Optical properties of single wall carbon nanotubes dispersed in biopolymers

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Abstract
In this paper we report the optical studies of single wall carbon nanotubes dispersed in biomaterials. We have obtained very stable suspensions of SWNTs, which allowed us to get good photoluminescence signal from the individually dispersed nanotubes. These new hybrid systems may find some applications in bionanocomposites with photoluminescence properties and in biosensors. Furthermore, the dispersion of carbon nanotubes in these biocompatible materials is important for evaluating the toxicity of either isolated or lightly bundled single wall carbon nanotubes.

1. Introduction
Optical properties (absorption and photoluminescence) of individual single wall carbon nanotubes (SWCNTs) dispersed in aqueous solutions have been intensively studied [1]. The SWCNTs are suspended in water or organic solvents by surfactant wrapping thus forming colloidal suspensions of individual and/or small bundles of carbon nanotubes. This method lets nanotubes to stay isolated from each other preventing their bundling, which is responsible for quenching their photoluminescence. The environmental screening of the nanotubes affects their optical spectra and is responsible for changes in the relative intensities, shifts of the optical transition energies and broadening of the absorption and emission bands [2,3]. This sensitivity of the optical properties to the screening environment has opened a possibility of using carbon nanotubes for building of extremely sensitive sensors able to detect, for example, changes in the polymorphism of DNA upon extremely small concentration of Hg$^{+}$ counter ions [4].

Most of the surfactants used for dispersing carbon nanotubes are synthetic and there is an extensive list of different polymers used as a wrapping medium for carbon nanotubes [5]. Biocompatible dispersants such as Pluronic F108 and hydroxypropylcellulose as well as biomolecules, such as single strand DNA, RNA and surfactin, have also been used as the wrapping medium [6–10].

Bio surfactants belong to heterogeneous group of natural molecules with surface active properties. These molecules have amphiphilic nature and their polar head may be ionic, non-ionic or amphoteric, whereas their apolar tail often is a hydrocarbons chain [11]. The biosurfactants are synthesized by living organisms, such as bacteria, yeasts and fungi [12,13]. The chemical classes of biosurfactants include peptides, glicolipids, lipopeptides, lipoproteins, phospholipids, fatty acids, polymeric surfactants and surfactant particles. Many species of vertebrates also synthesize biosurfactants, like some frogs that deposit their eggs in stable foams, which are rich in protein surfactants. Protein surfactants are very important, mainly because of their biocompatibility and biodegradability that offer excellent opportunities for industrial and biomedical applications [14].

Some polymeric substances are also known due to their active surface properties, but they are not classified as surfactants. These biomolecules as well as surfactants have tendency to form micelles and increase the surface area of hydrophobic substrates, which increases their solubility in water [15]. The natural products with these peculiar characteristics include chitin, chitosan, carrageenan, galactomannans, pectin, cellulose and agar, which find a lot of applications in pharmaceutical, cosmetic and food industries [16].

Carbon nanotubes are attractive systems not only for their possible applications in electronic, optic and mechanical materials but also in biological applications, such as imaging, sensing and drug delivery [5,17,18]. However, carbon nanotubes usually take the form of aggregates that are insoluble in all solvents. Amphiphilic surfactants are excellent resources for preparation of...
water-soluble suspensions of nanotubes. In these suspensions the detergent chains interact with the surface of the nanotubes, which are very hydrophobic, via Van der Waals bonding, while the polar heads point outwards into the surrounding aqueous medium. In the case of proteins with surfactant property the organization of protein at the surface of nanotubes can be assisted by the presence of hydrophobic domains in the protein structure, which allow it to interact with the graphite layer of the carbon nanotubes. In both cases, the result is the dispersion of carbon nanotubes in a proper form for multiples applications in biotechnology. Therefore, the usage of biomolecules instead of synthetic surfactants for dispersing carbon nanostructures (graphene and carbon nanotubes) is an active research field and developing this area in turn will help the community to reach some challenges in the biosensors applications. Our present work isinserted in this scenario pointing out that some bio-molecules can be successfully used for dispersing carbon nanotubes at individual nanotube level and profit from their remarkable emission of light in the infrared region.

In this paper we report the dispersion of carbon nanotubes in a new wrapping medium composed of biosurfactants and biomolecules. We have obtained stable suspensions, which allowed us to get good photoluminescence signal from individually dispersed nanotubes. The obtained dispersions are promising materials for applications in bionanocomposites and sensors.

### 2. Experimental

#### 2.1. Materials

In this study different biomolecules were tested as dispersing agents for SWCNTs. We used a protein surfactant that was isolated from foam nests of the tropical frog *Leptodactylus vastus* [14]; a lipopetide surfactant from the bacterium *Bacillus subtilis* CE002 purified according to the methodology described by Yeh et al. [19]; chitosan, a polysaccharide of the shrimp shell (Polymar S.A, Ceara, Brazil) and the crude polysaccharides from the red seaweed *Hypnea musciformis* [20]. As a control we used Sodium Dodecyl Sulfate (SDS) (Sigma-Aldrich).

