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Coumarin-Conjugated Multiwalled Carbon Nanotubes for Potential Biological Applications: Development and Characterization

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This work presents a novel cascade of chemical functionalization of multiwalled carbon nanotubes which allows the conjugation with differently substituted coumarins. Aim of the present work is to synthesize new materials able to rescue cells from the adverse effect of CNT particles since pristine CNTs are practically insoluble and tend to accumulate inside cells, organs and tissues. Moreover, it was reported that single walled CNTs particles show an adverse effect on keratinocytes through an oxidative mechanism, leading to NF-*k*B activation. The conjugation with coumarins, known superoxide anion scavengers, could switch the cytotoxicity of the new materials. The cascade functionalization of MWCNTs by sequential steps of carboxylation, acylation, amine modification and finally, coumarin conjugation have been performed and the synthesis and the chemical properties of several *f*-MWCNTs-coumarins have been exploited.

Keywords: Multi Walled Carbon Nanotubes, Coumarins, Nanotoxicity, CCVD, Chemical Functionalization.

RESEARCH ARTICLE

1. INTRODUCTION

A great scientific and technological interest on carbon nanotubes (CNTs) has been recently registered as a result of their fascinating properties and their ability to serve as candidate for a variety of therapeutic and diagnostic applications.[1–4] Besides the ability of functionalized carbon nanotubes (f-CNT) to act as carriers for the delivery of a wide range of therapeutic agents, f-CNTs-drug can be also considered as a new entity with novel therapeutic or diagnostic properties.[5–7] For successful applications in the biomedical field, bioeffects and safety of CNTs to the environment and human health need to be addressed. In this context, research into the toxicity of nanomaterials, generally referred as "nanotoxicity," is being carried out, but at a far slower pace than research into how nanomaterials can be employed.[8–10]

It has been reported that single walled CNTs particles show an adverse effect on keratinocytes through an oxidative mechanism leading to NFkB activation.[11] Chemical modification of CNTs by functionalization is often required for more versatile suspension capabilities and realization of certain applications, the most important being their molecular interactions with biological systems.[12] Moreover functionalization remarkably reduces the cytotoxic effects of CNT.[13]

Coumarin derivatives exhibit a broad range of biological effects including anticoagulation, antibiotic, antifungal, antipsoriasis, cytotoxic, anti-HIV, anti-inflammatory activities.[14–16] In particular, substituted hydroxycoumarins are potent superoxide anion scavengers, are able to inhibit *in vitro* lipid peroxidation and act as NF-*k*B inhibitors.[17–18]

In order to synthesize new material able to rescue cells from the adverse effect of CNT particles, we have focused our attention on the synthesis of new f-CNTs linked to natural antioxidants with a coumarin structure. We have performed the cascade functionalization of MWCNTs by sequential steps of carboxylation, acylation, amine modification and finally, coumarin conjugation. The synthesis and the chemical properties of several f-CNTs-coumarins has been exploited (Fig. 1).

Coumarins are spectrophotometer detectable biologically active molecules and their conjugation with CNTs, beside the improvement of the biocompatibility, could

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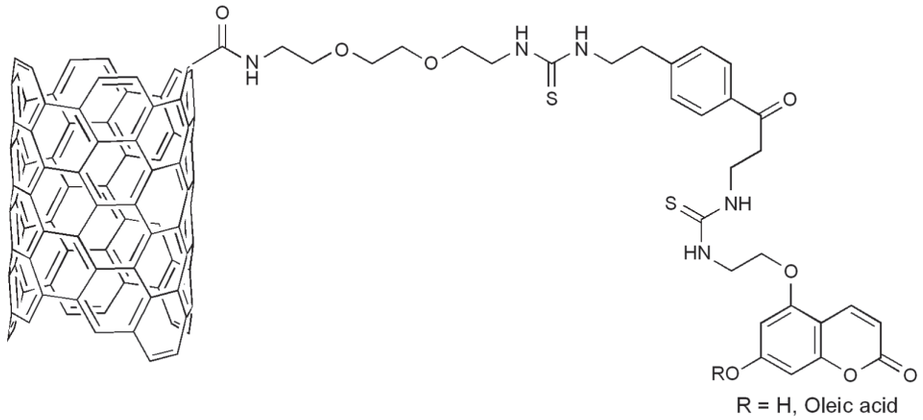

Fig. 1. Coumarin-conjugated MWCNTs.

allow an active recognition site at the surface of the nanocarrier.

2. EXPERIMENTAL DETAILS

2.1. Materials

Solvents were used as received from Carlo Erba Reagents; reagents were purchased from Sigma-Aldrich Co.; silica gel was purchased from Merck Chemicals. Sonication was carried out in a batch using Bandeling Sonorex Type RK52H equipment. Nuclear magnetic resonance spectra (^{1}H NMR recorded at 300 MHz, ^{13}C NMR recorded at 75 MHz) were obtained on Varian Instruments and are referenced in ppm relative to TMS or the solvent signal. Thin-layer chromatographic separations were performed on Merck silica gel 60-F_{254} precoated aluminum plates. Flash chromatography was accomplished on Merck silica gel (200–400 mesh). Preparative separations were carried out by MPLC Büchi C-601 using Merck silica gel 0.040–0.063 mm and the eluting solvents were delivered by a pump at the flow-rate of 3.5–7.0 mL min^{-1}.

The morphological characterization was carried out by SEM on a JEOL JSM 5600LV instrument, operating at 20 kV. HRTEM analysis was conducted on a 200 kV JEOL JEM 2010 analytical electron microscope (LaB6 electron gun) equipped with a Gatan 794 Multi-Scan CCD camera for digital imaging. UV spectra have been performed by Thermo Nicolet mod. Evolution 500 spectrophotometer. TGA analysis have been performed with a TA mod. SDTQ600 instrument. IR spectra were recorded on a Nicolet FT-IR Impact 400 D spectrometer.

