THE ANTIOXIDANT ACTIVITY OF THE BIOHYBRIDES BASED ON CARBOXYLATED / HYDROXYLATED CARBON NANOTUBES-FLAVONOID COMPOUNDS

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This study reveal the relationship between SWCNTs (SWCNTs, carboxylated respective, hydroxylated) with a series of flavonoids (quercetin, rutin) to design new bionanohybrids with higher antioxidant capacity. The chemiluminescence assay proved that the carbon nanotubes have antioxidant activity as electron donor or as proton donor (SWCNTs - hydroxylated and carboxylated) in the redox transition reaction with free radicals. The biohybrids "flavonoids- carbon nanotubes" shown a high antioxidant activity, evaluated by the amplification factor, ranging from 3 up to 4 times higher than single flavonoids. These results open the perspective to design functional carriers based on carbon nanotubes to transport low soluble antioxidants to specific target with improved scavenging rate against the free radicals (reactive oxygen species)

1. Introduction

The carbon nanomaterials once discovered they are seen as potential support in biomedicine, therapeutics, food, drug delivery, sensors. The oldest application of the carbon materials in medicine is the active carbon (medicinal charcoal), known by the ancient civilizations as absorbent against acidity or as food supplement. Each nanocarbon, after discovering, was used in bio-applications with hope to find solution to improve health, probes for the materials investigation or biocompatible nanodevices. So, since the discovery of fullerenes or 'buckyballs' (C60) in 1985 [1] and the carbon nanotubes (CNT, 1991) [2], a large class of applications were envisaged and their principles are demonstrated in biomedicine and pharmacology [3,4,5]. The uniqueness comes from its geometry and structure: single- or multi-rolled graphene (polyaromatic with large delocalized π-electron systems) reaching few nanometers in diameters and length up to a few micrometers. They could have unusual toxicological properties even though the data obtained *in vitro* systems are not conclusive [6,7,8]. For systems *in vivo* some acute inflammatory pulmonary effects in rodents were observed [9]. In the drug delivery, CNTs are used as carrier loaded with suitable drugs via functional groups. In this respect, new controlled drug delivery systems have devised such as delivery of therapeutic agents to the desired site, enhancing bioavailability and drug protection to name few in the nanotherapeutic area [10, 11, 12]. On the other hand new nanoscale materials have been investigated for drug delivery applications including: nanoparticles, nanotubes, nanofibers, dendrimers, liposomes, polymer micelles, nanogels, nanocrystals, viral vectors, and viruslike particles [13,14,15]. Despite of fast advances with nanomaterials in medicine, their toxicolo-

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gy remains a challenge to further developments in the evaluation of the adverse health effects and the environmental damage [16,17] or to improve the pharmacological profiles [5a, 12a, 18]. In re-thinking the nanotherapeutics with nanoparticles a special field is killing of the reactive oxygen species (ROS) using antioxidants to reduce oxidative stress against human body. The human body is exposed to an increasing oxidative stress, one of the harmful key issues in health care where excess of ROS (chemical species with unpaired electrons on the molecular orbitals, well-known free radicals) generated in various pathogenic processes are recognized as an indicator in citotoxiciy and cellular disorder [19]. At the intracellular level, ROS are balancing by the biochemical antioxidants such as Glutathione [20]. During the inflammatory processes from endogenous/ exogenous factors takes place overproduction and releasing of intracellular ROS with depletion in antioxidants. The supplements are taken from vegetables, which have a large complex of antioxidants, today used in the food processing or as nutraceuticals (commodities derived from food, used in the medicinal form of pills, capsules, potions and liquids with physiological benefits) [21]. To increase the antioxidant potential delivered to a target need carriers with molecular recognition and this could be the carbon nanostructures [12 22]. Earlier studies with fullerene shown a strong antioxidant activity [23] and this has been exploited in nanocosmetics for the skin care and dermatology [24]. Moving us toward carbon nanotubes it is possible to conceive new advanced antioxidants in combination with well-known natural polyphenols compounds which themselves have various antioxidant properties [25]. In the polyphenols class the flavonoids hold a privileged position. The pharmacological studies have proved that flavonoids possess many beneficial effects on the human health, including cardiovascular protection, anticancer activity, antiulcer effects, anti-allergic, antiviral, and anti-inflammatory properties [26]. In addition the flavonoids are important components in the human diet, although they are generally considered as non-nutrients. Sources of flavonoids are foods, beverages, different herbal drugs, and related phytomedicine [27]. Besides flavonoids exhibit various biological activities, however, most interest has been devoted to the antioxidant activity, which is due to their ability to scavenge free radicals involved in most of diseases [25a]. The important structure-activity relationships of the antioxidant activity and the capacity of flavonoids to act as antioxidants in vitro, have been established and related to the number of hydroxyl groups in their molecular structures [28].Regarding the free radicals inhibiting or direct interaction with DNA, enzymes and membrane receptors and hence the antioxidant efficacy of flavonoids in vivo, are aspects less documented, presumably because of the limited knowledge on the bioavailability and mechanisms of action in human body [29, 30]. Based on these pros and cons arguments in this study of the antioxidant activity related to the oxidative stress the opportunity to a deeper understanding via carbon nanotubes properties can be a good challenge. In a general scheme of the antioxidant activity deals with redox transitions from free radicals (R) to the antioxidant molecules (AH) by electron or hydrogen ($H^+ + e^-$) donating. SWCNTs, hydroxylated and carboxylated can perform these redox transitions by both mechanisms (scheme1 a) in similar way as AH or by neutralizing with forming of intermediate byproducts (SWCNT-COOR). When SWCNTs are functionalized with antioxidants, AH, the free radicals neutralizing can be performed by both compounds in a synergic way, here denoted with (SWCNT-OH-A)* or (SWCNT-COOH-A)* made of orto-quinone from AH and –C=O respective -COOR from nanotubes. In inset (scheme 1) are represented the direct redox transition for two representative flavonoids (quercetin and rutin) into a less active "orto-quinone" compound [31]. This contribution presents designing of biohybrids with quercetin (Q) and rutin (R) supported on SWCNTs, hydroxylated (SWCNT-OH), carboxylated (SWCNT-COOH) using high density ultrasonic field excitation with subsequent co-precipitation in dimethyl sulfoxide (DMSO- solvent compatible with the body fluids). DMSO is appropriate solvent for Q and R (Q is insoluble in water and R-partial soluble) and good dispersant for SWCNTs. In addition, in high density ultrasonic field takes place a large amount of bubbles from the cavitation effect where the solvent molecules gain high kinetics energy which by mechanical collision amplifies the nanotubes dispersion and the excitation of the solute molecules, favorable mechanisms to contribute to functionalizing without any intermediary process. If water is used instead of DMSO is possible to reduce carboxyl or hydroxyl groups due to generation in the cavitation bubbles very reactive free radicals such as –OH, dioxygen anion (O_2^-) and H^+ [32], deleterious for antioxidants and SWCNT-OH/COOH. The antioxidant activity for systems SWCNTs- Quercetin and Rutin was evaluated by chemiluminescence assay and first time demon-

