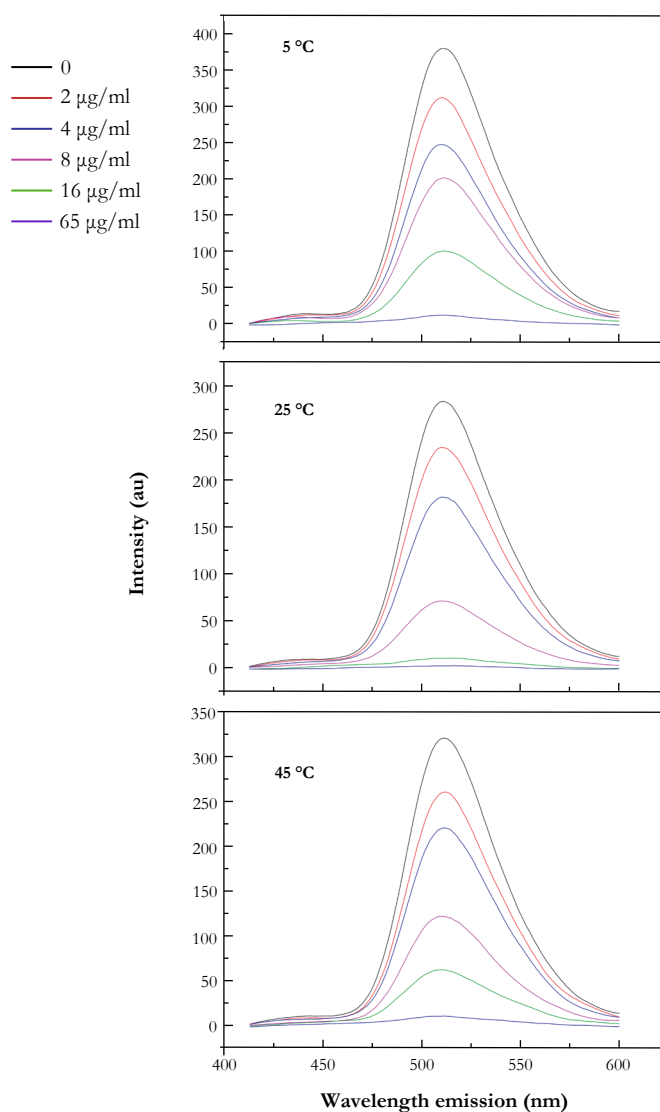


Figure 4. Fluorescence spectra of HPTS in the presence of SWCNT. The concentration of HPTS is 1mM.

The fluorescence quenching is described by the Stern-Volmer equation:

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \quad (1)$$

where I_0 and I are the fluorescence intensities of the probe in the absence and presence of the quencher, respectively. Several cases have documented the Stern-Volmer plots with a linear trend. However, other studies have shown a positive deviation from this linearity (Toprak *et al.* 2011). There are two possible reasons for the deviation from this linearity: the first is due to the simultaneous presence of dynamic and static quenching; the second is due to the formation of a chemical complex (static quenching) or by excited-state collisions (dynamic quenching) (Adenier *et al.* 2002).

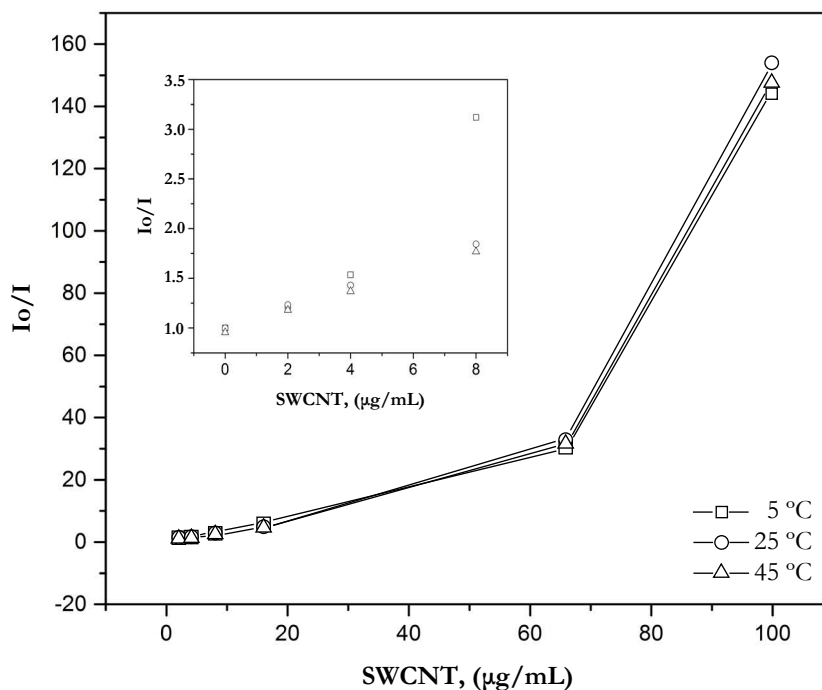


Figure 5. Stern-Volmer plots of HPTS quenching with SWCNT at different temperatures. Inset: Stern-Volmer plots at low concentrations of SWCNT.