2.2. Preparation of MWCNTs Acetyl Chlorides

2.2.1. Preparation of Pristine MWCNTs

MWCNTs were produced by the catalytic carbon vapor deposition (CCVD) and were subjected to purification according to previously reported procedure.[19] Briefly, after synthesis the silica support was removed by a solution of KOH (1 M) at 105 °C and the resulting material, washed with distilled water, was treated with a solution of HCl (1 M) in order to remove the remaining iron particles and subsequently washed with distilled water and dried at 200 °C to obtain the pristine MWCNTs wich were used in the subsequently functionalizations. The purity of pristine nanotubes was quantified by thermogravimetric analysis: pristine MWCNTs are stable below 800 °C with a weight loss ≈2%; thus the purity of synthesized CNTs was extimated >95% . All the diagnostics analyses were performed on purified samples.

2.2.2. Carboxylation of Purified MWCNTs

500 mg of pristine MWCNTs, sonicated in a water bath (60 W, 35 kHz), in 100 mL of sulfuric acid/nitric acid (3:1 v/v, 98% and 69% respectively) were stirred at 60 °C for 6 h. 500 mL of deionized water was then added and the mixture was decanted for 2 h and separated from the heavy MWCNT material. Then the supernatant (∼300 mL) was recovered, filtered under vacuum on a 0.1 μm Millipore membrane, carefully washed with deionized water until pH became neutral and dried. Light oxidized MWCNTs were obtained in a yield of 40%. Heavy oxidated MWCNT were recovered with a yield of 50%. Carboxylated MWCNTs were titrated to determine the concentration of acidic sites present according to literature method.[20] Briefly, MWCNTs were heated at 100 °C to remove carbon dioxide and water. Further carboxylated MWCNTs were added into 0.01 N sodium hydroxide and stirred for 48 h. The sample was centrifuged at 10,000 rpm for 15 minutes; unreacted NaOH was titrated with 0.01 N hydrochloridric acid. The number of free OH groups was found to be 1.8 mmol/g.

2.2.3. Acylation of Carboxylated MWCNTs

Carboxylated light MWCNTs (100 mg) were heated in 30 mL of neat oxalyl chloride at reflux for 48 h; the excess

of volatile reagent was removed under reduced pressure to give the corresponding MWCNTs acetyl chloride which were used without further purification.

2.3. Preparation of the Linker 5

2.3.1. Synthesis of 2-(1,3-dioxoisoindolin-2-yl)ethyl 4-(2-aminoethyl)benzoate 2

To a solution of 4-(2-aminoethyl)benzoic acid in a 1:1 mixture dioxane/water (10 mL) and in the presence of NaOH 1 M, di-*tert*-butyl dicarbonate was added and the mixture was left to stir for 1 h at r.t. After purification by flash chromatography using a mixture $CHCl_3$/MeOH (98/2), the protected compound was reacted with carbonyldiimidazole (CDI) in dioxane for 2 h at 100 °C; then,1 eq. of 2-(2-hydroxyethyl) isoindoline-1,3-dione was added and the mixture was stirred under reflux for 12 h. When the TLC revealed the absence of the starting compound, the solution was acidified at pH 5 using HCl 1 M. The mixture was dried under vacuo and the residue was washed with a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried and the residue was purified by MPLC using a mixture $CHCl_3$/MeOH 98/2 as eluent. The obtained compound as white solid (yield 70%) was deprotected using trifluoroacetic acid in CH_2Cl_2 for 12 h at r.t. The residue was then dried under vacuo and purified using a mixture CH_3Cl/MeOH 9 :1. **2-(1,3-dioxoisoindolin-2-yl) ethyl4-(2-aminoethyl) benzoate 2**, yellow oil, 70% yield. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.12 (t, 2H, $J = 6.5$ Hz), 3.28 (t, 2H, $J = 6.5$ Hz), 4.23 (t, 2H, $J = 4.2$ Hz), 4.69 (t, 2H, $J = 4.2$ Hz), 7.50–8.07 (m, 8H, Ar).

2.3.2. Synthesis of tert-butyl 2-(2-(2-isothiocyanato-ethoxy)ethoxy)ethylcarbamate 4

To a solution of diamine **3** (5 eq.) in dioxane (10 mL), 1 eq. of di-*tert*-butyl dicarbonate was added dropwise and the mixture was left to stir for 2 h in an ice bath. Then, water and chloroform were added; the organic layer was concentrated *in vacuo* and the residue was subjected to flash chromatography using as eluent a mixture $CHCl_3$/MeOH (98/2). The obtained monoprotected derivative (95% yield) was dissolved in ethanol (5 mL) and reacted with carbon sulfide (10 eq) in the presence of triethylamine (1 eq) for 30 min at 0 °C. Then, the mixture was cooled in an ice bath, added with 1 eq of di-*tert*-butyl dicarbonate and 2 mol% of DMAP and left under stirring at r.t for 1 h. The solvent was then removed under vacuo to obtain compound **4**, in quantitative yield which was used without further purification.

2.3.3. Synthesis of Linker 5

To a solution of compound **2**, in ethanol (10 mL) 1 eq. of compound **4** was added and the mixture was stirred at r.t. for 12 h, in the presence of 1 eq. of triethylamine. When the TLC revealed the absence of the starting materials, the mixture was filtered, the residue was washed with ethyl acetate and subjected to purification by MPLC using a mixture of $CHCl_3$/MeOH (98 :2) as eluent. The obtained N-protected compound was deprotected with TFA following the procedure reported for the synthesis of compound **2**. Linker **5** was purified by MPLC using a mixture $CHCl_3$/MeOH (9 :1).

