Dispersion of carbon nanotubes by single-stranded DNA wrapping for advanced biomedical applications

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Abstract. Carbon nanotubes (CNT) are novel materials with unique electrical, thermal, and structural proprieties, which make them attractive for applications in composites, medicine, biology, electronics, and energy management. There are more and more attempts to use CNT, especially single-walled carbon nanotubes (SWNT), in various biomedical applications, which require highly purified samples, with no contents of catalyst residual metal particles, good dispersion in an aqueous solution at relatively high concentrations. This report presents all these three main requirements as proved by Raman spectroscopy, UV-Vis-NIR spectroscopy and thermogravimetical analysis. The maximum achieved concentration of SWNTs dispersed in water when wrapped with single-stranded DNA (ss-DNA) was of 400 mg/l.

1. Introduction
Carbon nanotubes (CNTs) are novel structures formed from a hexagonal network of carbon atoms, rolled into a shape of a perfect cylinder, with diameters from 0.7 to a few nanometers. CNTs may have one, two or more walls, of one atom thick graphitic layers, depending on the synthesis conditions used. Due to their unique electronic and mechanical proprieties [1] and size, they have attracted considerable attention over the past decade, for their potential applications in various areas such as: biosensors [2], hydrogen storage media [3], composites [4] and actuators [5]. Recently [6], the possibility of photo-thermal destruction of cancer cells was demonstrated by using SWNTs. This potential biomedical application branches from the property of SWNTs to heat rapidly by absorption of infra-red radiation without harming the human tissue because it is transparent to infra-red radiation.

In biomedical applications it is imperative to examine their potential toxicity before further use. In vitro and in vivo tests for SWNTs cytotoxicity require aqueous media of well dispersed nanotubes which is difficult because of their hydrophobic nature and poor solubility in organic solvents [7]. Moreover, they are organized in bundles held together by strong van der Waals interactions so their individual separation and dispersion still remains a challenge. Several covalent [8] and non-covalent [9] surface functionalization methods have been used to make SWNTs water soluble and suitable for
biological and medical research, especially in what concerns surfactants toxicity. The non-covalent approaches are more proper because it does not suppose the CNTs lattices interruption. Until now, stable individual dispersion of SWNT have been obtained with surfactants like: Triton X-100, NaDDBS, SDS [10] or biomolecules like BSA or chitosan [11], but the best results have been obtained when wrapped with DNA fragments [12]. Zheng et al [13], revealed that single-stranded DNA (ss-DNA) binding to CNT is extremely effective and facile at low concentrations.

The purpose of this paper is to obtain stable homogeneous and highly concentrated aqueous dispersions of individual SWNT by wrapping them with ss-DNA.

2. Materials and methods

SWNTs were synthesized using an original version of catalytic chemical vapor deposition method based on induction heating (RF-CCVD), previously developed by our group [14], from the pyrolysis of acetylene, at 850°C, over a bi-metallic catalyst system Fe–Mo supported on MgO. The Fe–Mo/MgO catalyst with the chemical weight ratio composition of Fe:Mo:MgO = 1.4:0.14:98.46 (%wt), was produced by using the co-precipitation method [15]. The carbonaceous products obtained were purified in a Soxhlet apparatus with HCl (1:1) for 24 h, washed with distilled water, and dried overnight at 150°C. After the purification, the SWNTs morphology was investigated by Raman spectroscopy, which was performed at room temperature using the Jasco NRS-3300 Raman Spectrometer with a 633 nm laser. Thermogravimetrical analyses (SDT Q600 TA Instruments) were performed in air at a flow rate of 100 ml/min and a heat rate of 5°C/min.

The ss-DNA from salmon testes, in water, was purchased from Sigma-Aldrich. Dispersion of SWNTs by ss-DNA was carried out as described previously [16]. Briefly, 5 mg purified SWNT was mixed with 5 ml ss-DNA 0.1% solution and sonicated with a cup-horn sonicator (Sonics, Vibra-Cell VC505, 500 W, 20 kHz) on an ice-water bath for 15 min at 30% amplitude. The mixture was then centrifugated (Sigma) 1 h at 4000 g, in order to remove undispersed SWNTs, and the supernatant was collected and kept for some time. Optical absorption measurements were recorded using a Jasco V-570 UV-Vis-NIR Spectrophotometer, in 1 cm quarts cuvette, at a dilution of 1/40, for both, ss-DNA 0.1% solution and the collected supernatant.

