

# SCIENTIFIC REPORT

## Dendrimer-carbon nanostructure conjugates as drug delivery support

Phase 5 - period december 2014 – december 2015

### Phase IV. Synthesis and physico-chemical characterization of dendrimer-carbon nanostructure-dendrimer complexes - partial

**Activity:** *Optimization of reaction conditions*

The objective of this phase is to optimize the reaction conditions for dendritic structures obtained previously and in achieving carbon-dendrimer complex nanostructures. The structures were characterized in terms of physico-chemical properties.

Some of the activities in this stage, has focused on obtaining the metal-dendrimer complex structure and test their activity in the catalytic reduction reaction of p-nitrophenol to p-aminophenol.

In order to achieve the objective of this phase were carried out the following activities:

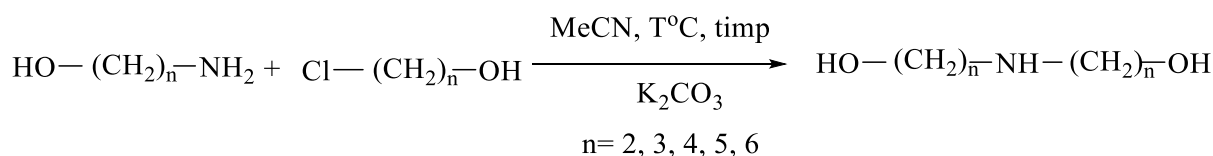
1. Optimization of the reaction conditions
  - a. optimization of the reaction conditions for obtaining dialkanolamines
  - b. optimization of the reaction conditions for obtaining dendrons
  - c. optimization of the reaction conditions for obtaining dendrimers
2. Obtaining dendrimer-nanotube complexes
3. Obtaining dendrimer-graphene complexes
4. Testing catalytic activity

#### 1. Optimization of the reaction conditions

Optimization of the reaction conditions had as goal to enestablish optimal parameters for maximum yield and purity. In order to optimize were taking into account three parameters: temperature, reaction time and type of solvent used.

##### *a. optimization of reaction conditions for obtaining dialkanolamines*

The general reaction in the case of dialkanolamines is shown in the following diagram:



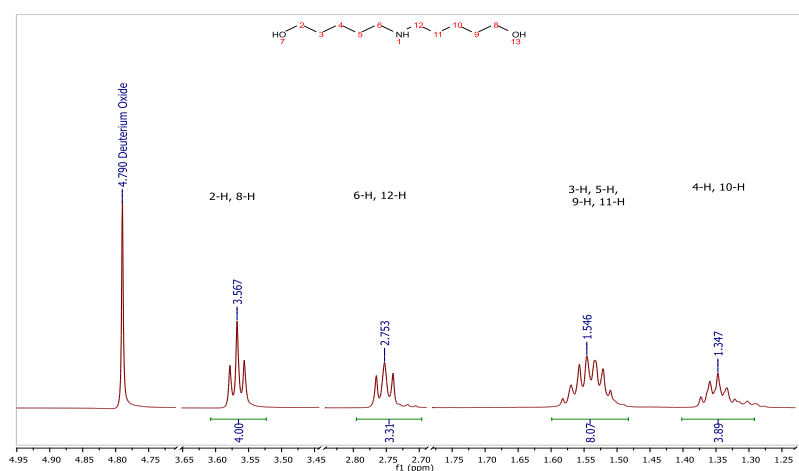
**Scheme 1.** General reaction for obtaining dialkanolamines.

#### **Procedure:**

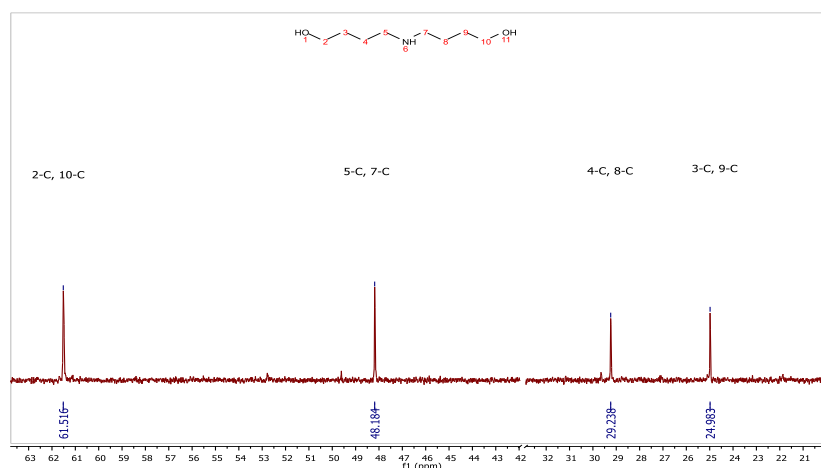
In a 25ml round bottom flask with continuously stirring is mixed an 1: 2.2 = chloroalkanol: aminoalkanol dissolved in acetonitrile (MeCN) or 1,4-dioxane (C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>), is added in minimal

excess potassium carbonate for acid neutralization resulted from reaction. The reactions were performed at different temperatures (60 and 80 in MeCN, 100°C in C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>) and different reaction times (6, 12, 24 h). The reaction mixture was brought to room temperature, the resulting KCl and unreacted K<sub>2</sub>CO<sub>3</sub> was removed by vacuum filtration. The filtrate was evaporated to dryness in a rotary evaporator. The resulting reaction product is in the form of viscous yellow liquid. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>).

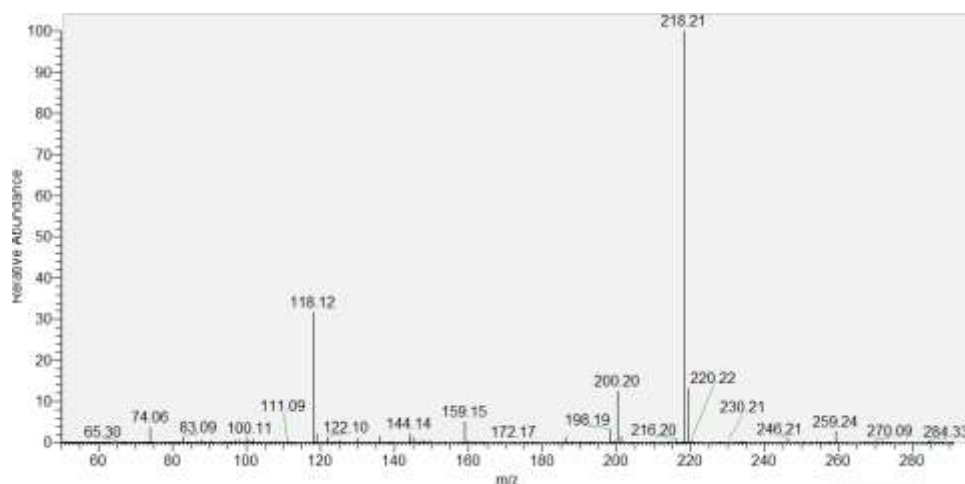
The products were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz), elemental analysis (Vario Micro Cube, CNH) (Figure 1-3 ).