The critical micellar concentration (CMC) of the biomolecules and SDS was obtained from conductivity measurements. The experiment was performed by preparing a serial dilution using a stock solution (1%) of the working sample. The electrical conductivity (using a conductivimeter electrode) for each dilution point was measured. Using these values we plot conductivity versus concentration curves, which was used for determining the critical micelle concentration values of the samples work [21]. Measurements were performed in triplicate.

#### 2.2. Techniques

The absorption spectra were measured in the 400–900 nm range using the Thermo Scientific-Genesy 6 equipment. Photoluminescence excitation (PLE) maps were obtained using a Horiba Nanogol fluorometer equipped with a liquid N$_2$-cooled InGaAs detector. The maps were recorded in the excitation range from 450 to 750 nm (coming from a Xe lamp) and emission range from 900 to 1400 nm. The resolution in the excitation and emission range was 5 and 1 nm, respectively.

### 3. Results and discussion

Micelles are defined as molecular-size colloidal aggregates, thermodynamically stable, spontaneously formed by amphiphilic compounds above a certain concentration called Critical Micellar Concentration (CMC). Below the CMC, the surfactant is predominantly in the form of monomers. The CMC depends on the structure of the molecule (size of the hydrocarbon chain) and the environmental conditions (ion concentration, temperature) [23]. The CMC, conductivity and surfactants used for dispersing the SWCNTs are listed in Table 1.

Among the biomolecules studied only the protein and lipopetide biosurfactants show two CMCs values. As the molecules are inserted in a low concentration, they begin to associate in such a way that the hydrophobic and hydrophilic segments interact with each other leading to the formation of the first micelles. However, because of the complexity of these molecules, the exposition of hydrophobic cores may result in a secondary aggregation order, thus forming larger and more stable micelles. Thus, the lower CMC value is attributed to the regime of intramolecular interactions while the higher CMC value is associated with intermolecular interactions since this process requires a higher concentration of biosurfactants [24,25]. Furthermore, the CMC values for the proteins are lower than that for lipopeptides. The CMCs for these two biosurfactants are also lower than that of SDS, which is considered a good synthetic surfactant. The lipopeptide from *B. subtilis* CE002 also shows the highest electrical conductivity, which confirms that it is a good dispersing agent. In fact, the genus *Bacillus* is a producer of lipopeptide biosurfactants and among them *B. subtilis* produces surfacin, the most potent biosurfactant known so far.

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The polysaccharides, chitosan and algal carbohydrates, show CMC and conductivity values quite similar, despite their different chemical compositions. They are also more stable than the low molecular weight surfactants (Table 1).

<table>
<thead>
<tr>
<th>Dispersing agent</th>
<th>CMC (mg/mL)</th>
<th>Conductivity (µS/cm) (at CMC)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>2.0</td>
<td>428 ± 8.1</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Lipopeptide</td>
<td>0.2</td>
<td>1079 ± 39.3</td>
<td><em>Bacillus subtilis</em> CE002</td>
</tr>
<tr>
<td>Protein</td>
<td>0.1</td>
<td>184 ± 13.4</td>
<td>Frog foam nest</td>
</tr>
<tr>
<td>Chitosan</td>
<td>2.5</td>
<td>174 ± 21.3</td>
<td>Shrimp shells</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>1.9</td>
<td>181 ± 16.2</td>
<td>Red seaweed (<em>Hypnea musciformis</em>)</td>
</tr>
</tbody>
</table>

* CMC—Critical Micellar Concentration.
Chitosan used in this work showed CMC values higher than that those reported in the literature, which are between 0.011 and 0.079 mg/ml [23]. The difference between these values can be explained by such factors as solvent used, molecular weight and deacetylation degree [26,27]. These factors are very important for the CMC, because they directly affect the formation of polymer aggregates [28,29]. As we used a chitosan with 78% of deacetylation, which indicates the presence of a large number of chitin waste, this polymer requires a larger amount of molecules to initiate the formation of the first micelles and therefore presents greater CMC. In general, all the suspensions obtained in this study were stable for months as unveiled by photoluminescence measurements.

Fig. 1A–E shows the aspects of the SWCNT dispersions obtained with synthetic and natural surfactants. Most of the dispersions were black, which may be due to the presence of SWCNT bundles with large diameters. On the other hand, the clear dispersion formed by the protein biosurfactant may be attributed to small diameter bundles (probably with one nanotube). After diluting the dispersions, we could observe that the suspensions were of good quality and did not show neither cloudy aspects nor signs of sedimented material. These results will be better discussed through the absorption and photoluminescence spectra.

Fig. 2 shows the optical absorption spectra of SWCNTs dispersed using different wrapping media after baseline subtraction. The spectra show the well-known pattern related to the second electronic transition of semiconducting carbon nanotubes except for the protein biosurfactant, which has a strong interference with absorption of the wrapping medium. Such peak structure indicates that the nanotubes are either individualized or they are in very small bundles. We identify the peaks below 600 nm as being attributed to the first electronic transition of metallic carbon nanotubes while the peaks above 600 nm are due to light absorption of the second electronic transitions for semiconducting nanotubes. The presence of both metallic and semiconducting nanotubes indicates that there is no selectivity of the wrapping media towards the nanotubes category. Next, we focused on the emission spectra, because they are much more sensitive to changes in the SWCNT environment [30].