strated *in vitro* the capability of the nanotubes to amplify the scavenging rate related to a given free radical generator (-OH) from the hydrogen peroxide decomposition.
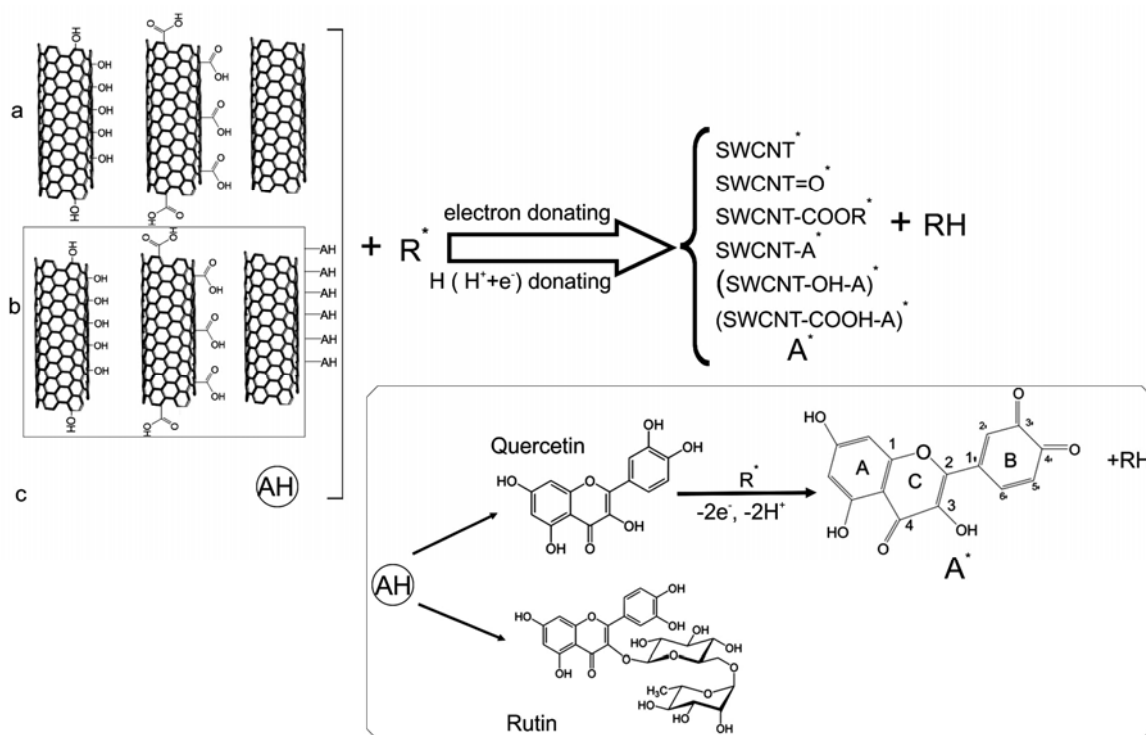
Scheme 1 The principle of the scavenging free radicals (R*) by redox transition reaction via electron donating or H (H$^+$+ e$^-$) donating from a) SWCNTs, SWCNT-COOH, SWCNT-OH b) SWCNTs decorated with antioxidants (AH) c) AH- antioxidants. Inset: two flavonoid representatives, Quercetin and Rutin (a glycoside between flavanol quercetin, the aglycon, and disaccharide-rutinose [33]). The mechanisms of the scavenging radicals as explained in [31] with conversion in less reactive orto-quinone (see comments in text).

