

SCIENTIFIC REPORT

Dendrimer-carbon nanostructure conjugates as drug delivery support

Phase I - period October 2011– December 2011

Phase I. Study of methods and procedures for dendrimer-carbon nanostructure complexes and coupling reactions

1.1. Study and documentation on the methods of coupling of carbon nanostructure-dendrimer conjugates

Until the 1980s, the only known about carbon allotropes was diamond and graphite. In 1985 and 1991 respectively, was discovered two newly form of trigonal carbon (sp^2 hybridized): fullerenes and nanotubes. Carbon nanotubes (CNT) are allotropes of carbon with a nanostructure that can have a length - diameter greater than 10000. These cylindrical carbon molecules exhibit that make them useful in many applications in nanotechnology, electronics, optics and other areas of materials science.

Surfaces functionalized carbon nanotubes have often been studied in biological applications. Dendritic architecture is undoubtedly one of the most common topologies universally observed in all biological systems. Attaching dendrimer on the surface of carbon nanotube (CNT) to obtain a functionalized composite material remains a big challenge. It is obvious that dendrimers with such of ramifications modify the structure of the nanotube offer unique functional and interfacial performance for biological applications.

It made a comprehensive study on the types of coupling reactions having as basis the following reaction scheme (Figure 1):

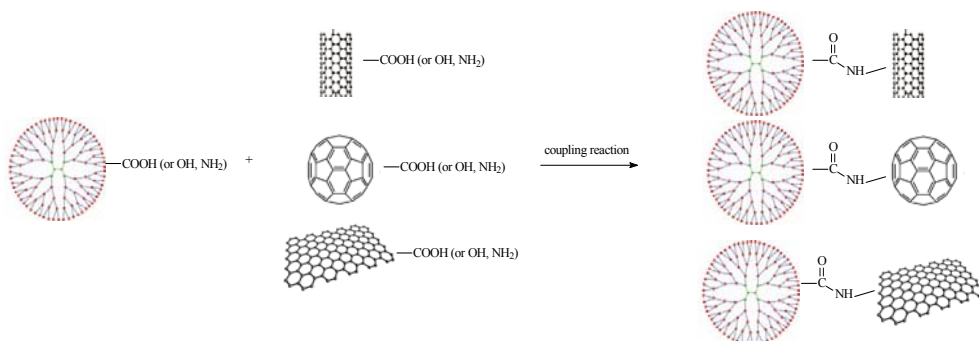


Figure 1. Main reactions.

The uniqueness of CNT as molecular transport began to be intensively developed. Biocompatibility, mechanical of properties, optical, electrical and physical prove to be the basis for new classes of materials, in protein and gene transport.

There are few empirical studies to achieve dendrimer - carbon nanostructures complexes. The further coupling reactions are based on grafting on the surface of a carbon nanotube (SWNT, DWNT or MWNT) of different dendritic structures. In this respect it is necessary to follow several steps.

The first phase consists of the nanotube surface preparation for coupling, by functionalization with groups hydroxyl, carboxyl or amino, depending on the type of dendrimer used (Figure 2).

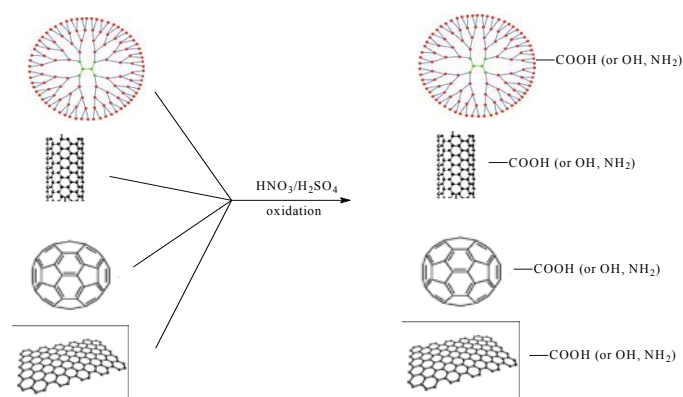


Figure 2. Base structures functionalizations.

Dendrimer surface coupling reactions can be divided into:

- Coupling reactions by amide
- Coupling reactions of the ester bonds
- Coupling reactions ether bond

Depending on the position functional groups there are 2 cases: 1) at the end nanostructure (in the case of carbon nanostructures or graphenes); 2) on the nanostructure surface.