2-(1,3-dioxoisoindolin-2-yl)ethyl 4-(2-(3-(2-(2-(2-aminoethoxy)ethoxy)ethyl)thioureido)ethyl) benzoate 5, light yellow oil, 74% yield. ^{1}H NMR (CD_3OD, 300 MHz) δ 2.94 (t, 2H, $J = 7.5$ Hz), 3.13 (t, 2H, $J = 5.0$ Hz), 3.58–3.75 (m, 12H), 4.06 (t, 2H, $J = 5.0$ Hz), 4.52 (t, 2H, $J = 5.0$ Hz), 7.30 (d, 2H, $J = 8$ Hz), 7.78–7.86 (m, 6H, Ar). ^{13}C NMR (CD_3OD, 75 MHz) δ 34.9, 36.7, 39.3, 45.5, 45.9, 61.9, 66.4, 69.1, 69.9, 70.5, 110.7, 114.7, 118.5, 122.8, 127.6, 128.7, 129.3, 129.4, 130.1, 130.9, 134.1, 145.7, 158.9, 165.3, 167.5, 168.0 (See Figs. 7 and 8).

2.4. Preparation of Coumarins 8 and 9

2.4.1. Synthesis of 5-oxyethyl- tert-butyl carbamate, 7-hydroxy coumarin 7

To a solution of 5,7-dihydroxy-coumarin[22] **6** in THF (20 mL), 2 eq of K_2CO_3 was added and the mixture was left to stir at r.t for 15 min; then, 1eq of *tert*-butyl-2-bromo-ethylcarbamate was added and the mixture stirred under reflux for 12 h. The solvent was then removed and the residue was subjected to purification by MPLC using a mixture of 98/2 $CHCl_3$/MeOH. **Tert-butyl 2-(7-hydroxy-2-oxo-2H-chromen-5-yloxy)ethylcarbamate 7**, yellow oil, 40% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.62 (s, 9H), 3.65 (t, 2H, $J = 5.5$ Hz), 4.22 (t, 2H, $J = 5.5$ Hz), 5. 20 (bs, 1H), 5.80 (bs, 1H), 6.25 (d, 1H, $J = 8.5$ Hz), 6.50 (s, 1H), 6.60 (s, 1H), 8.18 (d, 1H, $J = 8.5$)

2.4.2. Synthesis of 5-isothiocyanatoethoxy-7-hydroxy coumarin 8

Compound **7** was deprotected by reaction with 6 eq of trifluoroacetic acid in CH_2Cl_2 for 12 h at r.t. The solvent was then removed and the residue was subjected to flash chromatography using a 9 :1 mixture of CH_3Cl/MeOH. **5-(2-aminoethoxy)-7-hydroxy-2H-chromen-2-one**, yellow oil, 80% yield. ^{1}H NMR (CD_3OD, 300 MHz) δ 3.75 (t, 2H, $J = 5.5$ Hz), 4.62 (t, 2H, $J = 5.5$ Hz), 6.25 (d, 1H, $J = 8.5$ Hz), 6.60 (s, 1H), 6.62 (s, 1H), 8.45 (d, 1H, $J = 8.5$).

The purified deprotected compound was dissolved in ethanol (10 mL), treated with 10 eq of carbon sulfide in the presence of 1 eq of triethylamine and the mixture was stirred for 30 min at 0 °C. The mixture was then cooled on an ice bath and then, 1 eq of di- *tert*-butyl dicarbonate, dissoved in absolute ethanol (1 mL) and 2 mol% of DMAP was added. The mixture was left to stir for 1 h at r.t. Then, the solvent was removed under vacuum and the residue, represented by isothiocyanatoethoxy coumarin **8**

and obtained in quantitative yield was used without further purification.

2.4.3. Synthesis of 5-isothiocyanatoethoxy-7-oleate coumarin 9

To a solution of compound **7** in chloroform (20 mL) 1.2 eq. of Oleoyl chloride and 1.2 eq. of triethylamine were added and the mixture was then stirred for 12 h at r.t. The solvent was, then removed and the residue was subjected to purification by MPLC using a 98/2 mixture of $CHCl_3$/MeOH to obtain **(9Z)-5-(2-aminoethoxy)-2-oxo-2H-chromen-7-yl octadec-9-enoate** (*N*-Protected with tert-butyl carbamate), yellow oil, 70% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 3H, $J = 7.5$ Hz), 1.27 (m, 20H), 1.45 (s, 9H), 1.73 (t, 2H, $J = 7.2$ Hz), 2.01 (m, 4H), 2.18 (t, 2H, $J = 7.2$ Hz), 3.56 (t, 2H, $J = 4.2$ Hz), 4.10 (t, 2H, $J = 4.2$ Hz), 5.01 (bs, 1H), 5.35 (m, 2H), 6.29 (d, 1H, $J = 9.5$ Hz), 6.48 (s, 1H), 6.69 (s, 1H), 8.04 (d, 1H, $J = 9.5$ Hz). The obtained compound was deprotected with trifluoroacetic acid to give **(9Z)-5-(2-aminoethoxy)-2-oxo-2H-chromen-7-yl octadec-9-enoate**. ^{1}H NMR (CD_3OD, 500 MHz) δ 0.89 (t, 3H, $J = 6.9$ Hz), 1.27 (m, 20H), 1.75 (qt, 2H, $J = 7.2$ Hz), 2.05 (m, 4H), 2.60 (t, 2H, $J = 7.2$ Hz), 3.55 (t, 2H, $J = 4.2$ Hz), 4.20 (t, 2H, $J = 4.2$ Hz), 5.35 (m, 2H), 6.34 (d, 1H, $J = 10.0$ Hz), 6.77 (s, 1H), 6.81 (s, 1H), 8.32 (d, 1H, $J = 10.0$ Hz). ^{13}C NMR (CD_3OD, 125 MHz) δ 36.3, 38.1, 40.7, 63.3, 67.9, 70.5, 71.3, 71.4, 124.1, 124.3, 129.2, 130.1, 130.2, 130.8, 130.9, 133.3, 135.4, 135.5, 146.6, 167.8, 169.7 (See Figs. 9 and 10). The reaction with carbon sulfide, according to the above reported procedure, gave the corresponding isothiocyanatoethoxy coumarin **8**.

