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Evaluation of concentration and dispersion of functionalized carbon nanotubes in aqueous media by means of Low Field Nuclear Magnetic Resonance

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ABSTRACT

Dispersions of functionalized carbon nanotubes (f_x-CNTs) have been analyzed by means of Low Field Nuclear Magnetic Resonance measurements (LF-NMR). This technique showed that the presence of the f_x-CNTs strongly influences the structuring of the water molecules. Thereupon, the water transversal relaxation rate (r_2) can be used to get information about the concentration of the CNTs in aqueous dispersion and their aggregation tendency. Finally, the effect of the addition of an anionic polyelectrolyte, namely alginate, on aqueous dispersion of CNTs was explored by using LF-NMR, which revealed different dependence of r_2 from CNT concentration, depending on the type and charge of f_x-CNTs.

1. Introduction

Several research groups have proposed the use of carbon nanotubes (CNTs) - and in general of carbon nanostructures (CNSs) - for biomedical and tissue engineering applications, in particular for the preparation of biocompatible nanocomposite biomaterials in the form of solutions, hydrogels or scaffolds [1–4]. In order to optimize the contribution of CNTs to the nanocomposite materials, it is very important to achieve a good dispersion of CNTs [5,6] and to investigate how CNTs are dispersed within the biomaterial matrix and how they interact with the surrounding environment, namely with water molecules, polymeric chains and cells [2,7].

In particular, the interaction between water molecules and CNTs modifies several properties of the material - like wettability - consequently affecting protein adsorption and cell adhesion [8,9]. For example, it has been showed that depending on their surface tension, single and multi-walled CNTs are able to affect, in a different way, the structural changes of proteins, the damages to cell membranes and the mechanism of cell death [10]; moreover, depending on the oxidation degree, multi-walled CNTs can affect cell proliferation [11].

However, the final aggregation state within the matrix is affected by the dispersion and stability of the initial aqueous CNT systems. Several techniques have been employed to evaluate the dispersibility of CNTs [7], the effects of salts and dispersing agents and the interactions between CNTs and polymer chains, such as transmission and scanning electron microscopy [8], scattering techniques, like dynamic light scattering or small angle x-ray scattering [12,13], as well as, especially for single-walled CNTs, molecular dynamic simulations [14,15]. Moreover, it has been recently demonstrated that the presence of CNTs in solution alters its echographic response and therefore they have been proposed as potential contrast agents [16].

An interesting aspect, investigated by molecular dynamic simulations on single-walled CNTs, is the fact that depending on the chemical characteristics of CNTs surface, these are able to interact in a specific way with water molecules [17,18] and, therefore, to influence their relaxivity behavior [19,20]. Moreover, it has been

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observed by Baranowska-Korczyc et al., that the improved dispersibility of PEG-MWNCTs/Fe, after non-covalent functionalization, can be associated with the increasing of water relaxivity [21]. These findings have focused our attention on the possibility to use the Low Field Nuclear Magnetic Resonance (LF-NMR), to investigate how the water molecules can be affected by the presence of CNTs.

Accordingly to these premises, in this work LF-NMR was used for the characterization of aqueous dispersions of multi-walled CNTs, pristine or covalently functionalized with different chemical groups (f_x-CNTs) [22,23]. It was found the transversal relaxation rate, r_2, of water molecules can be used to evaluate both the concentration and the dispersion of CNTs as a consequence of a very strong effect of CNTs on the structuring of water molecules in solution. However, the presence of a (natural) anionic polyelectrolyte, namely alginate, may notably alter the effect of the CNTs in solution.

2. Experimental

2.1. Materials

Sodium alginate samples isolated from *Laminaria hyperborea* were provided by Kerry Group (Ireland). The relative molecular mass ("molecular weight", MW) was found to be 186 600 ± 1 100, as determined by capillary viscosimetry [24]. The mannuronic acid (M) and guluronic acid (G) fractional composition was determined by means of ^{1}H NMR [25,26] and resulted to be $F_G = 0.71$, $F_M = 0.29$, $F_{GG} = 0.55$, $F_{GM+MG} = 0.31$, $F_{MM} = 0.13$, $N_{G>1} = 12.2$. F_X is the compositional mole fraction of monomer X, F_{XX} (or F_{XY}) likewise is the compositional mole fraction of nearest-neighbor dyads of monomer X (or X *and* Y), $N_{G>1}$ is the number-average number ($\overline{n}_n$) of monomer G in homopolymeric sequences having $\overline{n}_n \geq 2$. Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), penicillin streptomycin 100X, L-glutamine 100X were purchased from EuroClone, S.p.A., Italy.