1) Functionalization at the end of nanostructures

Because the nanostructure surface is devoid of any group nanostructure and it is energetically stable, this leads to a high stability against various reagents. In order the functionalization need to use strong reaction condition to create functional groups. For this purpose, functionalization using chemical methods, such as strong oxidation with a mixture of $\text{HNO}_3/\text{H}_2\text{SO}_4$, by sonication, resulting in surface modification of nanostructures grafted groups such as carboxyl, carbonyl or hydroxyl.

2) Functionalization on the surface of the nanostructures

There are various methods with which nanostructures can be achieved wall functionalization: fluorination, nucleophilic addition, electrophilic addition and cycloaddition. For example, using azomethine ilydes (condensed amino acids with aldehyde) 1,3-dipolar cycloaddition leads through the nanotube functionalization, increasing its solubility in various organic solvents. These methods can attach bioactive peptides nanostructure surface with use in immunology.

Most coupling reactions are based on obtaining stable bonds between the two substructures usually amide bond. This is achieved primarily by the reaction between a carboxyl and an amino group in the presence of a coupling agent (EDC, NHS). Such a reaction is shown in Figure 3.

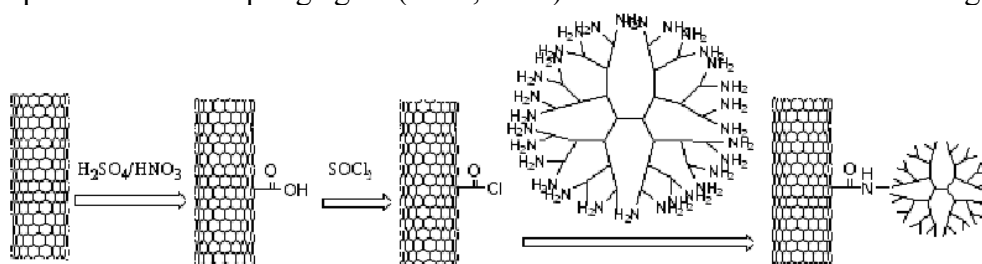


Figure 3. Nanotube-dendrimer coupling reaction.

Coupling the dendrimer on the surface of carbon nanotubes can be done in several steps. The first step is to realize the nanotube surface preparation coupling amine groups of the dendrimer. The method of reaction is as follows: nanotubes is suspended in paraformaldehyde in dimethylformaldehyde as solvent and refluxed for 5 days at 115°C , sonicated 10 min. at each addition. The obtained product was centrifugated, washed with DMF, dichloromethane and diethyl ether and dried under vacuum. Such functionalized nanotube is added to the dendrimer solution in

DMF. In order to coupling are used EDC as coupling agent, N- [3- (dimethylamino) propyl] -N-ethylcarbodiimide hydrochloride, triethylamine and anhydrous hidroxibenzthiazol. Stirring for 12h, filtered off and washed with DMF, dichloromethane and diethyl ether (Figure 4). The product thus obtained was characterized by IR, elemental analysis, SEM and TEM microscopy, Raman spectroscopy.

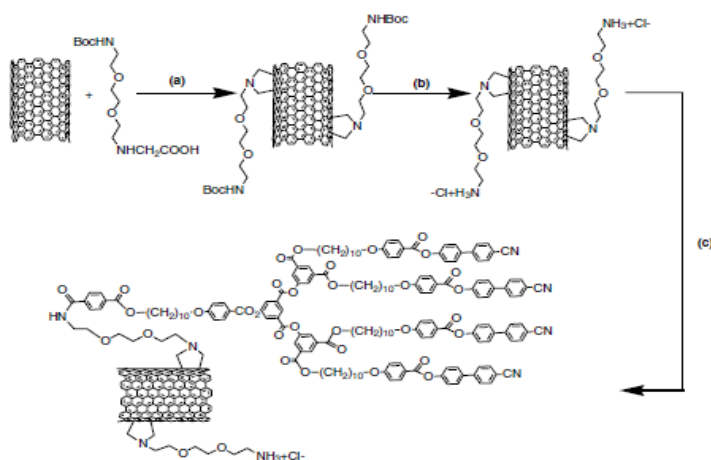


Figure 4. Reaction steps for carbon nanotube surface functionalization with dendrons.