2.5. Preparation of Coumarin-Conjugated MWCNTs

To a mixture of MWCNTs acetyl chlorides (50 mg) in THF (20 mL), 10 eq of the linker **5** (calculated by Kaiser test[21] on MWCNTs-NH_2) and 10 eq of triethylamine were added and the mixture was sonicated for 15 min and then left to stir at reflux for 48 h. The solvent was then removed and the functionalized MWCNTs were filtered by using Durapore Membrane Filter 0.1 μm and washed

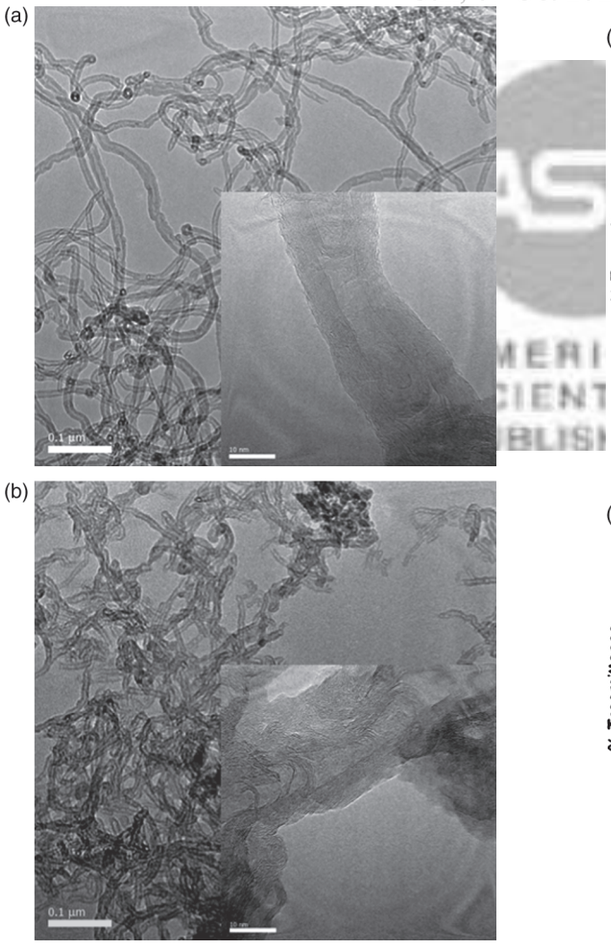

Fig. 2. Representative HRTEM images of a) pristine MWCNTs and b) oxidized MWCNTs.

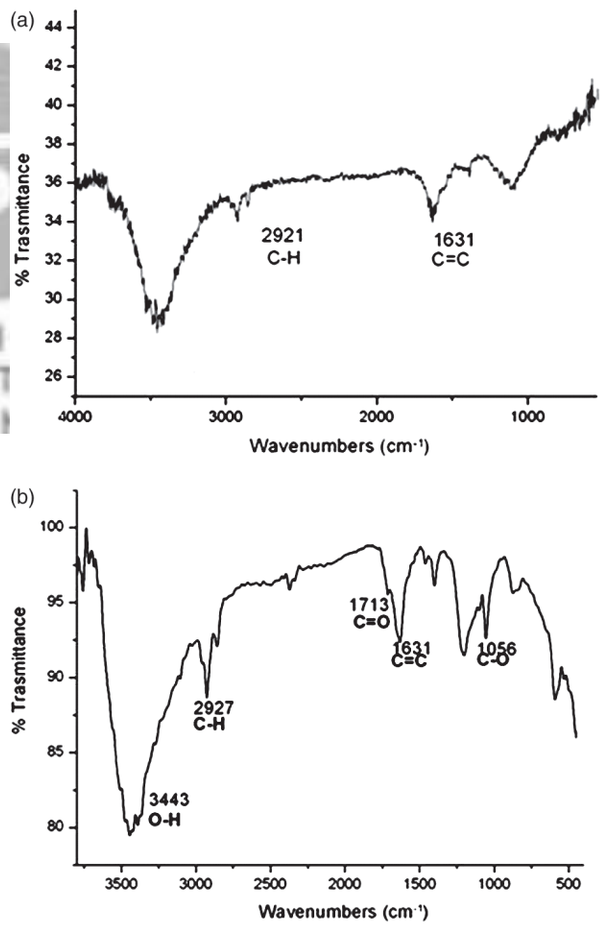

Fig. 3. FTIR Spectra of a) pristine MWCNTs and b) oxidized MWCNTs.

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with MeOH and $CHCl_3$ till disappearance of the starting material. The so functionalized MWCNTs (50 mg) were suspended in ethanol (20 mL) and then added with 10 eq of hydrated hydrazine and the mixture was stirred at reflux for 24 h. MWCNTs-linker-NH_2 dispersed in THF dry (20 mL) added with 10 eq of coumarin **8** or **9** were sonicated for 30 min and then left to stir at reflux for two days. The solvent was then removed and the functionalized MWCNTs were filtered by using Durapore Membrane Filter 0.1 μm and washed with MeOH and $CHCl_3$ till disappearance of the starting material.

3. RESULTS AND DISCUSSION

MWCNTs, produced by catalytic carbon vapor deposition (CCVD), after purification according to a previously reported procedure (purity >95%),[19] exhibit a clear multiwalled tube structure of 15–20 layers and the graphite layers do not stay always continuous along the growth orientation in some regions, which results from point defects and faults between graphitic carbon planes . Moreover, the HRTEM analysis revealed an average length of 11–20 μm, with a diameter close to 15–20 nm.