**Fig. 1.** <sup>1</sup>H-NMR spectrum for dipentanol-amine (600 MHz, 298 K, D<sub>2</sub>O)



**Fig. 2.** <sup>13</sup>C-NMR spectrum for dipentanol-amine (150 MHz, 298 K, D<sub>2</sub>O)



**Fig. 3.** Mass spectrum for dihexanol-amine (Mw= 217.2 g/mol)

After optimization of reaction conditions were obtained the following results (Table 1):

**Table 1.** Reaction conditions optimization

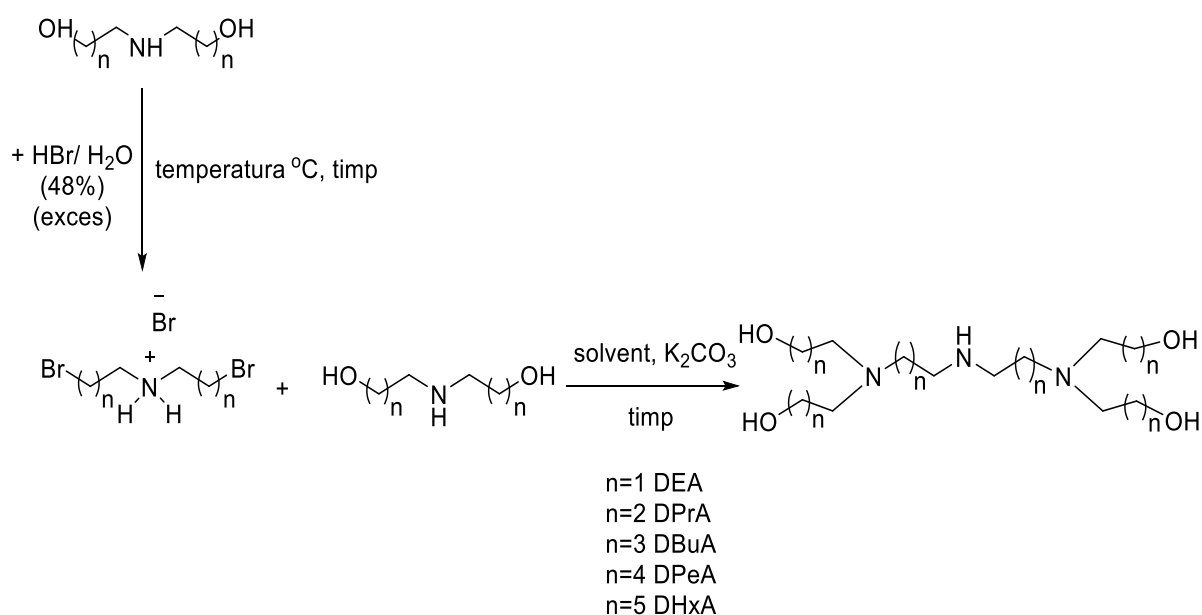
No	Temperature (°C)	Reaction time (h)	Yield (dialkanolamine) $A_n = \% (n=2,3,4,5,6)$
1	60	6	$A_2 = 75$ $A_3 = 72$ $A_4 = 78$ $A_5 = 78$ $A_6 = 76$
2	80	6	$A_2 = 80$ $A_3 = 82$ $A_4 = 82$ $A_5 = 80$ $A_6 = 81$
3	100	6	$A_2 = 85$ $A_3 = 86$ $A_4 = 81$ $A_5 = 82$ $A_6 = 82$
4	60	12	$A_2 = 76$ $A_3 = 75$ $A_4 = 78$ $A_5 = 79$ $A_6 = 78$
5	80	12	$A_2 = 80$ $A_3 = 81$ $A_4 = 81$ $A_5 = 82$ $A_6 = 81$
6	100	12	$A_2 = 89$ $A_3 = 87$ $A_4 = 88$ $A_5 = 87$ $A_6 = 86$

No	Temperature (°C)	Reaction time (h)	Yield (dialkanolamine) A <sub>n</sub> = % (n=2,3,4,5,6)
7	60	24	A <sub>2</sub> = 80 A <sub>3</sub> = 78 A <sub>4</sub> = 79 A <sub>5</sub> = 80 A <sub>6</sub> = 80
8	80	24	A <sub>2</sub> = 90 A <sub>3</sub> = 89 A <sub>4</sub> = 88 A <sub>5</sub> = 88 A <sub>6</sub> = 87
9	100	24	A <sub>2</sub> = 92 A <sub>3</sub> = 89 A <sub>4</sub> = 91 A <sub>5</sub> = 89 A <sub>6</sub> = 90

Based on the obtained analysis results are found that the most optimal in terms of yield, reaction time and temperature is version 9 (temperature = 100 ° C for 24 h, yield 90%) using dioxane as solvent.

*a. Optimization of reaction conditions for obtaining dendrons*

The next activity consisted in the reaction conditions optimization for obtaining dendrons. The reaction for obtaining are shown in Figure 2. In the first phase were obtained 0 generation dendrons, and by repeating the reactions are obtained higher generations.