Another functionalization method is achieved with PAMAM type dendrons. The first step is in activating the surface of the dendrimer using a 1,3-dipolar cycloaddition reaction of azomethine ylides. The compound thus obtained is suspended in methanol, glycidyl trimethyl ammonium chloride, and 1,2-diamino ethane. Reflux for 72 hours at 80°C. The resulting product is washed with DMF and dried under vacuum. The increase in the generation of the dendrimer can be achieved using alkylation reaction with glycidyl trimethyl ammonium chloride, and 1,2-diamino ethane (methanol, 40°C, 48 hours) (Figure 5).

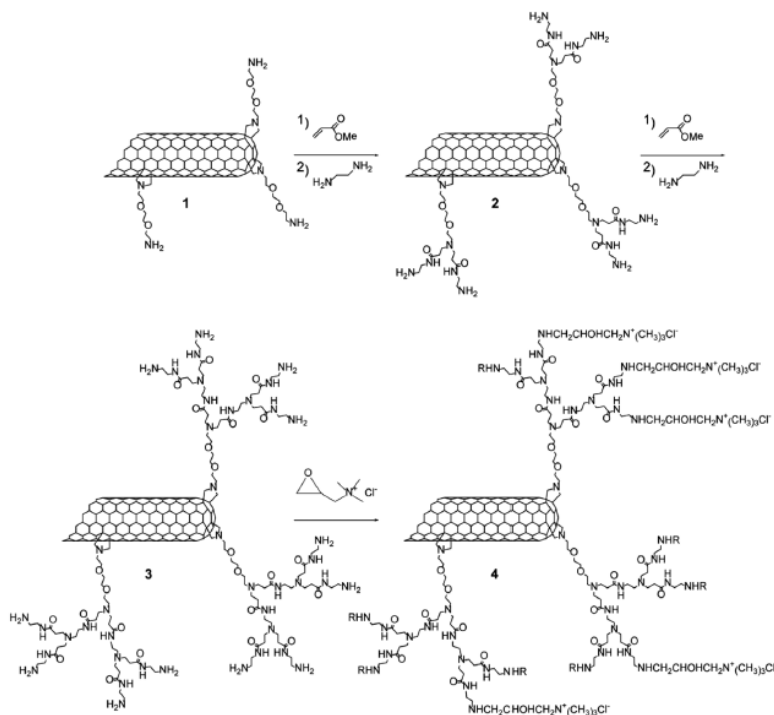


Figure 3. Reaction steps of carbon nanotube functionalization with PAMAM dendrimers.

Characterization is done by IR, elemental analysis, SEM and TEM microscopy, Raman spectroscopy (Figure 4).

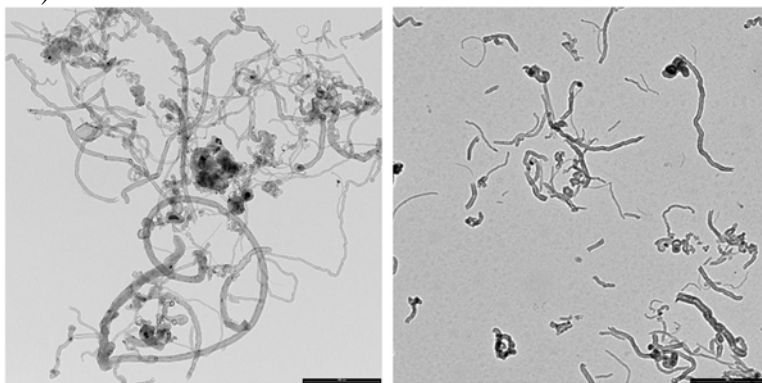


Figure 6. TEM microscopy: a) non-functionalized nanotube; b) dendrimer-functionalized nanotube.

1.2. Modeling carbon nanostructures and dendrimers

The initial configurations have been optimized using symmetry constraints using the PM6 semiempirical method, followed by harmonic vibrational analysis at the same level of theory. The obtained geometries have been re-optimized using the Hartree-Fock (HF) method in combination with the 3-21G and 6-31G(d,p) basis sets. All computations were performed using the Gaussian 09 rev. A01 quantum chemical software.