The pristine MWCNTs were oxidized with a mixture of sulfuric acid/nitric acid (3:1 v/v, 98% and 69% respectively) at 60 °C for 6 h. Then, deionized water was added and the supernatant was recovered, filtered under vacuum on a 0.1 μm Millipore membrane, carefully washed with deionized water until pH became neutral and dried. HRTEM images of pristine and oxidized MWCNTs show, for the latter, the presence of several damages on the side walls of nanotubes, as a consequence of the acid attack (Fig. 2(b)). Pristine and carboxylated MWCNTs were also characterized by FTIR spectra. The pristine MWCNTs show the C=C stretching vibration at 1631 cm^{-1} while for carboxylated MWCNTs representative additional C=O and C–O stretching vibrations

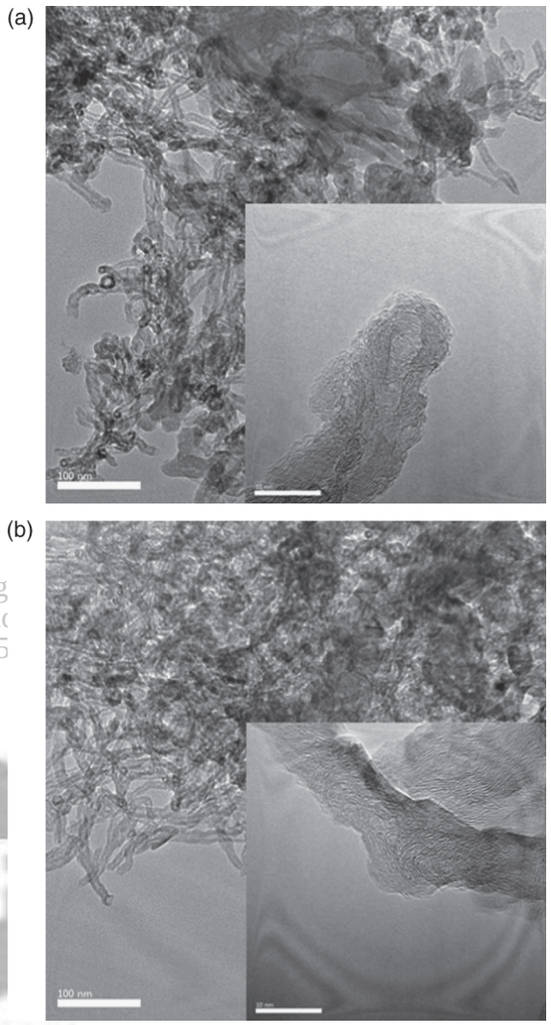


Fig. 5. Representative HRTEM analysis of MWCNTs conjugated with a) 7-hydroxy coumarin 8 and b) 7-oleate coumarin 9.

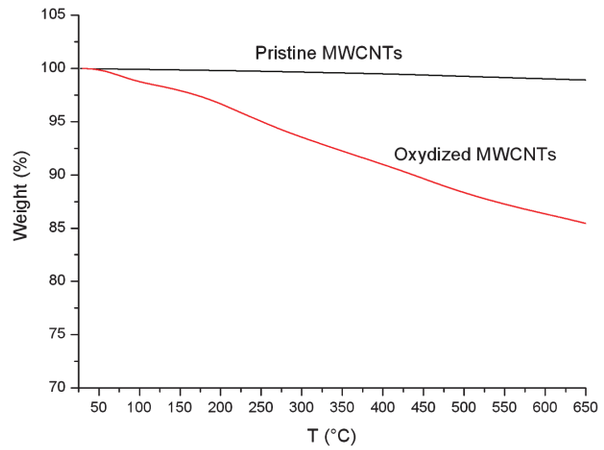


Fig. 4. TGA curves of pristineMCNTs, oxydized MWCNTs, MCNTs-Linker coumarin oleate conjugated MWCNTs and 7-OH-coumarin Conjugated MWCNTs.

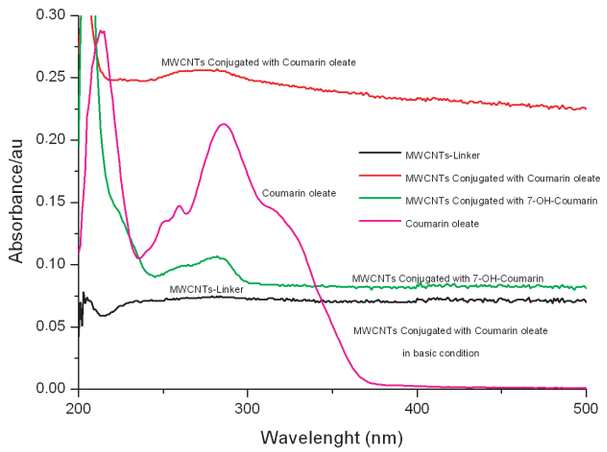


Fig. 6. UV spectra of MWCNTs conjugated with 7-oleate coumarin (red), MWCNTs conjugated with 7-hydroxy coumarin (green), MWCNTs-Linker (black) and 7-oleate coumarin (pink).