To identify the quenching mechanism as being dynamic or static, we collected fluorescence emission data at different temperatures. The spectra were recorded for various concentrations of SWCNT containing HPTS after incubating the mixture at various temperatures (5 °C, 25 °C and 45 °C).

Fig. 5. shows the S-V plots at different temperature. It can be seen from Figure 4 that the quenching efficiency correlates inversely with temperature, in the range between 0-8 μg/mL, strongly evidenced the presence of a static quenching mechanism. However, it is worth to mention that at a concentration of 66 μg/mL SWCNT there is a noticeable deviation from the linearity probably due the quenching is nearly completed. Also, the above concentration of 66 μg/mL, an opposite behavior, is observed, meaning probably the presence of a dynamic quenching. However, to confirm this observation further experiments with a narrow interval of concentrations between 60 μg/mL and 100 μg/mL are necessary to carry out.

At higher temperatures, the probability of a collision between the HPTS and carbon nanotubes will be increased leading to a decrease in the fluorescence.

The increase in temperature favors the equilibrium to shift towards the non-complexed form of the HPTS decreasing the fluorescence quenching efficiency. In a recent work, Ahmad *et al.* 2009 studied the mechanism between CNTs and Rhodamine B showing a static quenching mechanism between the dye and carbon nanotube.

This paper is the first study to determine the quenching mechanism between HPTS and SWCNTs opening new insights into the use of the system HPTS-SWCNT for sensor probes applications.

Conclusions

The fluorescence quenching of HPTS by single walled carbon nanotubes was analyzed by a fluorescence method. We have shown that SWCNTs quench HPTS by a static mechanism by forming a stable ground state complex which decreases the efficiency quenching at higher values of temperature. The use of SWCNT integrated to probe fluorescent as HPTS will facilitate the entry to the membrane cells opening new perspectives for creating novel pH intracellular biosensors for biomedical applications.

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Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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Estudio de la disminución de la fluorescencia del ácido 1-hidroxipireno-3,6,8-trisulfónico por medio de nanotubos de carbono de pared única

Resumen. Este artículo presenta el estudio de la desactivación de fluorescencia del ácido 1-hidroxipireno-3,6,8-trisulfónico (HPTS) en presencia de nanotubos de carbono de pared única (SWCNT). Para investigar el mecanismo de desactivación (dinámico o estático) del HPTS se evaluaron nanotubos de carbono de pared única a diferentes temperaturas y se analizaron por medio de gráficas Stern-Volmer. La desviación positiva de la linealidad en las gráficas Stern-Volmer sugiere que los nanotubos de carbono de pared única actúan por medio de un mecanismo de desactivación estático, que se evidencia también por la formación de un complejo estable en estado fundamental. Los resultados presentados aquí nos ayudan a aclarar el mecanismo de desactivación de fluorescencia cuando tiene lugar la interacción entre un colorante derivado del pireno y nanotubos de carbono. Este estudio abre nuevas posibilidades para el uso de conjugados formados por SWCNT y HPTS en la fabricación de un biosensor basado en sondas fluorescentes intracelulares.

Palabras clave: Nanotubos de carbono de pared única; ácido 1-hidroxipireno-3,6,8-trisulfónico; desactivación estática; fluorescencia.

Estudo da diminuição da fluorescência do ácido 1-hidroxipireno-3,6,8-trisulfônico por meio de nanotubos de carbono de parede simples

Resumo. Este artigo apresenta estudo da diminuição da fluorescência do ácido 1-hidroxipireno-3,6,8-trisulfônico (HPTS) em presença de nanotubos de carbono de parede simples (SWCNT). Para investigar o mecanismo da desativação (dinâmico ou estático) do HPTS se avaliaram nanotubos de parede simples em diferentes temperaturas e se analisaram por meio de gráficos Stern-Volmer. O desvio positivo de linearidade nos gráficos Stern-Volmer sugere que os nanotubos de carbono de parede simples atuam por meio de um mecanismo de desativação estática, que também se evidencia pela formação de um complexo estável fundamental. Os resultados apresentados aqui nos ajudam a aclarar o mecanismo de desativação de fluorescência quando ocorre a interação entre um corante derivado do pireno e nanotubos de carbono. Este estudo abre novas possibilidades para o uso de conjugados formados por SWCNT e HPTS na fabricação de um biosensor baseado em sondas fluorescentes intracelulares.

Palavras-chave: Nanotubos de carbono de parede simples; ácido 1-hidroxipireno-3,6,8-trisulfônico; desativação estática; fluorescência.

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Chemist and Doctor in chemistry from Universidad Industrial de Santander (Assistant Professor) with a Postdoctorate in nanotechnology from Denmark Technical University. He is author and co-author of 23 specialised high impact journals and his research is focused on biosensors, bioelectronics and nanosciences.

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