Oligo Methanofullerenes $C_{60}(CH_2)_n$ $n = 0, 1 \dots 6$

The structures have been built by the cyclopropanation of hh bonds (the shared bond between two hexagons) from fullerene $C_{60}-I_h$. Because of the equivalence of all [6,6] type bonds only one isomer of $C_{60}CH_2$ exists with C_{2v} symmetry (Figure 8). The next cyclopropanation step results 8 regioisomers $C_{60}(CH_2)_2$. The obtained theoretical results for the set of 8 isomers are presented in figure 7. It can be observed that the size of the basis set has no influence on the stability ordering of the clusters. The results indicate that both from thermodynamic and kinetic point of view their stability is very close.

The total energy decreases whereas the gap energy increases with the number of carbene fragments attached to fullerene C_{60} , as it can be seen in Table 1. Since the number of isomers is very large only the structures with high symmetry have been considered. In the case when n equals 3, 5 and 6, the preferred addition is in the equatorial belt of the fullerene. A remarkable stability was observed for the hexamethanofullerene with T_h symmetry.

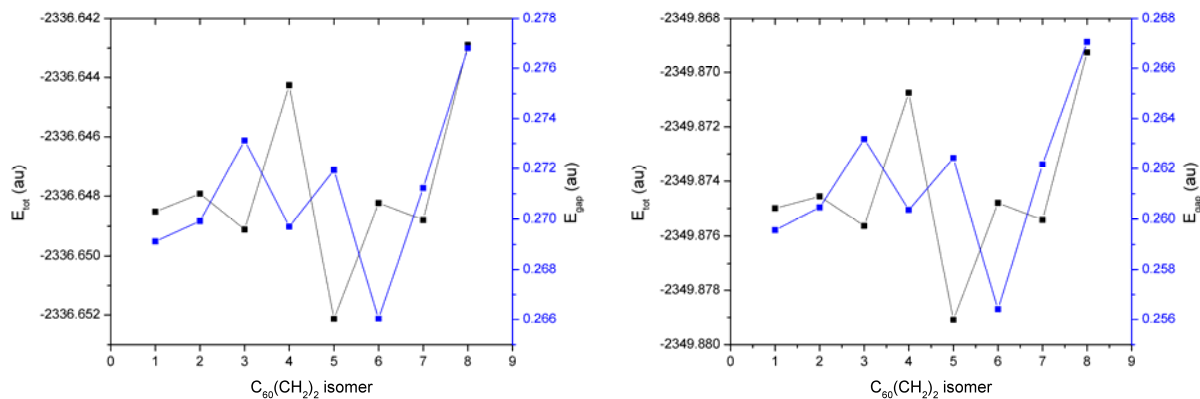


Figure 7. Plot of the total energy (E_{tot} in au) and HOMO-LUMO gap energy (E_{gap} in au) of the eight region isomers of fullerene $C_{60}(CH_2)_2$, evaluated at the HF/3-21G (left) and HF/6-31G(d,p) (right) level of theories. Isomer count corresponds to the one in Figure 2.

Table 1. Single point energy results obtained with the HF/6-31G(d,p) method.

Structure	Sym	ΔE_{tot} (au)	E_{tot}/N (au)	E_{gap} (au)
C_{60}	I_h	0	-37.8638	0.2727
$C_{60}CH_2$	C_{2v}	-39.0226	-37.8828	0.2624
(1) $C_{60}(CH_2)_3$	C_{3v}	-117.0645	-37.9189	0.2630
(2) $C_{60}(CH_2)_3$	C_3	-117.0679	-37.9190	0.2888
(3) $C_{60}(CH_2)_3$	D_3	-117.0671	-37.9190	0.2774
(1) $C_{60}(CH_2)_4$	C_{2h}	-156.0781	-37.9361	0.2550
(2) $C_{60}(CH_2)_4$	D_{2h}	-156.0880	-37.9362	0.2576
(1) $C_{60}(CH_2)_5$	C_{5v}	-195.0712	-37.9523	0.2672
(2) $C_{60}(CH_2)_5$	D_5	-195.0891	-37.9526	0.2701
(1) $C_{60}(CH_2)_6$	D_{3d}	-234.093	-37.9685	0.3087
(2) $C_{60}(CH_2)_6$	D_{3d}	-234.1038	-37.9687	0.2662
(3) $C_{60}(CH_2)_6$	T_h	-234.1316	-37.9691	0.3223
$C_{60}CHCOOH$	C_s	-212.5759	-39.8650	0.273
$C_{60}C(COOH)_2$	C_2	-399.1262	-38.8188	0.2727