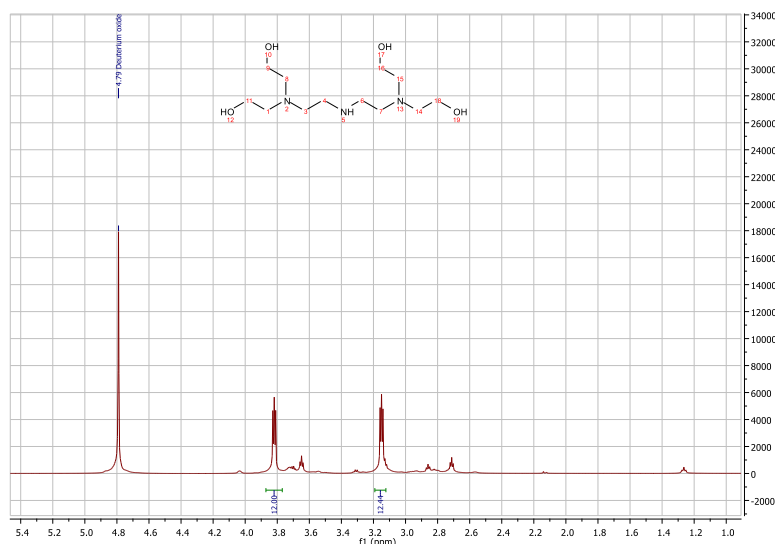


**Scheme 2. General reaction of obtaining dendrons**

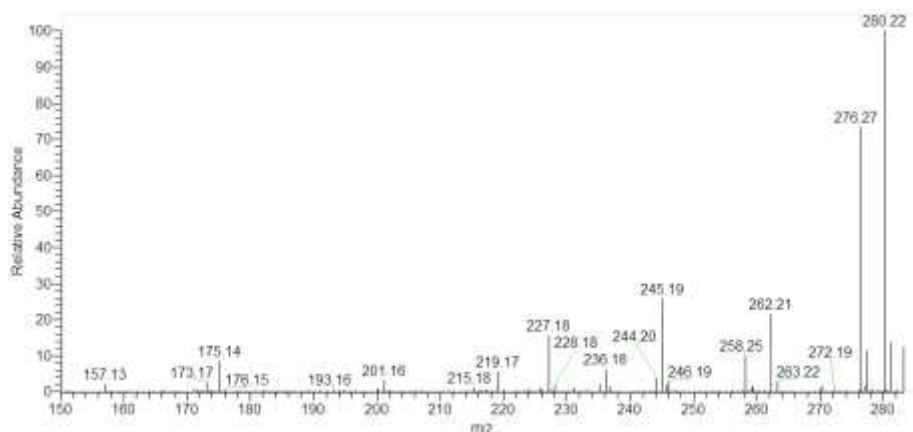
## Procedure

In a round bottom flask of 50 ml under continuous stirring was added 25 mL solvent (acetonitrile for the reactions conducted at 60 and 80 ° C and 1,4-dioxane for reaction at 100° C), and dibromo derivative: amine dialkanol=1:3 and 5 parts of K<sub>2</sub>CO<sub>3</sub>. After the reaction period the mixture was brought to room temperature, KBr result that using excess potassium carbonate was removed by filtration. The filtrate was brought to dryness using a rotaevaporator. Dialkanol amine excess is removed by distillation in vacuo. The result is a pale yellow liquid to dark yellow. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>).

The products were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz), elemental analysis (Vario Micro Cube, CNH) (Figure 4-5).



**Fig. 4.** <sup>1</sup>H-NMR spectrum of the **DEA-1G** dendron (600 MHz, 298 K, D<sub>2</sub>O)



**Fig. 5.** Mass spectrum of the **DEA-1G** dendron (M<sub>w</sub>=279.38 g/mol)

Results for reaction conditions optimization are presented in table 2.

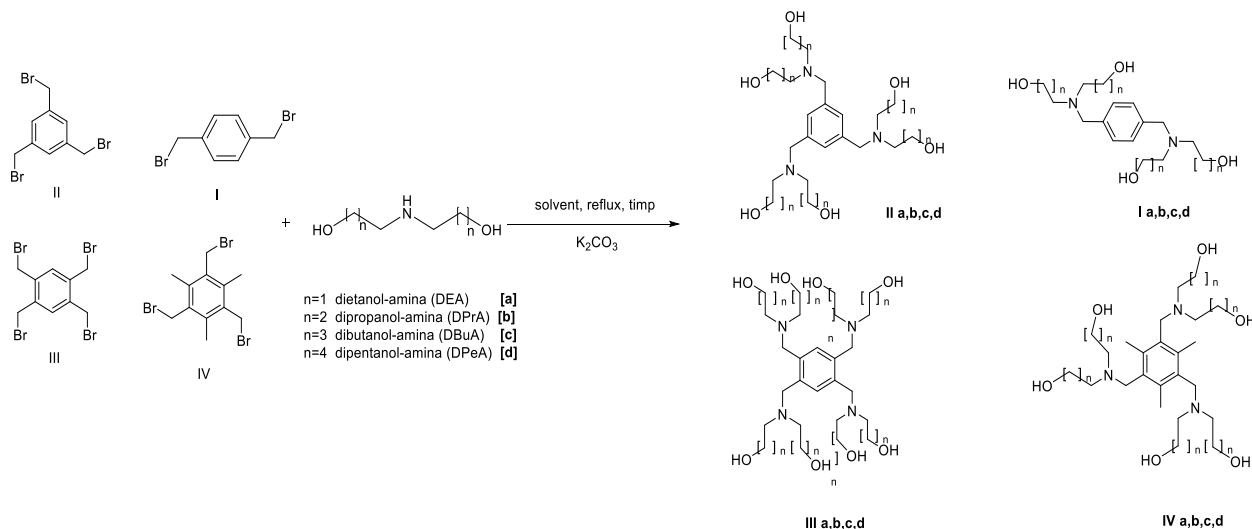
**Table 2.** Reaction conditions optimization

No	Temperature (°C)	Reaction time (h)	Yield (dialkanolamine) $A_n = \% (n=1,2,3,4,5,6)$
1	60	6	$A_1 = 37$ $A_2 = 33$ $A_3 = 35$ $A_4 = 33$ $A_5 = 39$
2	80	6	$A_2 = 39$ $A_3 = 39$ $A_4 = 40$ $A_5 = 41$ $A_6 = 38$
3	100	6	$A_2 = 42$ $A_3 = 41$ $A_4 = 40$ $A_5 = 41$ $A_6 = 40$
4	60	12	$A_1 = 42$ $A_2 = 41$ $A_3 = 40$ $A_4 = 44$ $A_5 = 41$
5	80	12	$A_2 = 60$ $A_3 = 59$ $A_4 = 61$ $A_5 = 58$ $A_6 = 60$
6	100	12	$A_2 = 59$ $A_3 = 60$ $A_4 = 65$ $A_5 = 66$ $A_6 = 66$
7	60	24	$A_1 = 44$ $A_2 = 46$ $A_3 = 44$ $A_4 = 49$ $A_5 = 47$
8	80	24	$A_2 = 53$ $A_3 = 55$ $A_4 = 52$ $A_5 = 52$ $A_6 = 50$
9	100	24	$A_2 = 67$ $A_3 = 69$ $A_4 = 68$ $A_5 = 69$ $A_6 = 67$

The best option for getting dendron is conducted at reflux in dioxane for 24 h (version 9 Table 2).