2. Experimental details and comments

2.1. Materials

Carbon nanotubes characteristics: SWCNTs, SWCNT-OH, SWCNT-COOH, purchased from Shenzhen Nanotech Port Co. Ltd (NTP) Physical characteristics and chemical composition are given in table 1.

Table 1: Physical and composition characteristics for SWCNTs (based on supplier data)

Products	Diameter	Length	Purity (%)	Ash (%)	Specific Surface Area (m^2/g)	Carboxyl ratio (%)wt	Hydroxyl ratio (%) wt
SWCNT	<2nm	5-15um	>95%	< 5%	500-700	N/A	N/A
SWCNT-COOH	<2nm	5-15um	>97	< 2	N/A	2.31*	N/A
SWCNT-OH	<2nm	5-15um	>97%	<2	N/A	N/A	2.97**

*The rate of surface carbon atom carboxylated : 8-14mol%
**The rate of surface carbon atom hydroxylated : 6-8mol%

Our supplemental investigation using TGA and Chemical analysis (CNOS) are in agreement with data supplied in table 1. Raman spectroscopy (Raman spectra were recorded by Jasco NRS 3100 equipment with dual laser beams, 532 and 785 nm, resolution 4 cm^{-1}, configuration , backscattering) shows SWCNTs with radial breathing mode at 260 cm^{-1} (close to 2nm diameter counted from ref [34] and figure 2 from AFM topography). G bands, associated with the tangential modes of vibrations in the aromatic rings (figure 1) are centered on 1584 cm^{-1} for all SWCNTs but D bands and other fingerprints are quite different. SWCNT has D band at 1220 cm^{-1} and a small peak at 1308 cm^{-1}. The D modes correspond to the first order resonance Raman scattering process that reflects the presence of defects on the nanotubes body [35]. The two bands reflect the presence of two types of defects which vanish for SWCNT-OH and reinforce only one type in SWCNT-COOH, at 1308 cm^{-1}. At low Raman shift wavelengths are several fingerprints assigned to other disorder carbon forms, also from defects sites which alter π-continuum structure. At the defect sites, bonds are formed through alteration of the carbon body structure with introduction of pentagons and heptagons, known as Haeckelites (in Raman spectra localized in region 1000- 1100 cm^{-1} [36]).

These defect sites usually come from cleaning process using sulfuric/ nitric acid wet chemistry. Then the carbon nanotubes become more reactive at these defect sites, consequently, more prone to functionalizing respective to catch the free radicals on their body. In SWCNT-OH the small band at 794 cm^{-1} can be assigned to the bending for –OH and 877 cm^{-1} in association with 680 cm^{-1} are assigned to –COOH groups (indexed with KnowITAll ® Informatics systems, BioRad Laboratory) in SWCNT-COOH. The Haeckelites sites are insignificant in carboxylated respective hydoxylated carbon nanotubes and can be associated with subsequent chemical treatments to induce carboxyls and hydroxyl groups.
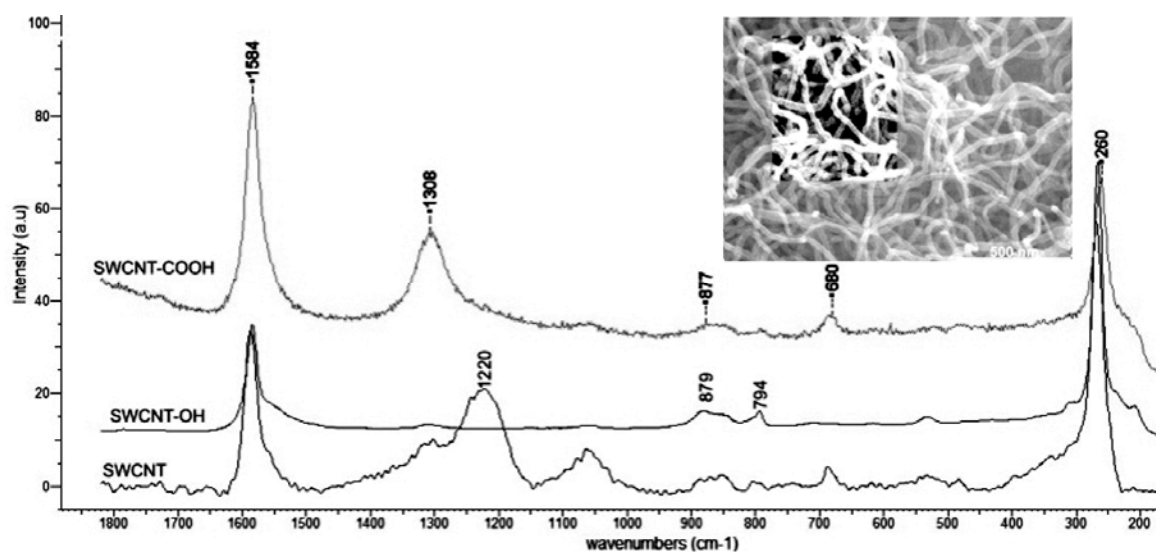

Fig. 1 RAMAN spectra for SWCNT, SWCNT-OH, SWCNT- COOH, in inset SEM micrograph (scale 500nm) for pristine SWCNTs as received. Haeckelites defects (see text) for SWCNTs are in range 1000-1100 cm^{-1}, hydroxyl groups assigned at 794 cm^{-1} (-O-H bending), carboxyl groups assigned at 877 cm^{-1} and 690 cm^{-1} (indexed with Know ITAll software).