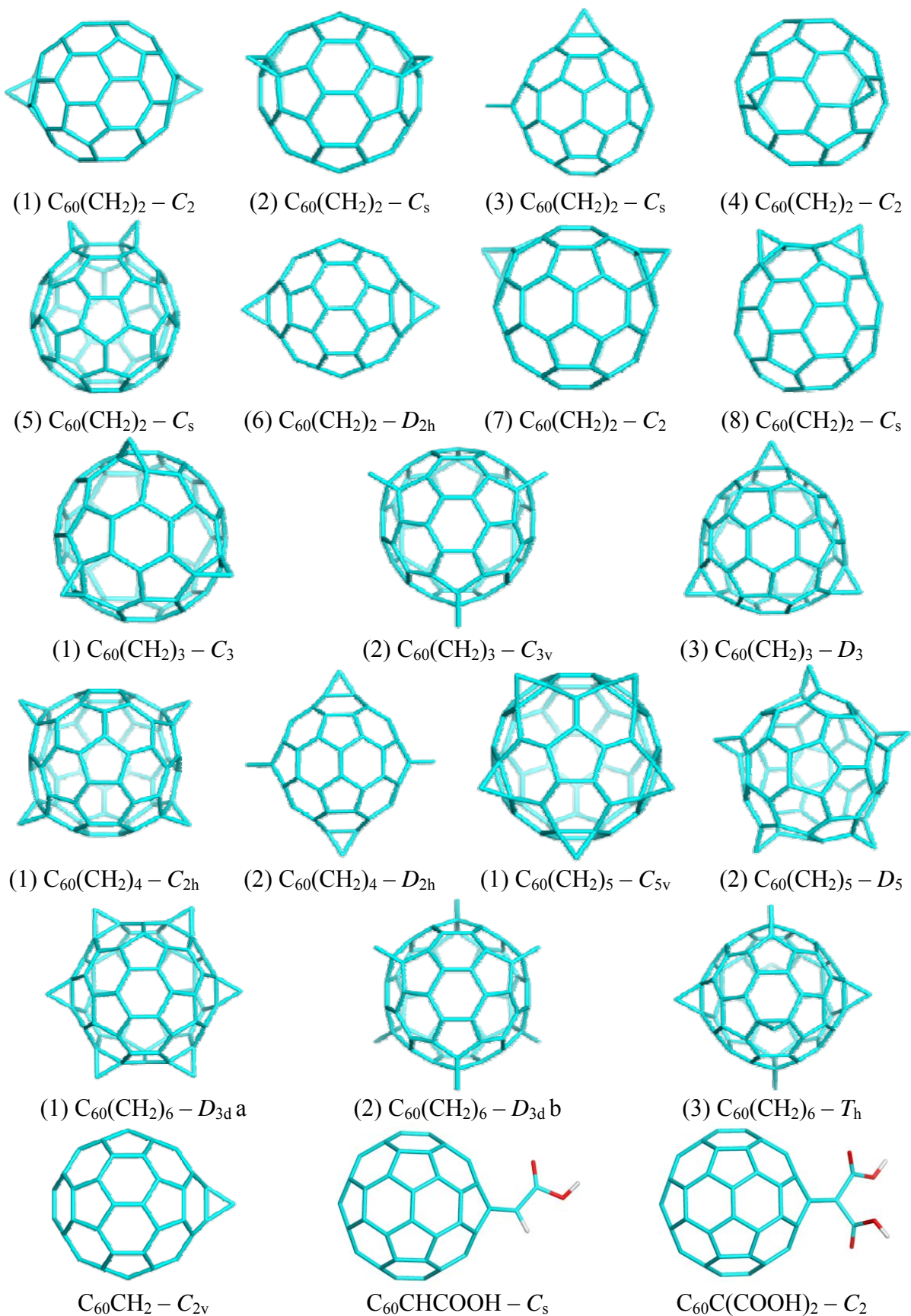


Figure 8. Optimized geometries at the HF/6-31G(d,p) level of theory: oligomethanofullerene $C_{60}(CH_2)_n$ ($n = 1, 2, \dots, 6$), and carboxy fullerenes $C_{60}CHCOOH$, and $C_{60}C(COOH)_2$.