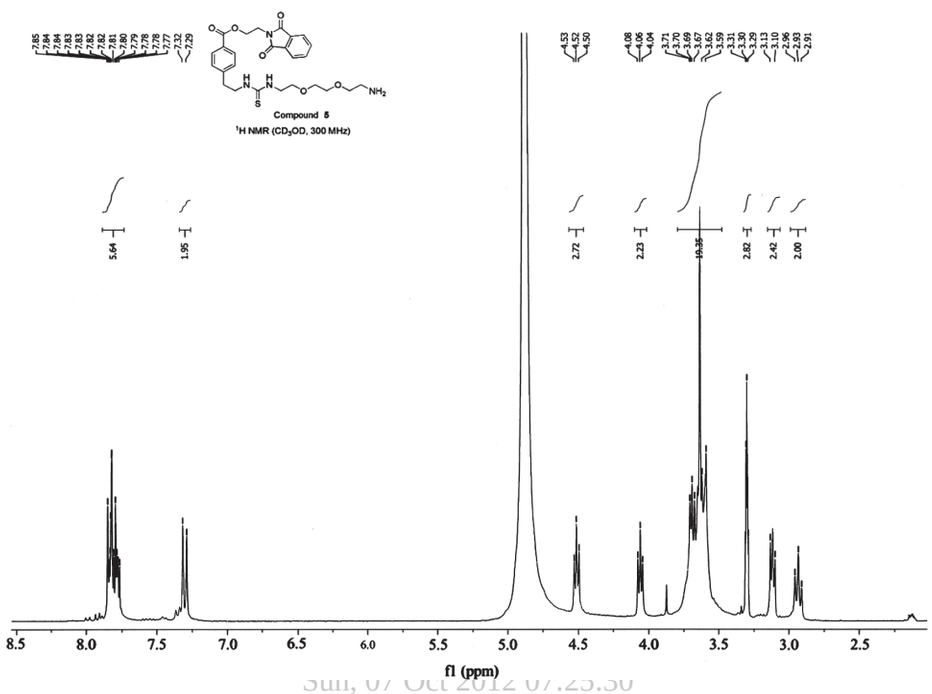


Fig. 7. ^{1}H NMR spectrum of compound 5.

are observed at 1713 and 1056 cm^{-1} respectively (Fig. 3). Moreover the peak at 3443 cm^{-1} could be ascribed to the O–H vibration (Fig. 3(b)).

The TGA results of oxidized MWCNTs reveal a weight loss attributable to the COOH groups, at 650 °C, under N_2 atmosphere, of ∼10%. The thermogravimetrical analysis of pristine and carboxylated MWCNTs was performed at 10 °C/min in temperature range from 30 to 650 °C. In the first stage, up to a temperature of 150 °C , a weight loss of approximatively 1% is detected for the highly hydrophilic acid-treated MWCNTs, which corresponds to the evaporation of the adsorbed water.

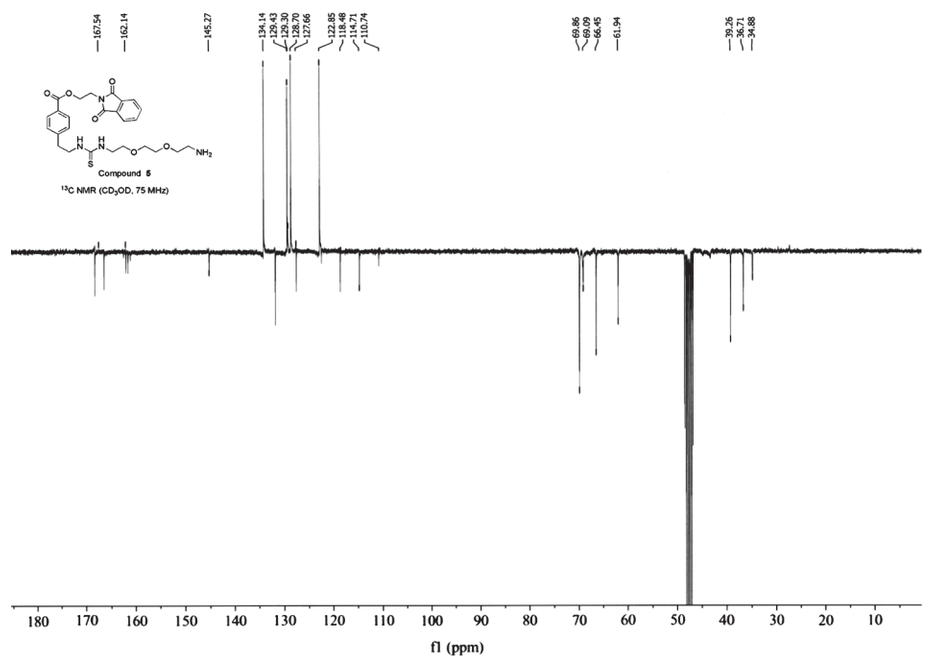
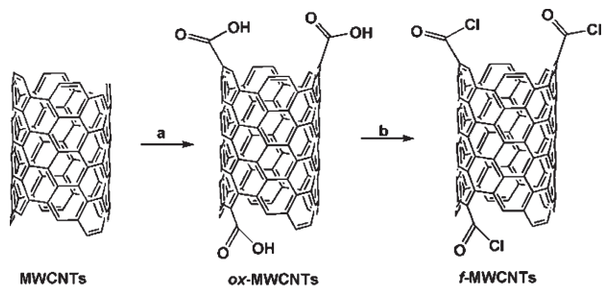


Fig. 8. ^{13}C NMR spectrum of compound 5.



Scheme 1. Reagents and conditions: (a) HNO_3/H_2SO_4 (1:3), 60 °C, 6 h; (b) $(COCl)_2$, reflux, 2d.