The efficient dispersion of the carbon nanotubes in the biocompatible dispersants was confirmed by the photoluminescence measurements. It is well established in the literature that only dispersed tubes show resolved photoluminescence spectra [1]. In Fig. 3A–E we show the emission vs. excitation photoluminescence maps for the nanotubes dispersed using SDS and the biomolecules. The PL intensity was comparable to that reported for other biomaterials, such as dispersion with DNA [31]. In panel (F) we show the PL map for suspension (C) aged for 2 months, which confirms good stability of the dispersion. The (n, m) nanotubes associated with the main peaks are identified and the first ($E_{11}$) and second ($E_{22}$) electronic transition values are listed in Table 2 for the six suspensions. The comparison of $E_{11}$ shows that depending on the surfactant there is a Stokes shift of the emission peaks. This effect is associated with the excitonic effects, whose energy depends on the environment around the nanotubes through the dielectric screening, whose detailed model is discussed in Ref. [32]. The analysis of the PLE maps for SDS shows this molecule is effective in wrapping SWCNTs. We can see clearly nine types of semiconducting nanotubes with indices (n, m): (8, 4), (8, 7), (7, 6), (9, 4), (10, 2), (10, 3), (11, 1), (7, 5) and (6, 5). The nanotube assignment was made based on the photoluminescence maps reported in the literature for a similar single wall carbon nanotube sample we used [1,3]. SDS is considered as a molecule that has no specificity for any kind of semiconducting nanotube conformation [33]. Functionalization of CNTs by SDS is due to its hydrophobic tail that can perform various types of guidance to the nanotubes. Probably, SDS molecules can bend over and even wrap around the nanotubes or they can reach the center of the nanotubes bundles exfoliating them and dispersing in water [1].

The biomolecules turned out to be as efficient in the individualization of CNTs as the SDS. The protein surfactant and

![Fig. 1. Aspects of SWCNTs dispersed in water using SDS (A), lipopeptide surfactin (B), protein biosurfactant (C), Chitosan (D) and crude polysaccharides from red seaweed (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)

![Fig. 2. Typical optical absorption spectra of SWCNTs dispersed by SDS (A), lipopeptide surfactin (B), protein biosurfactant (C), Chitosan (D) and crude polysaccharides from red seaweed (E).](image)
Chitosan were able to functionalize also the following nine types of semiconducting nanotubes: (8, 4), (8, 6), (8, 7), (7, 6), (9, 4), (7, 5), (10, 2), (10, 3) and (11, 1). The dispersion of carbon nanotubes in chitosan is associated with this polymer structure. Chitosan has amine groups, which are good electron donors and interact with the nanotubes that act as electron acceptors. It is expected that chitosan transfers charge to the carbon nanotubes [34].

The polysaccharides from seaweeds functionalize the nanotubes (8, 4), (8, 7), (7, 6), (9, 4), (6, 5), (7, 5), (10, 2) and (10, 3), while the lipopetide biosurfactant wrapped the same eight types of nanotubes plus the nanotubes (8, 6) and (11, 1) generating ten types of carbon nanotubes. It is believed that the surfactin is able to form cylindrical micelles with a β-sheet as its secondary structure, and this structure is responsible for the functionalization of SWCNTs [8].

The capacity to solubilize of the CNTs through other studied biomolecules appears to be related mainly to the size of the hydrophobic chain, which is the group with higher molecular weight and suspending more nanotubes. However, each biomolecule has its particular mechanism to CNTs dispersion.

Regardless of the excitation energy we did not observe the emission peak at 1150 nm, which is associated with carrier migration from large to lower diameter nanotubes when they are bundled [35]. This result gives an additional evidence that the suspensions studied here contained very small number of SWCNTs bundles.

One of the main drawbacks in evaluating toxic effects of the nanotubes using in vivo models is poor solubility of the nanotubes both in water and in biological fluids [36]. The suspension of carbon nanotubes in a biocompatible medium is very important.
for evaluating the ecotoxicology of carbon nanotubes and the biomaterials presented here have potential to be used in these applications. Biosurfactants have several advantages over some synthetic surfactants including lower toxicity and higher biodegradability as reported for chitosan.[37]

4. Conclusions

Single wall carbon nanotubes can be wrapped with different biopolymers and investigated by optical absorption and photoluminescence spectroscopies. All the biosurfactants evaluated in this work are suitable for dispersing single wall carbon nanotubes, that yields the possibility of preparing bionanocomposites with photoluminescent properties. The light emission energy from carbon nanotubes depends on the kind of biosurfactants and this is attributed to the environmental changes, which leads to screening effect of excitons. These new hybrid systems may find some applications, which demands stable and biocompatible suspensions of carbon nanotubes.

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