Armchair type carbon nanotubes

Series of armchair carbon nanotubes have been designed with chirality [3,3] up to [8,8] and length $n = 4, 5, \dots, 10$. The dangling bonds have been hydrogenated and the geometries were optimized by DFT using the B3LYP functional and 6-31G(d,p) basis set. The single point computational results are presented in Figures 9-10.

The thermodynamic stability depends on the length and diameter of the nanotube as it can be seen in Figure 9. The total energy, although very close in values it decreases with the increment in the nanotube diameter, in Figure 9 right it can be observed that the stability ordering is the same. The total energy decreases with the length of the nanotube, however per atom a small rise in energy can be observed.

The HOMO-LUMO gap indicates a periodicity as function of the nanotube length, however the stability ordering is different between the nanotube sets.

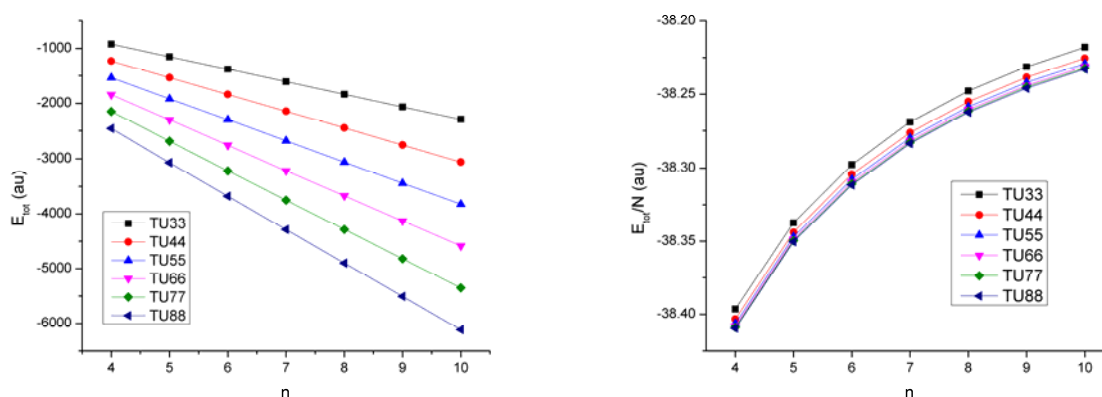


Figure 9. Variation of the total energy (E_{tot} in au, left) and total energy per number of carbon atoms (E_{tot}/N in au, right) as function of the nanotube length $n = 4, 5, \dots, 10$.

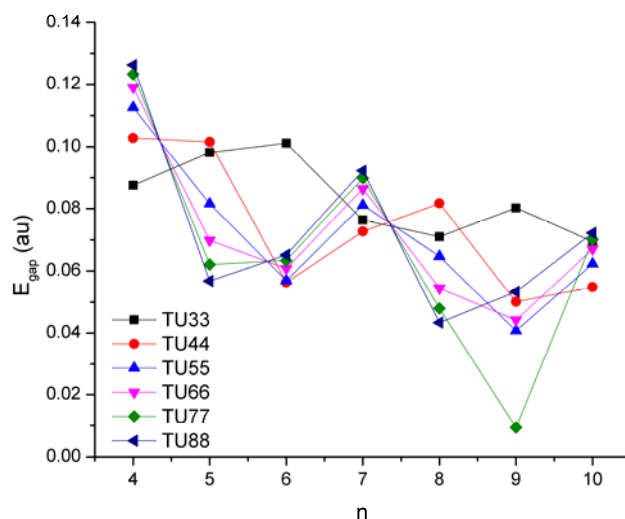


Figure 10. Variation of the HOMO-LUMO gap energy (E_{gap} in au) as function of the nanotube length $n = 4, 5, \dots, 10$.

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