Flavonoids: rutin, quercetin (Sigma-Aldrich);
Reagents for chemiluminescence: Luminol (disodium salt 5-amino-2,3-dihydro-1,4-phthalazine-dione), 3 wt % hydrogen peroxide, buffer TRIS-HCl, (Sigma Aldrich).
Solvents: DMSO (Sigma-Aldrich, analytical grade);
2.2 Equipments
Chemiluminometer (Sirius Luminometer Berthelot - GmbH Germany): for antioxidant activity mea-surements by chemiluminescence technique (CL).

Ultrasound system: Ultrasonic processor, UIP 1000W (Hielscher - Ultrasound Technology) frequency 20 kHz, sonotrode amplitudes up to 170 micron, liquid pressures up to 10 bars. The sonotrode horn with diameter 22 mm provides a high ultrasonic field density $\sim 260W/cm^3$ (measured by calorimetric method).

Atomic Force Microscopy: NTEGRA PRIMA Platform (NT-MDT) for study of topography at molecular scale, tip NGS01, tapping operating mode.

2.3 Methods

a) Functionalizing carbon nanotubes- flavonoids. The SWCNTs are functionalized with 10% wt flavonoids by excitation in ultrasonic field using DMSO as solvent in two steps. Briefly 0.9 mg SWCNTs in 5ml DMSO and 0.1 mg falvonoids in 5 ml DMSO are apart ultrasonicated for 4 min on cold water bath at 10^0C (to avoid overheating over $50\ ^0C$ and the biomolecules degradation). In the next stage the solutions are merged and ultrasonicated for another 4 min. The mixture was left overnight at room temperature in nitrogen-glove box to perform co-precipitation between nanotubes and flavonoids. The precipitate was extracted by centrifugation at 2000g and dried at 60^0C for few days up to constant weight under inert atmosphere.

All samples are indexed as SWCNT, SWCNT-OH (hydroxylated), SWCNT-COOH (carboxy- lated), for quercetin (Q, SWCNT-Q, SWCNT-OH-Q, SWCNT-COOH-Q) and rutin (R, SWCNT-R, SWCNT-OH-R, SWCNT-COOH-R).

b) The chemiluminescence assay. There are lots of generators of free radicals but the system luminol - H_2O_2 at pH>8.5 lead to high reactive oxygen species to be neutralized by the flavonoids at a given concentration. The rate of the chemiluminescence quenching is direct related with the flavonoid structure and its capacity of scavenging being a measure of the capacity to reduce free radicals.

The antioxidant activity counted by the scavenging rate (SR%) of the flavonoid- carbon nanotubes was evaluated by chemiluminescence assay [CL] using the method described elsewhere [37]. Briefly the chemiluminescent system is made of luminol- H_2O_2- TRIS-HCl and the scavenging rate is defined as:

$$\%SR = \frac{I_0 - I_s}{I_0} \cdot 100 \tag{1}$$

where: I_0 = CL intensity in the absence of samples at t = 5s; I_s = CL intensity for sample at t = 5s. In case of the biohybrids systems the active principles can be activated or inhibited depending of the carbon nanotube support. Based on this assumption was defined the amplification factor:

$$F_a = \frac{SR(\%)}{\dfrac{m_{SWCNT}}{m} SR_{SWCNT}(\%) + \dfrac{m_f}{m} SR_f(\%)} = \frac{SR}{SR^*} \tag{2}$$

where SR is the measured scavenging rate and SR*- the additive scavenging rate counted as individual contribution proportional with their concentration, m is the biohybrid weight, m_{SWCNT} & m_f are weights of SWCNTs and flavonoid.

3. Results and discussions

3.1. The Chemiluminescence assay

The antioxidant activities for SWCNTs and SWCNTs - flavonoid coumpounds are summa-rized in figure 2
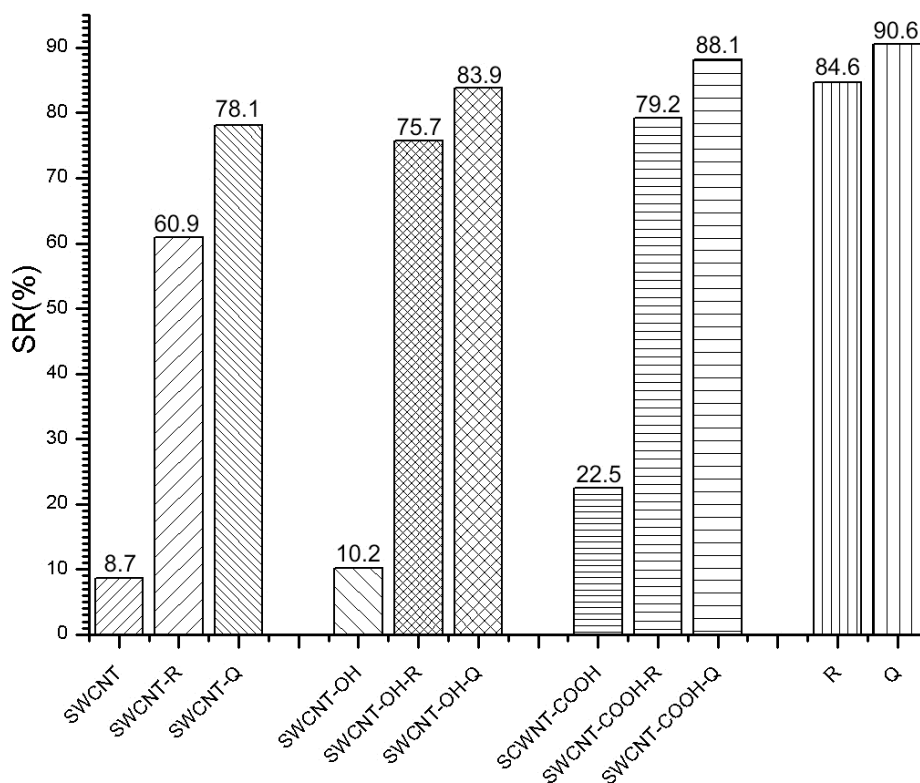
Fig. 2. The scavenging rate for SWCNTs and biohybrids with quercetin and rutin