## b. Optimization of reaction conditions for obtaining dendrimers

The reaction for obtaining dendrimers was based on the following scheme (Scheme 3):



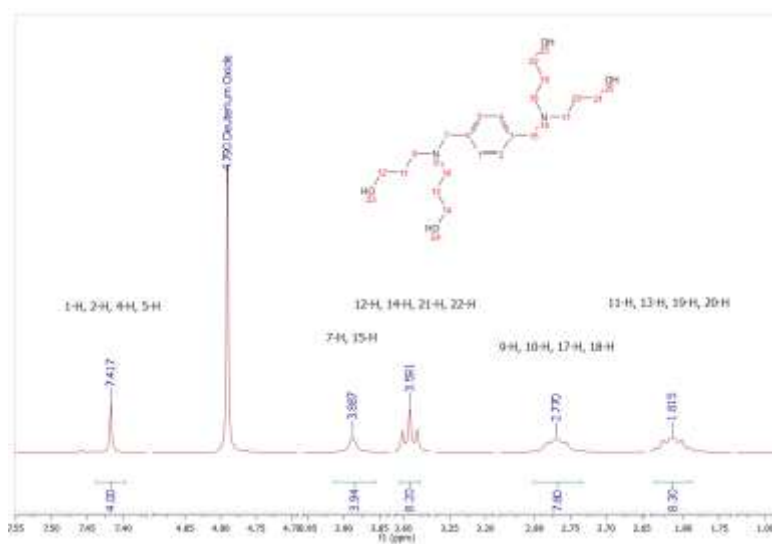
**Scheme 3.** General reaction of obtaining 0 generation dendrimer with aromatic ring

### Procedure

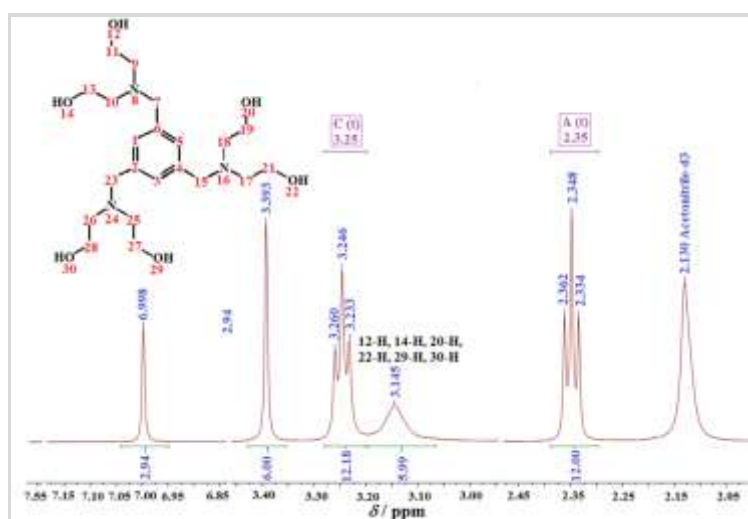
In a round bottom flask of 50 ml was dissolved under continuous stirring in approximately 30 mL of solvent, an aromatic ring, and dialkanolamines (a, b, c, d) in a ratio of 1: 2.2 for the core di-1: 3.3 to tri-substituted ring and 1: 4.4 for the core tetrasubstituted. Potassium carbonate is in 2.5, respectively 3.5, 4.5 parts. The reaction mixture was heated at different temperatures (40, 60 and 80°C) for 6, 12 and 24 hours. After completion of the reaction the mixture was cooled, filtered KBr resulting  $K_2CO_3$  respectively. It is washed with 3 x 20 ml methanol. The resulting filtrate was taken to dryness by rotary evaporation.

Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>). The products were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz), elemental analysis (Vario Micro Cube, CNH) (Figure 6-8 Table 3).

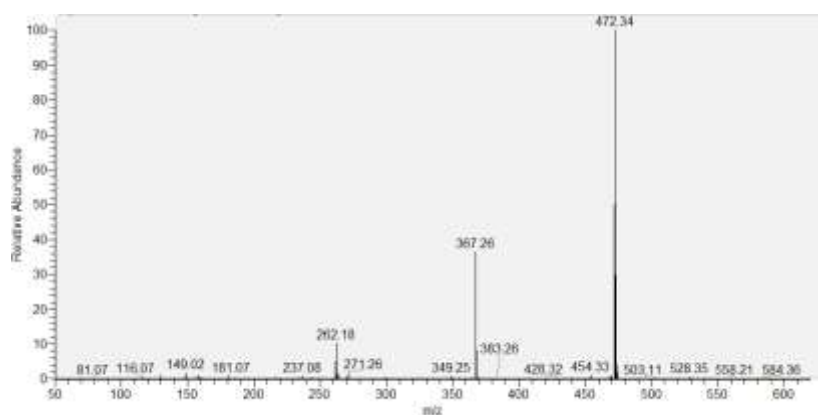
Results of optimizing reactions condition are shown in Table 4.



**Fig. 6.**  $^1\text{H-NMR}$  spectrum for **I b** compound (600 MHz, 298 K,  $\text{D}_2\text{O}$ )



**Fig. 7.**  $^1\text{H-NMR}$  spectrum for **II a** compound (400 MHz, 298 K,  $\text{CD}_3\text{CN}$ )



**Fig. 8.** Mass spectrum for **IV a** compound ( $M_w=471.33$ )



**Table 3.** Elemental analysis

Dendrimer	Calculated values (%)				Measured values (%)		
	C	H	N	O	C	H	N
I a	61.51	9.03	8.97	20.49	61.97	8.98	8.67
I b	65.19	9.85	7.60	17.37	64.96	9.78	7.49

**Tabel 4.** Reaction condition optimization

No	Solvent	Temperature (°C)	Reaction time (h)	Yield (dialkanolamine) A <sub>n</sub> = % (n=1,2,3,4)
1	Acetonitril	40	6	A <sub>1</sub> = 74 A <sub>2</sub> = 70 A <sub>3</sub> = 71 A <sub>4</sub> = 72
2	Dioxan	40	12	A <sub>1</sub> = 79 A <sub>2</sub> = 77 A <sub>3</sub> = 77 A <sub>4</sub> = 78
3	Acetonitril:dioxan= 2:1	40	24	A <sub>1</sub> = 80 A <sub>2</sub> = 81 A <sub>3</sub> = 80 A <sub>4</sub> = 80
4	Acetonitril	60	6	A <sub>1</sub> = 80 A <sub>2</sub> = 81 A <sub>3</sub> = 81 A <sub>4</sub> = 80
5	Dioxan	60	12	A <sub>1</sub> = 86 A <sub>2</sub> = 82 A <sub>3</sub> = 82 A <sub>4</sub> = 84
6	Acetonitril:dioxan= 2:1	60	24	A <sub>1</sub> = 88 A <sub>2</sub> = 85 A <sub>3</sub> = 88 A <sub>4</sub> = 89
7	Acetonitril	80	6	A <sub>1</sub> = 78 A <sub>2</sub> = 77 A <sub>3</sub> = 77 A <sub>4</sub> = 74
8	Dioxan	80	12	A <sub>1</sub> = 87 A <sub>2</sub> = 85 A <sub>3</sub> = 85 A <sub>4</sub> = 82
9	Acetonitril:dioxan= 2:1	80	24	A <sub>1</sub> = 89 A <sub>2</sub> = 90 A <sub>3</sub> = 91 A <sub>4</sub> = 90

The best option is for acetonitrile solvent mixture:dioxane = 2:1 at 80°C for 24 h (version 9).