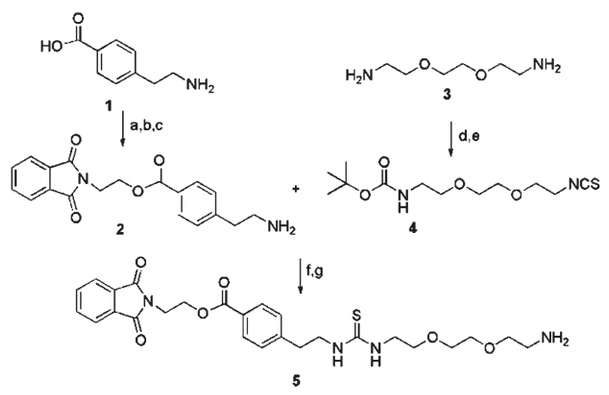
The second stage, from 150° to 350 °C is attributed to the decarboxylation of the carboxylic groups present on the MWCNTs walls. Thermal degradation in the range between 350 °C and 500 °C may be explained by the elimination of the hydroxyl functionalities. Finally, at the temperature higher than 500 °C, the observed degradation corresponds to the thermal oxidation of the remaining disordered carbon (Fig. 4).

Carboxylated MWCNTs were titrated to determine the concentration of the present acidic sites, according to literature method.[20] The number of free OH groups was found to be 1.8 mmol/g.

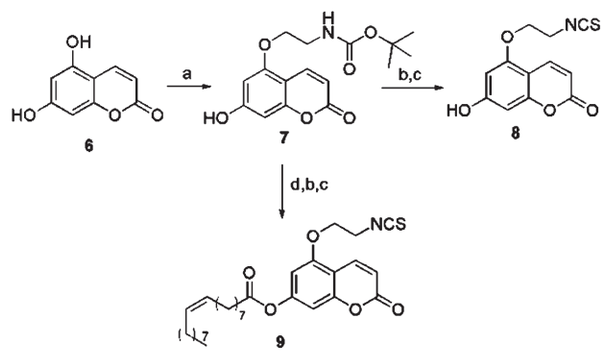
The purification and the subsequent oxidation of raw MWCNTs allowed the removal of catalyst impurities and graphite particulates, so allowing the effective organic functionalization on the selected carbon nanotubes.

Oxidized MWCNTs were heated in 30 mL of neat oxalyl chloride at reflux for 48 h in order to obtain the corresponding MWCNTs acetyl chlorides which were used without further purification (Scheme 1).

Coumarins were coupled with MWCNTs by means of the linker **5** which has an amino group able to react with acyl MWCNTs. The synthesis of the linker starts from 4-(2-aminoethyl)benzoic acid **1** according to the synthetic



Scheme 2. Reagents and conditions: (a) Boc_2O, dioxane/water, NaOH 1M, r.t. 1 h; (b) CDI, dioxane, 100 °C, 2 h, then 2-hydroxyethylphtalimide, 100 °C, 12 h; (c) TFA, CH_2Cl_2, r.t., 12 h; (d) Boc_2O, $CHCl_3$ dropwise for 2 h, then r.t, 24 h,; (e)CS_2, ETA, EtOH, Boc_2O, DMAP, r.t., 1 h; (f) EtOH, ETA, r.t., 12 h; (g) TFA, CH_2Cl_2, r.t., 12 h.



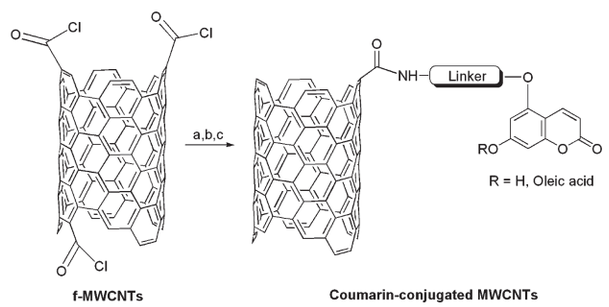
Scheme 3. Reagents and conditions: (a) *tert*-butyl 2-bromoethylcarbamate, K_2CO_3, reflux, 12 h; (b) TFA, CH_2Cl_2, r.t., 12 h, c) CS_2, ETA, EtOH, Boc_2O, DMAP, r.t., 1 h; (d) Oleoyl chloride, ETA, $CHCl_3$, r.t., 12 h.

procedure reported in Scheme 2. Coumarins **8** and **9**, where the C-5 position is substituted with the isothiocyanatoethoxy group, necessary for the reaction with the amino group of the linker, were synthesized from the 5,7-dihydroxy-coumarin **6**.[22]

Treatment of **6** with *tert*-butyl 2-bromo-ethylcarbamate affords the key intermediate **7** which was converted into the corresponding 5-isothiocyanatoethoxy-7-hydroxy coumarin **8** and 5-isothiocyanatoethoxy-7-oleate coumarin **9** following the procedure reported in Scheme 3.

Finally, MWCNTs acetyl chlorides were conjugated with coumarins **8** and **9** by means of the linker **5** (Scheme 4).

These two orthogonal protecting groups of the amino functionalities in compounds **2** and **4**, were removed and employed selectively by using different reaction conditions. The Boc group was cleaved after the coupling of **2** to **4** by treatment with HCl in dioxane. The obtained compound **5** was reacted with MWCNTs acetyl chlorides and then the Pht protecting group was removed using a solution of hydrazine in ethanol at room temperature. The quantity of the free amino groups (1.5 mmol/g) was measured with a quantitative Kaiser test.[21] The coupling of coumarins **8** or **9** with the NH_2 moiety presented on MWCNTs was performed by reaction in DMF for two days at room temperature. HRTEM analysis of



Scheme 4. Reagents and Conditions: (a) 5, ETA, THF, reflux, 48 h; (b) hydrazine, EtOH, reflux, 24 h; (c) 8 or 9, DMF, r.t., 2d.