At the first sight the carbon nanotubes in pristine form have a notable antioxidant activity increasing from SWCNT< SWCNT-OH< SWCNT-COOH. These results can be assigned to the – OH and – COOH groups which can perform the scavenging free radicals as proton donating. As well SWCNTs have an appreciable antioxidant activity (8.7 %, figure 2) and this can be assigned as electron donating from defect sites (SWCNT[*], scheme 1) or from "continuum π-conjugated structure". In this case SWCNTs can be seen as "collector of free radicals" via a single step reaction: SWCNT+R[*]$\rightarrow$ SWCNT-R. These results are in agreement with the basic concept related to the antioxidant activity of the flavonoids and polyphenolic compounds The antioxidants (AH) are involved in redox transitions with single electron donating (or H atom, equivalent with donating of a proton and an electron) to the free radical species (R*) in a general scheme: AH+ R*$\rightarrow$A*+RH, i.e the free radicals are neutralized resulting less or weakly active byproducts [38]. Unexpected results come from biohybrids: only 10 % flavonoids selfassembled on SWCNTs lead to a high antioxidant activity, close to the 100% rutin or quercetin scavenging rate. In table 2 are summarized the amplification factors for each biohybrid taken in account the additive contribution related to the experimental values (eq 2). The higher Fa values are for byohybrids made of SWCNTs and SWCNTs-OH with R and Q. That shows that the reaction kinetics to donate an electron is amplified by SWCNTs respective to donate protons is amplified by the hydroxyl groups and less by the acidic groups (-COOH). Therefore, the biohybrids work in synergic way bringing together all delocalized π-electron system to annihilate the hydroxyl radicals in case SWCNTs with R and Q. For SWCNTs-OH with R and Q it is expected as all –OH groups from molecules and nanotubes to be consumed in the free radicals annihilation.

Table 2- The amplification factor, F_a estimate with (2) using data from Fig. 2

Sample	SR* (%)	SR (%) (experimental value)	Fa
SWCNT-R	16.29	60.9	3.73
SWCNT-OH-R	17.64	75.71	4.29
SWCNT-COOH-R	28.71	79.21	2.75
SWCNT-Q	16.89	78.09	4.62
SWCNT-OH-Q	18.24	83.89	4.59
SWCNT-COOH-Q	29.31	88.11	3.006

*See equation 2

3.2 AFM Topography

Complementary techniques such as electron microscopy and atomic force microscopy are also very helpful in elucidating the structure and topography of the biohybrids or other drug delivery systems based on carbon nanotubes targeting even cancer therapy [39]. In this respect, AFM images reveal a series of specific biohybrid features: they are made of single tubes or in small bundles covered with flavonoids in a proper way due to specific interaction induced by the functionalized method. Function of type and level of defects existing in pristine CNT the droplets have various sizes but the tendency is to cover large area on its surface or to be localized on the specific sites.