2.2. Multi-walled carbon nanotubes functionalization and characterization

Carbon nanotubes have been modified through two different kinds of reactions in order to achieve differently charged moieties on the carbon sidewall. Pristine multi-walled CNTs (f_0-CNT; diameter 20–30 nm, length 0.5–2 μm) have been purchased from Nanostructured & Amorphous Materials Inc., with a degree of purity greater than 95%. The characterization of CNTs used in this work has been reported in the Supplementary Content. Pristine carbon nanotubes have been directly analyzed (f_0-CNTs) or functionalized through the diazonium salt-based arylation reaction [22] (f_1-CNT) and the 1,3-dipolar cycloaddition of azomethines ylides reaction [23] (f_2-CNT) to achieve the modified CNTs reported in Fig. 1.

2.3. Low field NMR relaxometry (LF-NMR)

LF-NMR characterization of CNTs dispersions was performed by means of a Bruker Minispec mq20 (0.47 T). Transverse relaxation time (T_2) measurements were performed at 25 °C according to CPMG (Carr-Purcell-Meiboom-Gill) sequence with a 90°–180° pulse separation of 0.25 ms (number of scans 4; delay 5 s, from 500 to 1000 time intervals). In the light of this setting, the T_2 measurement required approximately 1 min. The T_2 discrete distribution was determined by fitting the experimental time (t) decay of the signal, related to the extinction of the x-y component of the magnetization vector (M_{xy}), with the traditional multi-exponential function ($I(t)$):

$$I(t) = \sum_{i=1}^{m} A_i e^{-t/T_{2i}} \quad (1)$$

where A_i are the (dimensionless) pre-exponential factors proportional to the number of protons relaxing with the relaxation time T_{2i}. This fitting allowed determining the different relaxation times (T_{2i}) of the water molecules subpopulations in the sample with the respective spin density (A_i). The chosen number of subpopulations was identified with that minimizing the product χ^{2*}Np, where χ^2 is data fitting chi-square value, and Np is the number of model parameters used, recalling that each exponential function introduced requires two parameters [27]. $\overline{T}_2$ and $\overline{r}_2$ are defined as:

$$\overline{T}_2 = \frac{\sum_i A_i \cdot T_{2i}}{\sum_i A_i} \quad \overline{r_2} = \frac{1}{\overline{T}_2} \quad (2)$$

LF-NMR characterization has been performed on f_x-CNTs dispersed, at different concentrations, in deionized water, in complete culture medium (DMEM) and in salt-free aqueous alginate solution (2% w/V in deionized water). The complete culture medium has been prepared by adding to DMEM: heat-inactivated FBS (10% w/V) 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine.

In order to illustrate the procedure adopted for the determination of $\overline{T}_2$ and $\overline{r}_2$, let's focus the attention, for instance, on the relaxation behavior of the system f_1-CNT/H_2O after 32 days (46,080 min). Fig. 2 and the statistical F-test ($F(5, 624, 0.95) < 1.6 \times 107$) show the good agreement between the experimental data (extinction of the x-y component of the magnetization vector (I_s), open circles) and the Eq. (1) best fitting (solid line). The minimization of the product χ^{2*}Np allows to conclude that three exponential terms are needed and that the fitting parameters read: ($A_1 = 19.8 \pm 0.3$, $T_{21} = (190.2 \pm 0.8$ ms)), ($A_2 = 50.3 \pm 0.3$, $T_{22} = (94.7 \pm 0.4)$ ms) and ($A_3 = 4.6 \pm 0.1$, $T_{23} = (17.4 \pm 0.4)$ ms).

Dotted lines indicate, respectively, the contribution of the first (A_1, T_{21}), the second (A_2, T_{22}) and the third (A_3, T_{23}) exponential. The existence of three different relaxation modes of the water protons in the f_1-CNT/H_2O implies that water molecules interact in three different ways with CNTs. Although it is never simple attributing a precise physical meaning to the different relaxation modes, for sure, they are directly connected to the three-dimensional organization of CNT in the liquid environment. Borrowing what happens for the meshes of polymeric networks (the smaller the mesh size, the smaller the relaxation time) [28] and porous materials [28,29] (the smaller the pores, the smaller the relaxation time), we could argue that the smallest relaxation time (A_3, T_{23}) is associated to the presence of highly packed CNTs clusters, where the average distance among CNTs is small. The second (A_2, T_{22}) should correspond to less packed CNTs clusters, where the average distance among CNTs is bigger. Finally, the first relaxation time (A_1, T_{21}) should correspond to a uniform distribution of CNTs in the liquid phase characterized by a higher average distance among CNTs. The volumetric percentage of the water contained in each class of CNTs ($A_{i\%}$) can be evaluated on the basis of A_1, A_2 and A_3. Indeed, we have:

$$A_{i\%} = 100 \frac{A_i}{\sum_i A_i} \quad (3)$$

Accordingly, the first, second and third class contain, respectively, 26.5%, 67.3% and 6.2% of the volume of the whole water present in the system. The global effect of this CNTs organization, according to Eq. (2), is given by $\overline{T}_2 = (115 \pm 0.5)$ ms and $\overline{r}_2 = (8.7 \pm 0.04) \times 10^{-3}$ ms^{-1}. The standard deviations associated to $\overline{T}_2$ and $\overline{r}_2$ reflect the values typically found for all other systems