3. Results and discussion

Raman spectra for SWCNTs (figure 1) present bands in two characteristic spectral regions: at low frequency (100-400 cm\(^{-1}\)), or the radial breathing mode (RBM) region, and at high frequency D, G and G' bands. While D, G, and G' modes are also found in graphite, the RBM is a unique CNT mode. It is the real signature of the presence of carbon nanotubes in a sample and strongly depends on the diameter and chirality of the nanotubes. The RBM frequency (\(\omega\)) is inversely proportional to the CNT diameter that can be calculated with equation (1) [32].

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\omega(cm^{-1}) = \frac{223.5}{d(nm)} + 12.5
\] (1)

The differences, in Raman spectra, between SWNT after the purification step and SWNT dispersed with ss-DNA consist only in the RBM region. In RBM band picks intensity is proportional with existing quantity in the sample. The ss-DNA selectively extracts certain SWNT diameters. Spectral position and corresponding diameter values for our samples (SWNTs before and after dispersion) are represented in Table 1. D band (between1305 and 1330 cm\(^{-1}\)) is associated to the vacancies, defects, and carbonic impurities (amorphous carbon, glassy carbon) that destroy the symmetry, and in this case its intensity is low meaning that our SWNT contain almost no defects and no carbonic impurities [17].
Figure 1. Raman analysis spectra at 633 nm (a) and magnified RBM band (b) for SWNTs right after the purification (blue) and after the dispersion with ss-DNA (red).

In figure 2, the thermogravimetrical analysis (TGA) was performed to confirm SWNT’s purification step. The final purity of the SWNTs was of 96.97%. The TGA curves indicate the existence of a single mass loss profile for all reaction temperatures, which indicates that all the analyzed nanotubes samples are composed of a single carbonaceous type.

Table 1. Spectral position and corresponding diameter values from RBM band at \( \lambda_{\text{exc}} = 633 \) nm.

| SWNT after purification | SWNT dispersed with ss-DNA |
|-------------------------|---------------------------|
| \( \omega_{\text{RBM}} \) (cm\(^{-1}\)) | \( d \) (nm) | \( \omega_{\text{RBM}} \) (cm\(^{-1}\)) | \( d \) (nm) |
| 192.4 | 1.24 | 195.6 | 1.22 |
| 216.6 | 1.09 | 219.3 | 1.08 |
| 229.1 | 1.03 | - | - |
| 242.1 | 0.97 | - | - |
| 250.7 | 0.94 | 254.6 | 0.92 |
| 261.3 | 0.90 | - | - |
| 281.9 | 0.83 | 283.7 | 0.82 |
| 294.7 | 0.79 | - | - |

Figure 2. The TGA and the derivative TGA (dW/dT) profiles of the purified SWNTs.

The SWNTs concentration in the collected supernatants aqua solution after the dispersion process can be evaluated from the Beer-Lambert equation by deeming the absorbance [18, 19]. Concentration evaluation from the absorbance of the ~320 nm pick is not possible because it is due not only to the CNT absorption but also to the absorption of the \( \pi \) plasmons and the ss-DNA. As it can be seen in the absorption spectra of ss-DNA (figure 3), the ss-DNA absorbs at the same wavelength range of 200-320 nm [19]. The concentration obtained, from the absorption spectra of SWNT dispersed with ss-DNA (figure 3), in agreement with Ryabenko et al [19], is 400 mg/l for SWNT dispersed with ss-DNA.

The SWNT identified from the absorption spectra of ss-DNA dispersed SWNTs (figure 4), following the method described in literature [19, 20], have the diameters: 0.8 nm, 0.95 nm, 1.0 nm, 1.20 nm. Larger diameters can be identified from the absorption spectra but those tubes diameters do not appear in the Raman spectra because are not resonant at 633 nm laser frequency.
Figure 3. Absorption spectra for ss-DNA dispersed SWNTs (left). Fragment from the absorption spectra of ss-DNA dispersed SWNTs (center). Absorption spectra for ss-DNA (right).

4. Conclusions
The purpose of this research was to obtain stable homogeneous and concentrated aqueous dispersions of individual SWNT. The reported concentration of SWNTs dispersed in water with ss-DNA was found to be 400 mg/l and the suspension was stable for several months when stored at 4°C. It is currently being used to determine the SWNT potential toxicity and oxidative stress on culture cells and animal models. The SWNTs purity was demonstrated by thermo-gravimetical analyses and confirmed once again by the low intensity of the D band in the Raman spectra.

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