Figs. 3 and 4 show the quercetin and rutin distribution around of "nanotube wires". Quercetin selfassembled on SWCNT is distributed in small aggregate droplets in specific locations along nanotube. which can be associated with defect sites (figure 3 SWCNT-Q). Also rutin selfassembled on SWCNTs is aligned in larger droplets along of tubes located on defect sites. The difference is in that quercetin is more disperse along carbon nanotubes and rutin is more localized due to the steric interactions with glycosides from aglycon. For SWCNT-OH,: quercetin and rutin are distributed in randomized droplets due to electrostatic interaction between hydroxyl groups but still with a high coverage on the carbon nanotubes. SWCNT –OH-Q (Figure 3) has a distribution along of nanotubes but very localized on the defect sites and on the ends of carbon nanotubes. SWCNT-OH-R is larger distributed along of nanotubes in very thin droplets encompassing the carbon nanotubes. By comparison with figure 2 where the scavenging rate is higher for quercetin than rutin a simple conclusion come from synergic activity between electron donating and proton donating mediated by hydroxyl groups. In case SWCNTs-COOH the main feature is aggregates of droplets (Figure 3 and 4) with distinctive features: Q-droplets are well localized and R- droplets are arranged in forms of stacked scales.
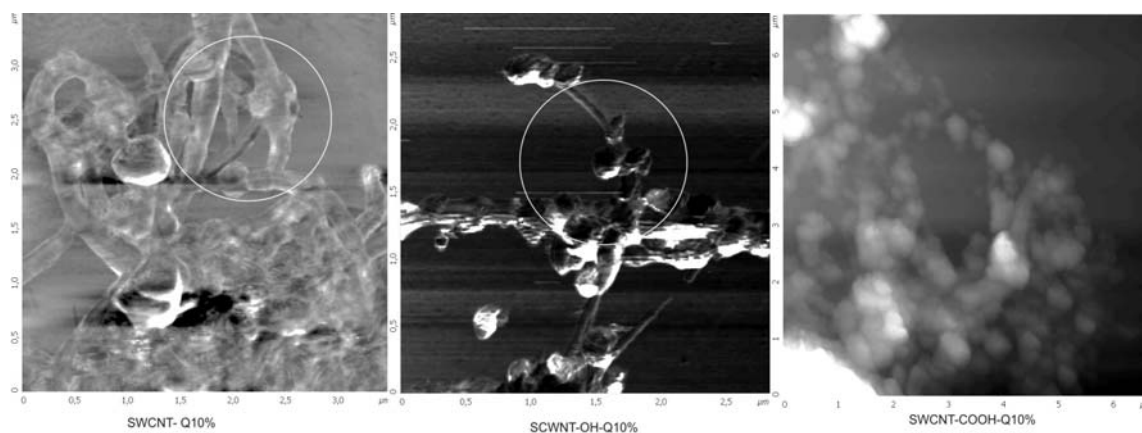
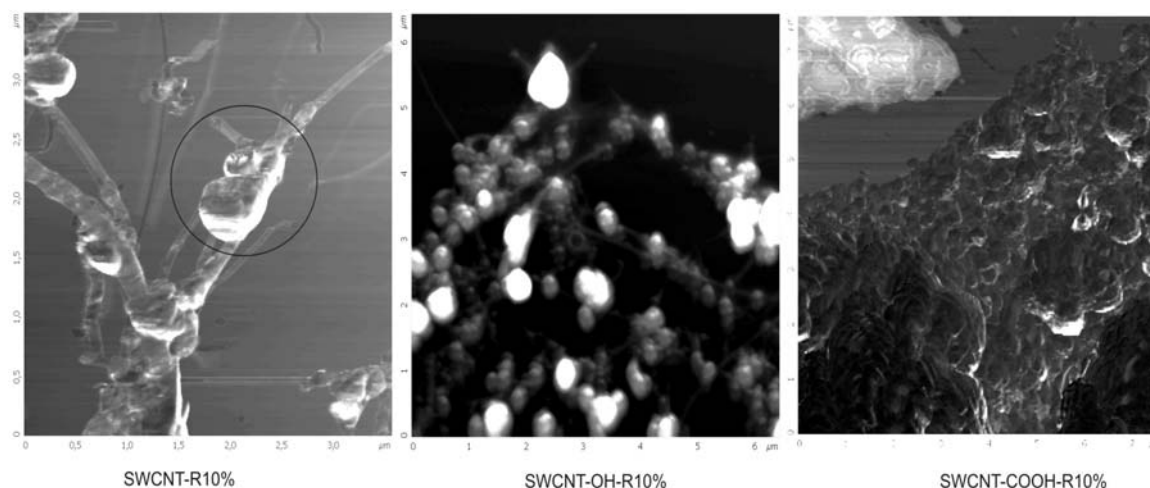*Fig. 3. SWCNTs decorated with Quercetin 10%*

Fig. 4 SWCNTs decorated with Rutin

These features reinforce several general assumptions: a) The biomolecules are localized on the defect sites via π-π bonding followed of Vander-Waals stacking in small bubble aggregates; b) High specific surface area is a dominant factor to increase the scavenging rate in combination with synergic contribution from delocalized electronic system and –OH functional groups; c) The acidic groups seems to improve the nanotube contribution to the amplification factor playing a role of proton donating; d) The conformational behavior, molecular geometry and electronic structure of quercetin and rutin as well the steric effects are contributors to the synergic antioxidant activity nanotubes- flavonoids; e) Quercetin has a nonplanar molecular structure, with cross-conjugation occurring at the C ring (see scheme 1) which in combination with π- electron conjugation system from carbon nanotubes can work as amplified redox transitions; f) The OH groups from Q and R (ring B, scheme 1) are in the same levels of energies therefore, the antioxidative process mechanisms, exerted by Q and R as a free radical scavenger, relies on two isoenergetic radicals (scheme 1) with extended electronic delocalization between adjacent rings from carbon nanotubes [30d]. g) –OH groups are very important in the scavenging rates as proton donating but are not a universal conjecture in the antioxidant activity. The –OH position in flavonoids and its strain by the other functional groups in whole biohybrids can contribute to the antioxidant activity amplification.

The earlier studies show that the number of phenolic –OH groups is not always the only determining factor on the antioxidant activity. The structure of an antioxidant molecule, the positions of phenolic –OH groups, presence of other functional groups in the whole molecule, such as double bonds and their conjugation to –OH groups and ketonic groups, also play important roles in antioxidant activities [40]. The structure-antioxidant activity relationships of flavonoids and phenolic acids, in general, are influenced by the state of –OH groups. The strain of the phenolic –OH groups is increasing in the presence of the acidic groups or by other external factors such as defect sites on the carbon nanotubes, hydrophobicity and polarity of the environment already proved in different experiments with other polyphenols (rosmarinic acid, sesamol, carnosic acid, caffeic acid to name few [41]) where phenolic groups are strained by carboxylic or oxygen.