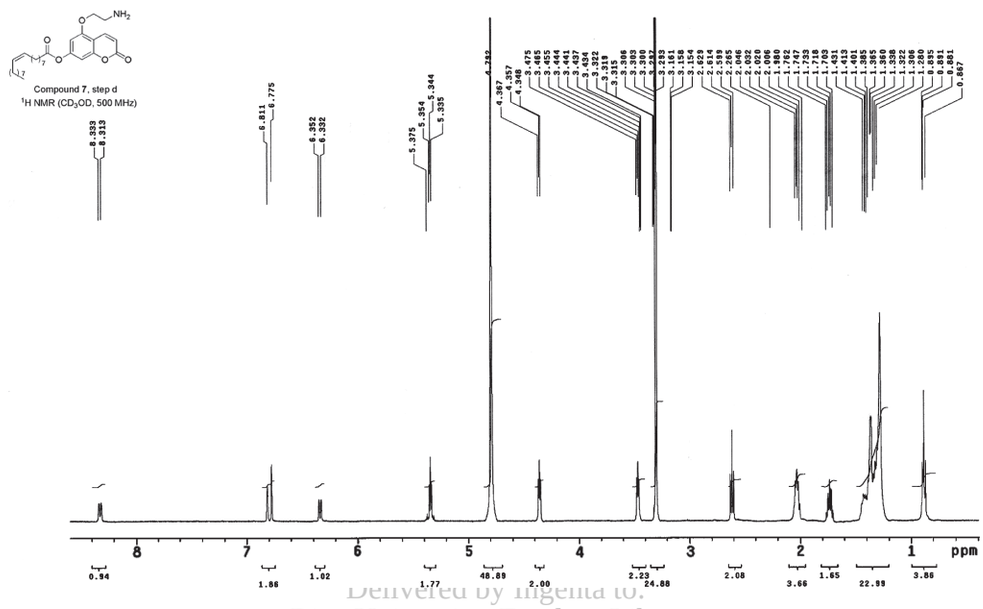


Fig. 9. ^{1}H NMR spectrum of compound 7 (step d).

coumarin-conjugated multiwalled carbon nanotubes show the presence of an additional material on the surface of nanotubes (Fig. 5). This new material is reasonably attributable to the presence of new molecular aggregates covalently bonded to the nanotubes. This material is more considerable for MWCNTs conjugated with coumarin **9**, because of the presence of the oleoyl moieties.

The UV spectra confirm the presence of coumarins covalently bonded to the nanotubes. MWCNTs conjugated with 7-oleate coumarin and 7-hydroxy coumarin show an absorbance at 240–300 nm, while no absorbance was detected for the MWCNTs functionalized with the linker; a different spectrum was registered for isothiocyanatoethoxy-7-oleate coumarin **9** (Fig. 6).

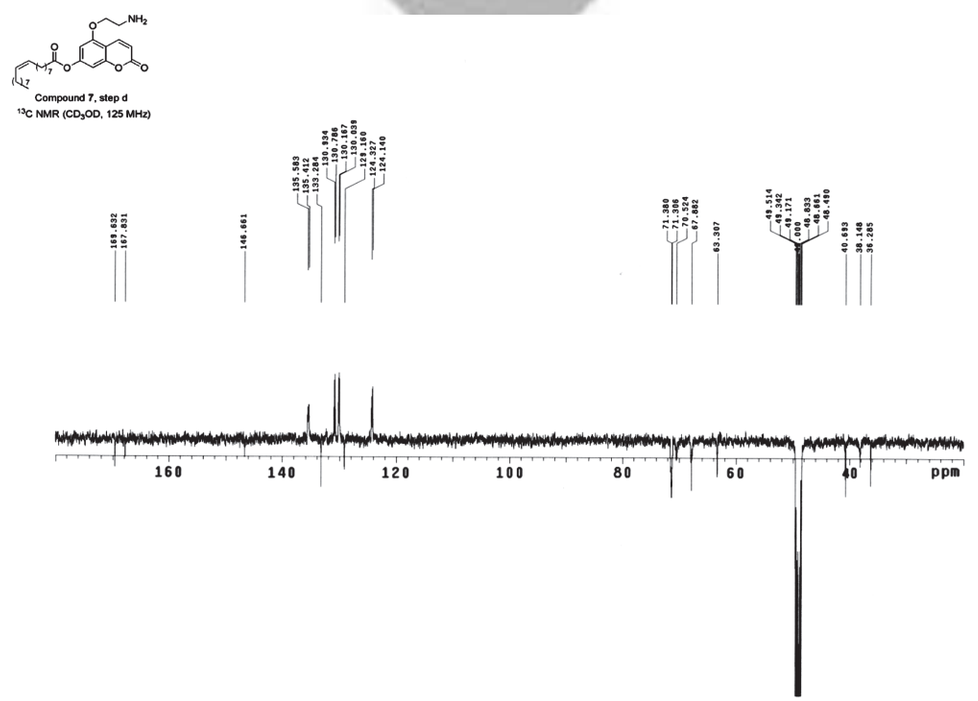


Fig. 10. ^{13}C NMR spectrum of compound 7 (step d).

4. CONCLUSIONS

The use in biomedical applications of CNTs requires a clear assessment of safety and bioeffects of CNTs to the environment and human health. Pristine CNTs are practically insoluble and tend to accumulate inside cells, organs ant tissues and, in addition, it was reported that single walled CNTs show an adverse effect on keratinocytes through an oxidative mechanism, leading to NF-kB activation. A useful strategy appears to be the effective functionalization of CNTs with coumarins, known superoxide anion scavengers, in order to overcome the poor dispersibility and toxicity of pristine carbon nanotubes.

The current work present a rational approach to functionalize MWCNTs that could be further used in biomedical applications. The work summarizes sequential functionalization of MWCNTs via purification, carboxylation, acylation, amine modification and, finally, coumarin conjugation. The various data (TEM, TGA, FTIR, UV/Vis, etc.) proved that successful functionalization has been carried out. Moreover, being coumarins spectrophotometer detectable, their conjugation with CNTs, beside the improvement of the biocompatibility, could allow an active recognition site at the surface of the nanocarrier.

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