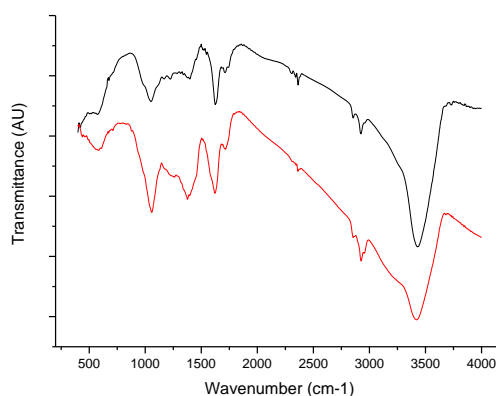
## 2. Obtaining carbon nanotubes dendrimer complexes

Dendrimer-nanotube complexes are made for use as support material in transport biologically active compounds, and more.

### Procedure

Single-walled carbon nanotubes with a carboxyl group (SWCNT-COOH, 150 mg) was suspended in dichloromethane (15 ml) and was sonicated in the ultrasonic bath for 10 minutes. For activation of the carboxyl group, in the resulting suspension was added 4-dimethylaminopyridine (DMAP, 15 mg) or the aromatic ring dendrimer generation 0 (300 mg) dissolved in DMF (5 ml). The resulting mixture was sonicated for 5 minutes after which was added N, N'-dicyclohexylcarbodiimide (DCC, 150 mg) with stirring in an ice bath. Vigorous stirring was continued for another 3 h at room temperature. Filtered and washed repeatedly with dichloromethane (3x20 ml), water (3x25 ml) or methanol (2x40 ml) to remove both reagents and for removing covalently unbound dendrimer on nanotube. Dendrimer-CNT complexes thus obtained are dried in air for 48 hours.

Dendrimer carbon nanotube complexes were tested in catalytic hydrogenation reaction as support for metal catalyst, in this case Cu(0) nanoparticles. Hydrogenation reaction model was 4-nitrophenol to 4-aminophenol in the presence of excess  $\text{BH}_4$ . The reaction product was analyzed by FTIR spectroscopy, observing the appearance of signal around  $1400\text{cm}^{-1}$ , which confirms the emergence of the complex (Figure 9).



**Fig. 9.** FT-IR spectrum for dendrimer II a – CNT complexes (black line =reference, red line =sample).

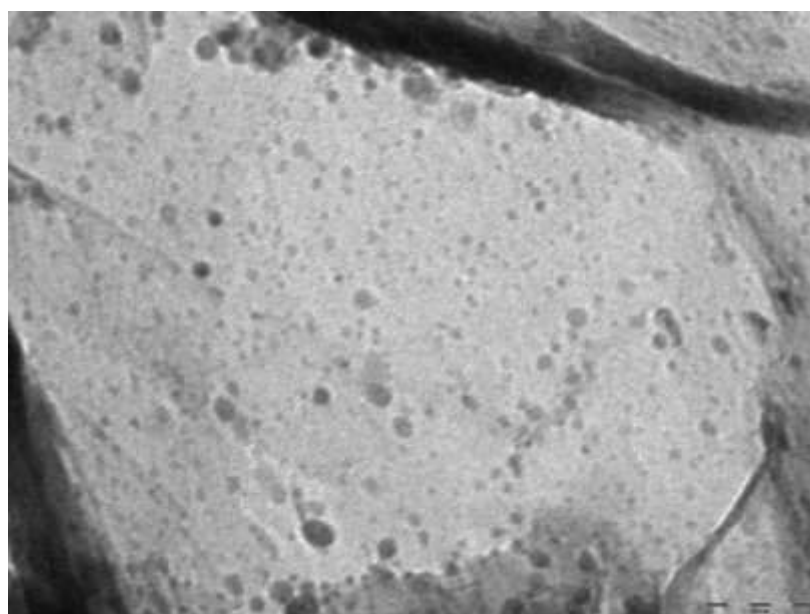
### ***3. Obtaining dendrimer-graphene complexes***

Also, as substrate, they were made graphene-dendrimer complexes.

#### ***Procedure***

Graphene oxide (GRF-COOH, 150 mg) was suspended in dichloromethane (15 ml) and sonicated in the ultrasonic bath for 10 minutes. To the resulting suspension was added 4-dimethylaminopyridine (DMAP, 15 mg) or the aromatic ring dendrimer generation 0 (300 mg) dissolved in DMF (5 ml). The resulting mixture was sonicated for 5 minutes after which was added N, N'-dicyclohexylcarbodiimide (DCC, 150 mg) with stirring at 0°C. Vigorous stirring is maintained at room temperature an additional 3h. Filtered and washed repeatedly with dichloromethane (3x20 ml), water (3x25 ml) or methanol (2x40 ml) to remove both reagents, as well as covalently unbound dendrimer on graphene surface. Dendrimer graphene complexes thus obtained are dried in air for 48 hours.

Graphene-dendrimer complexes were tested as support material for the Cu(0) nanoparticles in catalytic reaction of reduction the p-nitrophenol to p-aminophenol. The following figure shows the distribution of the nanoparticles (NP) of Cu (0) on the surface of graphene (Figure 10).



**Fig. 10.** Graphen-dendrimer-NP Cu(0) complex.

#### 4. Testing catalytic activity

In order to test the usefulness of the obtained new structures were studied catalytic activity of complexes with Cu (0) in the reduction reaction of p-nitrophenol (PNP) to p-aminophenol (PAP).