In this respect, the carbon nanotubes revealed multiple facets of the antioxidant mechanisms: 1) A π- conjugated electronic system has antioxidant activity as electron donor but not so efficient to kill free radicals even they carry unpaired electrons (see figure 2, SWCNTs); 2) A proton donating system coupled with electron donor, i.e. a hydrogen transfer to the free radicals is more efficient (SWCNT-OH and SWCNT-COOH); 3) Flavonoids (Q&R) perform antioxidant activity via proton donating converting to quinone like compounds (scheme 1) with extension of conjugation on adjoining rings including also carbon nanotubes.

4. Conclusions

It is reported a new route to design biohybrids based SWCNTs (carboxylated, respective hydroxylated) and flavonoid compounds using high mechanical excitation in ultrasonic field. The excitation in high density ultrasonic field can mediate reactions and functionalizing without wet chemistry. Therefore, this method is affordable in designing hybrid biosystems for biomedicine and drugs in clean conditions. The antioxidants - carbon nanotubes are biohybrids with an improved scavenging rate encompassing a more efficient redox transitions via electron-proton donating to the free radicals. SWCNTs simple or hydroxylated and carboxylated show an intrinsic antioxidant activity in increasing sequence SWCNT-COOH> SWCNT-OH>SWCNT lead to that proton donating is more efficient than electron donating in the mechanisms of free radicals scavenging. There is an optimum combination between electron-proton donating when the flavonoids are selfassembled on carbon nanotubes. The amplification factor reaches high values for SWCNT-Q (F_a= 4.62) and SWCNT-OH-Q (F_a= 4.59), intermediate values for SWCNT-OH-R (F_a=4.29) and SWCNT-R

(F_a=3.73). These results can be assigned to the synergic cooperation between –OH groups from flavonoids and the π- electronic conjugate system of the carbon nanotubes. The steric effects of the glycosides in rutin lead to lower values in the antioxidant activity. The carboxylic groups increase the antioxidant activity but the amplification factor reduced from F_a ~3 for SWCNT-COOH-Q to F_a=2.75 for SWCNT-COOH-R. These phenomena can be assigned to the specific interaction between hydroxyls- carboxyls- π- conjugated system. These results open new approaches in understanding the antioxidant mechanisms as well in combination with chemiluminescence could be developed new strategies in signal monitoring of the free radicals involved in different disease. SWCNT-OH and SWCNT-COOH are more soluble and it is expected to be more compatible with the body fluids, therefore they could be used as nontoxic drug carriers.

References

[1] H. W. Kroto, J. R. Heath, O'Brien, S. C. O'Brien, Curl, R. F. Curl, R. E. Smalley, Nature **318**, 162 (1985).

[2] S. Iijima, Nature **354**, 56 (1991).

[3] R. H. Baughman, A. A. Zakhidov, W. A. De Heer, Science, **297**, 787 (2002).

[4] C. R. Martin, P. Kohli, Nat. Rev. Drug Discov. **2**, 29 (2003).

[5] a) Z. Liu, S. Tabakman, Welsher K. Welsher, H. Dai, Nano Res. **2**, 85 (2009);
b) Z. Liu, S.M. Tabakman, Z. Chen, H. Dai, Nat Protoc. **4**, 1372 (2009);
c) K. Kostarelos, A. Bianco, M. Prato, Nat. Nanotechnol. **4**, 627 (2009).

[6] Yoshinori Sato, Atsuro Yokoyama, Ken-Ichiro Shibata, Yuki Akimoto, Shin-Ichi Ogino, Yoshinobu Nodasaka, Takao Kohgo, Kazuchika Tamura, Tsukasa Akasaka, Motohiro Uo, Kenichi Motomiya, Balachandran Jeyadevan, Mikio Ishiguro, Rikizo Hatakeyama, Fumio Watari, Kazuyuki Tohji, Mol. BioSyst. **1**, 176, (2005).

[7] D. Cui, F. Tian, C. S. Ozkan, M. Wang, H. Gao, Tox. Lett. **155**, 73 (2005).

[8] J. M. Worle-Knirsch, K. Pulskamp, H. F. Krug, Nano Letters **6**, 1261 (2006).

[9] D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. M. Reynolds, T. R. Webb, Toxicol. Sci. **77**, 117-125, (2004).

454

[10] M. M. Amiji, Nanomedicine **2**, 299, (2006).

[11] O. Kayser, A. Lemke, N.H. Trejo, Curr. Pharm. Biotechnol.**6**, 3 (2005).

[12] a) A. Bianco, K. Kostarelos, M. Prato, Curr Opin Chem Biol. **9**, 674 (2005);
   b) R. Partha, J.L. Conyers, Int J Nanomed. **4**, 261 (2009);
   c) C. Tripisciano, K. Kraemer, A. Taylor, E. Borowiak-Palen, Chem Phys Lett. **478**, 200 (2009).

[13] C. Wei, W. Wei, M. Michael, M. Eisaku, G. Mikhail, T. Donald, Med Clin N Am. **91**, 863 (2007).

[14] G.A. Hughes, Nanomedicine **1**, 22, (2005).

[15] M. Vallet-Regí, F. Balas, D. Arcos, Angew Chem Int Edit. **46**, 7548 (2007).

[16] a) V.L.Colvin, Nature Biotech. **21**,1166 (2003);
   b) R. Dagani, Chem Eng News. **81**, 30 (2003).

[17] Z.Liu, C.Davis, W.Cai, L.He, X.Chen, H. Dai, Proc Natl Acad Sci USA **105**, 1410 (2008).

[18] E. Katz, I. Willner, Chem Phys Chem. **5**, 1084–1104, (2004).

[19] a) J.M McCord, Science, **185**, 529 (1974);
   b) B.M. Babior, Blood, **93**, p. 1464 (1999);
   c) P. T. Schumacker, Cancer Cell, **10**, 175 (2006);
   d) H.Wiseman, B. Halliwell, Biochemistry **313**, 17 (1996)

[20] S. Nuttall, U. Martin, A. Sinclair, M. Kendall, The Lancet, **351**(9103), 645 (1998)

[21] V. Fogliano, P.Vitaglione, Molecular Nutrition & Food Research, **49**, 256 (2005)

[22] M. Foldvari, M.Bagonluri, Nanomedicine, **4**, 173 (2008)

[23] R. Bakry, R. M. Vallant, M. Najam-ul-Haq, Matthias Rainer, Z. Szabo, C. W. Huck, G. K. Bonn, Int J Nanomedicine **2**, 639–649,(2007).

[24] Ruy Beck, Silvia Guterres, Adriana Pohlmann (Eds.) Nanocosmetics and Nanomedicines New Approaches for Skin Care, Springer-Verlag Berlin Heidelberg (2011)

[25] a) B. Halliwell, J. Gutteridge, Free radicals in biology and medicine. 4 ed. Oxford: Oxford University Press (2007);
   b) C.A. Kaliora, G.V Dedoussis,Pharmacological Research, **56**, 99-109 (2007);
   c) H. Kaya-Celiker, K. Mallikarjunan, Food Eng Rev, **4**, 114-123 (2012)

[26] J. Ezzati Nazhad Dolatabadi, Int. J Biol Macromol. **48**, 227 (2011).

[27] a) S. Aherne Aisling, N.M O'Brien, Nutrition, **18**, 75 (2002);
   b) S. Burda, W. Oleszek, Journal of Agricultural and Food Chemistry, **49**, 2774 (2001)

[28] Cesar G. Fraga, Monica Galleano, Sandra V. Verstraeten, Patricia I. Oteiza, Molecular Aspects of Medicine **31,** 435 (2010)

[29] P.G. Pietta, J. Nat. Prod. **63**, 1035 (2011).

[30] a) S.A. Payán-Gómez, N.Flores-Holguín, A. Pérez-Hernández, M. Piñón-Miramontes, Chemistry Central Journa, **4**, 12 (2010);
   b) A.Scalbert, G.Williamson, J Nutr. **130**, 2073 (2010);
   c) S.Erkoc, F.Erkoc, N.Keskin, Mol Struct-Theochem. **631**, 141 (2003);
   d) N. Russo, M. Toscano, N. Uccella, J Agric Food Chem. **48**, 3232 (2000).

[31] a) Ana Maria Oliveira Brett, Mariana-Emilia Ghica, Electroanalysis,
    15(22), 1745 (2003); b) Paola Montoro, Alessandra Braca, Cosimo Pizza, Nunziatina
    De Tommasi, Food Chemistry, **92,** 349 (2005); c) Ana K. Timbola, Cristine D. de
    Souza, Cristiano Giacomelli, Almir Spinelli, J. Braz. Chem. Soc.,
    17(1), 139 (2006); d) Martina Medvidović-Kosanović, Marijan Šeruga, Lidija Jako-
  bek, Ivana Novak, Croat. Chem. Acta **83**(2), 197 (2010)
[32] a) Takashii Kondo, Louis J. Kirschenbaum, Heosook Kimm, Peter Riesz J. Phys.
    Chem., **97**, 522-527 (1993); b) N.H. Ince, G. Tezcanli, R.K. Belen, I.G. Apikyan,
     Applied Catalysis B: Environmental **29**,167 (2001)
[33] Nathalia Lucci, Paulo Mazzafera, Canadian Journal of Plant Science,
    89 , 895 (2009)
[34] M.S. Dresselhaus, G. Dresselhaus, R. Saito, A. Jorio, Physics Reports
    409, 47 (2005)
[35] R. Saito, A. Gruneis, G.G. Samsonidze, V. W. Brar, G. Dresselhaus,
    M. S. Dresselhaus, A. Jorio, L.G. Cancado, C.Fantini, M.Pimenta, A.G.Souza Filho,
    New J.Phys. **5**, 157 (2003).
[36] Jamal Talla, Donghui Zhang, Madhuvanthi Kandadai, Aditya Avadhanula, Seamus
    Curran, Physica B, **405**, 4570 (2010)
[37] Cornalia Nichita, I. Stamatin, Adriana Balan-Andronie, Mihaela Zaulet , Digest
    Journal of Nanomaterials and Biostructures **6**, 1851 (2011).
[38] E. Cadenas, BioFactors **6**, 391 (1997).
[39] A. Bianco, K.Kostarelos, C. D. Partidos, M. Prato, Chem. Commun.
    5, 571 (2005).
[40] C. A Rice-Evans, N.J. Miller, G. Paganga, Free Radical Biology & Medicine,
    20, 933 (1996).
[41] a) C.R Wu, M.Y Huang, Y.T Lin, H.Y Ju, H. Ching, Food Chem.,**104**, 1464 (2007).
    b) M. El-Deib Kamal, Z. Ahmed Nermien, Nature and Science, **9**, 90 (2011).