##### *Reaction for obtaining dendrimer-Cu(0) nanoparticles complexes*

##### **Procedure**

Prepare two solutions:

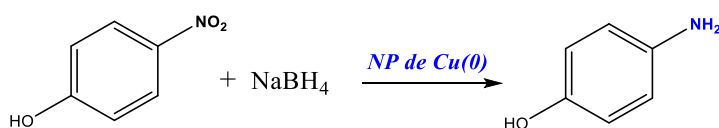
Solution 1: In a 1.5 ml eppendorf was dissolved in methanol (1 ml) the aromatic nucleus containing 0 generation dendrimer (I-Va, 100 mg)

Solution 2: 5% solution of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  in methanol.

In a round bottom flask (10 ml) add 5 ml of solution 2 over which was added with continued stirring at room temperature Solution 1 in 5 portions at intervals of 5 minutes, after which the resulting mixture was allowed to reflux for 24 hours thus obtaining dendrimer- $\text{Cu}^{2+}$  complexes.

In the second step, the reaction mixture brought to room temperature was added  $\text{NaBH}_4$  (70 mg) in small quantities in order to obtain dendrimer-Cu(0) complexes. At the end of the reduction reaction the resulting solid particles are removed by vacuum filtration through PTFE membrane of porosity 0.2 $\mu\text{m}$ . The filtrate obtained is green and represents the catalyst. Dendrimer-Cu(0) complexes activity was tested using standard reaction described below (scheme 4).

##### *Standard catalytic reduction reaction*

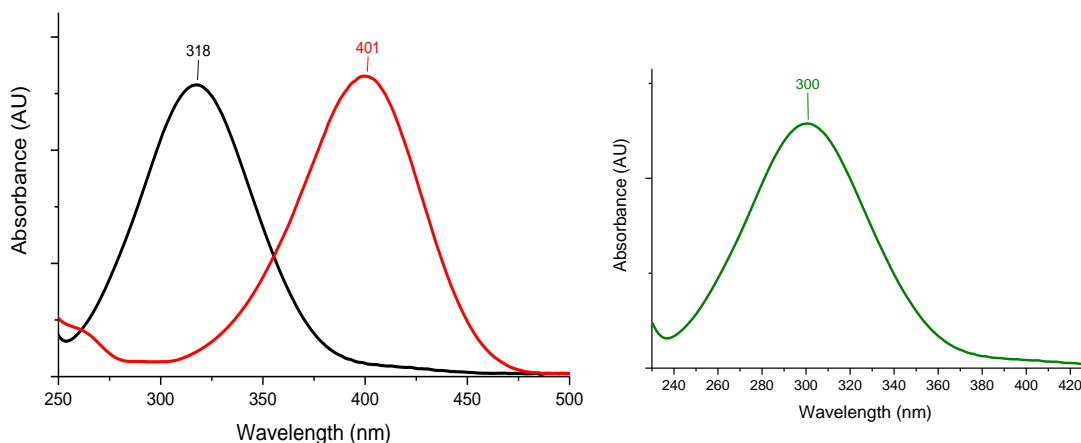


**Scheme 4.** Reduction reaction of p-nitrophenol to p-aminophenol.

To the excess sodium borohydride solution was added 4-nitrophenol in the presence of Cu(0) nanoparticles resulting the formation of the hydrogenated derivative of 4-aminophenol. Catalytic reaction monitoring was done by UV-Vis spectrophotometer (Agilent 8453 spectrophotometer, ambient temperature, quartz cuvette 3 ml capacity, 1 cm diameter; measurement time 500 seconds, stirring 480 rpm).

Aqueous solutions were prepared using ultrapure water only. Thus prepared two solutions of 1.2 mM p-nitrophenol, respectively of 15 mM sodium borohydride.

The aqueous solution of p-nitrophenol (180  $\mu$ l + 2.62 ml of 1.2 mM  $H_2O$ ) absorbs at 318nm (black line spectrum below). By adding sodium borohydride solution (180  $\mu$ l 1.2 mM + 1 mL of 15 mM + 1.62 mL  $H_2O$ ) absorption maximum occurs at 401 nm wavelength (red line). Hydrogenated (p-amino phenol) has an absorption maximum at 300 nm (green line) (Figure 11).

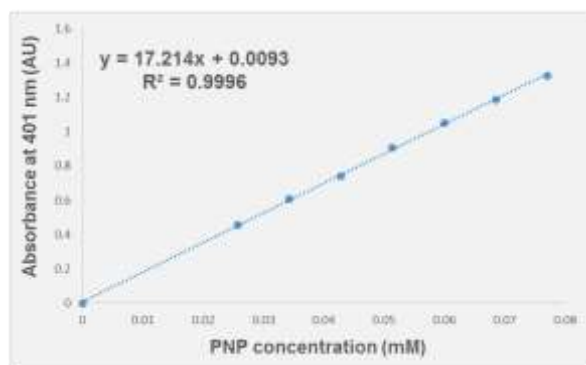


**Fig. 11.** UV-Vis spectra (PNP, PNP+NaBH<sub>4</sub>, PAP)

### *Molar extinction coefficient determination*

For the determination of the molar extinction coefficient of the equation of Lambert-Beer  $\epsilon$  ( $A = \epsilon * c * l$ ) used later in determining the catalytic activity is necessary to perform calibration curve of standard solutions of known concentration of PNP. In all cases the concentration of sodium borohydride was identical (1 ml of 15 mM to a final volume of 2.7 ml of solution).

The image below is representative graphical calibration curve obtained from which, molar extinction coefficient that is 17 214 ( $l * \text{mmol}^{-1} * \text{cm}^{-1}$ ) (Figure 12).



**Fig. 12.** Standard calibration curve.

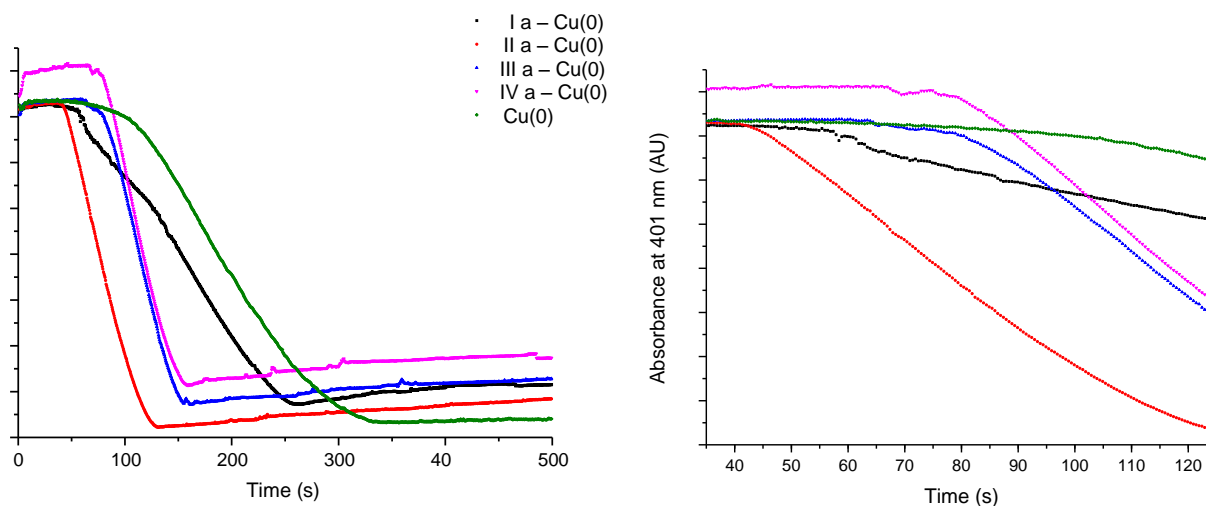
### *Catalytic activity testing of the dendrimer-Cu(0) nanoparticle complexes*

To test the catalytic activity of the obtained dendrimer-Cu (0) complexes in UV-V measurement is used:

- *Sample (final volum 2.7 ml)* PNP (180 ul 1.2 mM)  
NaBH<sub>4</sub> (1000 ul 15 mM)  
H<sub>2</sub>O (1620 ul)
- *Catalyst:* dendrimer-Cu(0) 30 ul

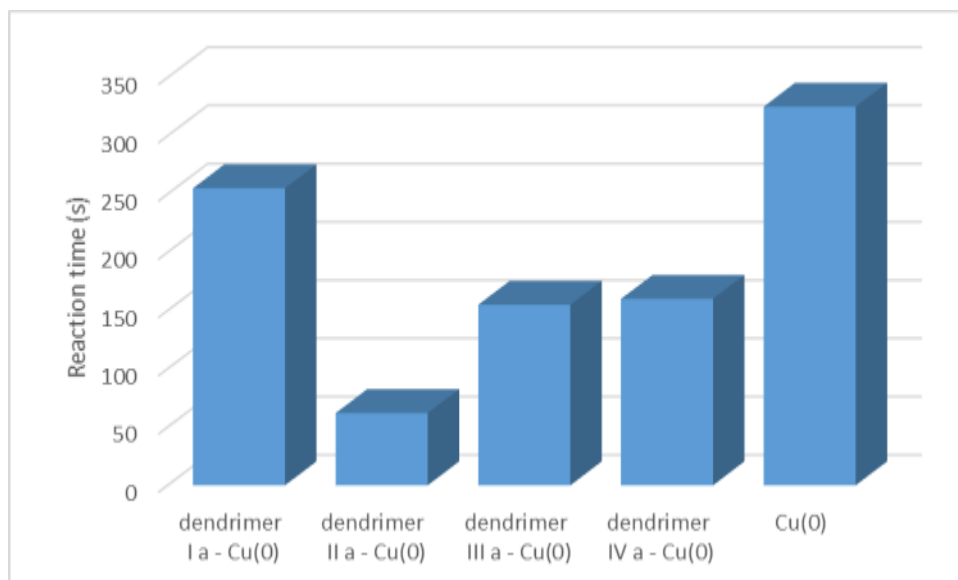
For reference Cu(0) nanoparticles were prepared from 5 ml methanolic solution with 5% CuCl<sub>2</sub>\*2H<sub>2</sub>O to which was added 70 mg of sodium borohydride. The nanoparticles thus obtained were separated by filtration and dried in air.

Figure 13 shows the absorbance at 401 nm depending on the time.



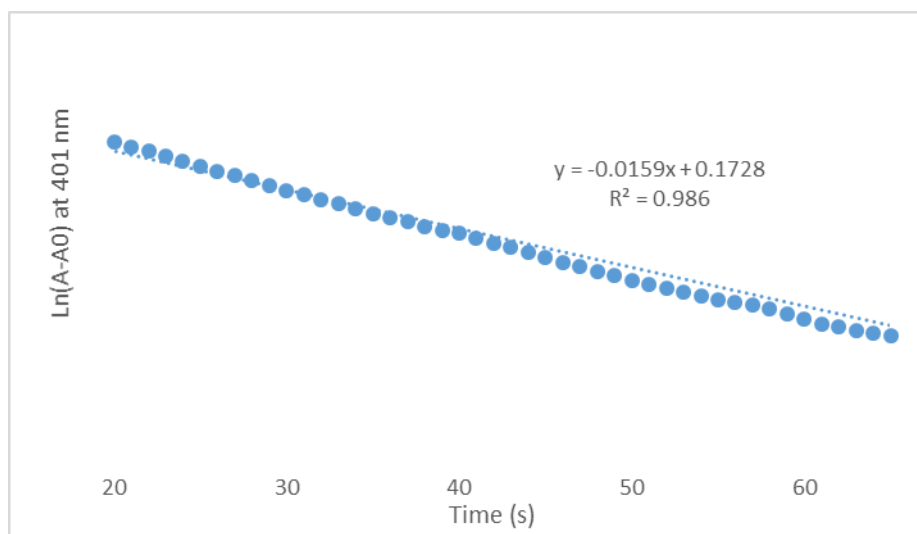
**Fig. 13.** Catalytic efficiency for dendrimer-Cu(0) nanoparticle complexes

All dendrimer-Cu (0) complexes exhibited catalytic activity superior to Cu (0) nanoparticles, which leads to the fact that, by using a dendrimer containing terminal hydroxyl groups reduces the reaction time necessary for nitro derivatives hydrogenation (Figure 14 ).



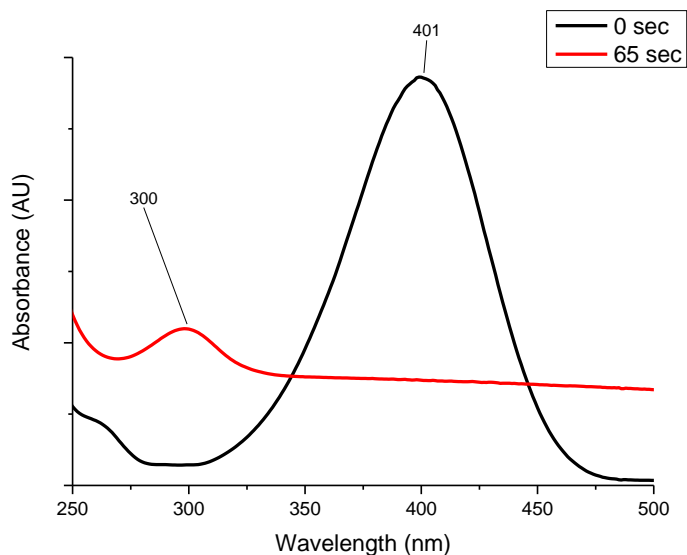
**Fig. 14.** Total time for reducing quantitatively of the p-nitrophenol

In order to calculate the reaction rate were chosen the data obtained for the shortest reaction time, with the catalyst-dendrimer **II** Cu(0) complexes. For constant speed calculation first order reaction was considered:  $\ln(A) = -k_{\text{obs}}*t + \ln A_0$



**Fig. 15.** Calculation of the speed constant.

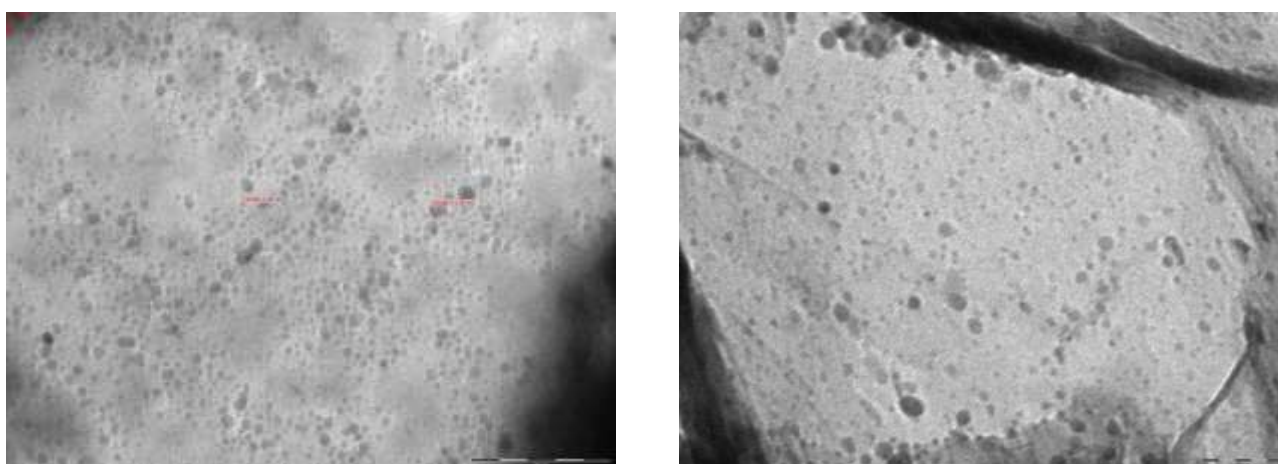
From regression equation ( $R^2 = 0.986$ ), with high correlation coefficient, speed constant are  $k_{\text{obs}} = 0.0159 \text{ s}^{-1}$ .



**Fig. 16.** Catalytic reaction in the presence of the dendrimer **II a**-Cu(0)

In Figure 16 presented above the black spectrum line was recorded at zero reaction time after adding the catalyst. As expected, we have a maximum absorption at a wavelength characteristic mixture PNP + NaBH<sub>4</sub>. The red line represents the absorption spectrum recorded after 65 seconds of reaction monitoring. Observe a maximum absorption wavelength at 300nm characteristic for hydrogenated product (PAP) and the disappearance of the absorption peak at 401nm which indicating that the p-nitrophenol was consumed.

Also, the complexes with Cu (0) thus obtained were analyzed by TEM microscopy. The data obtained show that the size of the nanoparticles of Cu (0) is between 2-13 nm (Figure 17).



**Fig. 17.** TEM Micrograf– left : dendrimer-Cu(0) nanoparticle complex; - right: graphene -Cu(0) nanoparticle complex.



The results of this phase have materialized in these ISI articles and international conferences:

1. M. V. Diudea, B. Szeffler, Cluj and Omega Polynomials in PAHs and Fullerenes, *CURRENT ORGANIC CHEMISTRY*, **2015**, 19 (4), 311-330.
2. M. E. Fustos, M. V. Diudea, G. Katona, Catalytic reduction of 4-nitrophenol using new Cu(0)/aromatic core dendrimer complexes, *Studia Universitatis Babes-Bolyai Chemia*, **2015**, in press.
3. M. Diudea, Multi-shell clusters in 3D and higher dimensional spaces, *Studia Universitatis Babes-Bolyai Chemia*, **2015**, in press.
4. M. E. Fustos, M. V. Diudea, G. Katona, Catalytic reduction of 4-nitrophenol using new Cu(0)/aromatic core dendrimer complexes, Nanoscience in Chemistry, Physics, Biology and Mathematics, NANOMATHCHEM 2015, 12-14 november, **2015**, Cluj-Napoca, Romania.
5. M. V. Diudea, Multi-shell clusters in 3D and higher dimensional spaces, Nanoscience in Chemistry, Physics, Biology and Mathematics, NANOMATHCHEM 2015, 12-14 november, **2015**, Cluj-Napoca, Romania.
6. K. Nagy, C. L. Nagy, M. V. Diudea, Theoretical investigation of three-terminal junctions, Nanoscience in Chemistry, Physics, Biology and Mathematics, NANOMATHCHEM 2015, 12-14 november, **2015**, Cluj-Napoca, Romania.
7. C. L. Nagy, K. Nagy, M. V. Diudea, Closed-shell octahedral carbon nanoclusters, Nanoscience in Chemistry, Physics, Biology and Mathematics, NANOMATHCHEM 2015, 12-14 november, **2015**, Cluj-Napoca, Romania.
8. K. Nagy, M. V. Diudea, C.L. Nagy, Theoretical investigation of symmetrical three-terminal junctions, *Studia Universitatis Babes-Bolyai Chemia*, **2015**, in press.
9. M. V. Diudea, O. Ponta, K. Zahra, Multi Shell Cluster Building a topological approach, 3rd International Congress on Nano Science & Nano Technology (ICNT 2015), 02-03 july **2015**, Istanbul, Turcia.
10. C. L. Nagy, K. Nagy, Corrugated carbon nanotubes – a DFT study, 17th Frühjahrssymposium 2015 (FJS), 25-28 march **2015**, Munster, Germania.
11. C. L. Nagy, K. Nagy, M. Diudea, Three terminal armchair carbon nanotube junctions, 17th Frühjahrssymposium 2015 (FJS), 25-28 march **2015**, Munster, Germania.
12. K. Nagy, C. L. Nagy, Theoretical study of six-terminal armchair carbon nanotube junctions, 21st Internațional Conference on Chemistry, 23-27 september **2015**, Sumuleu-Ciuc, Romania.