FULERENIC MATERIALS WITH BIOMEDICAL APPLICATIONS

Radu Claudiu FIERASCU[1], Rodica Mariana ION[2], Irina DUMITRIU[3]

Rezumat: *Derivații solubili de tip fuleneric sunt esențiali numeroaselor tehnici biomedicale care exploatează structura chimică unică şi proprietățile fizice ale nanosferei de carbon. Toxicitatea lor, demonstrată in vitro şi in vivo, este importantă pentru caracterizarea şi limitarea acestor aplicații. Fototoxicitate unor molecule fulerene a fost identificată ca un instrument terapeutic viitor. Alte studii, axate pe reducerea fototoxicității fulerenelor hidrosolubile urmăreşte utilizarea acestor compuşi ca sisteme de livrare de droguri sau folosirea lor în domeniul protecției mediului. Pornind de la caracteristicile acestor compuşi, care pot fi ei □nşişi citotoxici, sau ar putea deveni în timpul iradierii (fotosintetizatori) am încercat să obținem noi materiale bazate pe fullerene şi diade/triade fullerene/porphyrines sau fullerene/complexe calixarenes. Complexele obținute au fost caracterizate prin spectroscopie UV Vis şi IR.*

Abstract: *Soluble fullerenic derivates are essential for numerous biomedical techniques that exploit the unique structural chemical and physical properties of carbon nanospheres. Their toxicity, demonstrated in vitro and in vivo is important for the characterization and limitation of those applications. The phototoxicity of some fullerene molecules was identified as a future therapeutical instrument. Other studies focused on the decrease of the phototoxicity of hydrosoluble fullerenes follow the use of those compounds as drug delivery systems or their use in environment protection. Starting from the characteristics of those compounds, which can be by themeselves cytotoxic, or could become during irradiation (photosensitizers) we have tried to obtain new materials based on fullerenes and diads/triads fullerene/porphyrines or fullerenes/calixarenes.The obtained complexes were characterized by UV Vis and IR spectroscopy.*

Keywords: fullerene, porphyrines, calixarenes, biomedical applications

1. Introduction

Soluble fullerenic derivates are essential for numerous biomedical techniques that exploit the unique structural chemical and physical properties of carbon nanospheres [1-5]. Their toxicity, demonstrated in vitro and in vivo is important for the characterization and limitation of those applications. The phototoxicity of some fullerene molecules was identified as a future therapeutical instrument.

[1]Scientifical Researcher, PhD student, ICECHIM Bucharest / Valahia University, FIMMR Faculty, Romania (radu_claudiu_fierascu@yahoo.com)
[2]Prof., PhD, Scientifical Researcher I, Valahia University, FIMMR Faculty / ICECHIM Bucharest, Romania (rodica_ion2000@yahoo.co.uk).
[3]Scientifical Researcher III, PhD student, ICECHIM Bucharest / Valahia University, FIMMR Faculty, Romania (dumitriu.irina@yahoo.com).

Other studies focused on the decrease of the phototoxicity of hydrosoluble fullerenes follow the use of those compounds as drug delivery systems or their use in environment protection. Starting from the characteristics of those compounds, which can be by themeselves cytotoxic, or could become during irradiation (photosensitizers) we tried to study in vitro C60 and C70 fullerenes, and some of their functionalized derivates, on experimental models in vitro with normal and tumoral cells, and the investigation of their toxicological and pharmacological profile, in order to identify new anti-neoplasic therapeutical instruments [6]. C60 and C70 fullerenes were prepared and purified, as well as their complexes with PVP (poly-vinyl-pyrolidone) and with the oxo-dimer $(Fe\text{-}O)_2TPP$.

2. Experimental

In the present report, is presented the synthesis and the characterization of some C60 and C70 complexes: μ-oxobis [5, 10, 15, 20-tetraarylporphyrinatoiron (III)] $(TXP\text{-}Fe)_2O$, where X= phenyl, naphthyl, tolyl, and μ-oxobis [5, 10, 15, 20-tetraarylporphyrinatomanganese (III)] $(TXP\text{-}Mn)_2O$, where aryl is phenyl or naphthyl. The Fe-μ-oxo-dimer-porphyrin was obtained by the typical method. After dissolving 0.5 g TPPFeCl in 60 ml $CHCl_3$ (1 hour reflux), 50 ml 25% KOH solution was added. The $(TPP\text{-}Fe)_2\text{-}O$ compound was separated by chromatography on alumina column and eluted with chloroform.

This method can be followed for $(TPP\text{-}Fe)_2\text{-}O$, $(TNP\text{-}Fe)_2\text{-}O$ and $(TTP\text{-}Fe)_2\text{-}O$. The same synthesis method is suitable for the Mn compounds. Similar recipe can be used for $(TXP\text{-}Fe)_2$•C70 complexes. The crystals of $(TXP\text{-}Fe)_2$•C60 complex were obtained by slow evaporation of toluene solution containing 20 mg of C60 and 38 mg of $(TPP\text{-}Fe)_2\text{-}O$ (1:1 molar ratio) under argon flow for a week (yield 90%). The complexes were characterized by UV-VIS and IR spectroscopy.

3. Results and discussions

The stoichiometry of those complexes is 1:1. μ-oxo-dimer complexes of trivalent metals (Mn, Fe) with different ligands – tetraphenylporphyrin (TPP) and tetranaphtylporphyrin (TNP) have similar structures. In the fullerene- μ-oxo-dimer complexes, the fullerene molecule is embraced in a pocket built by porphyrins (figure 1).

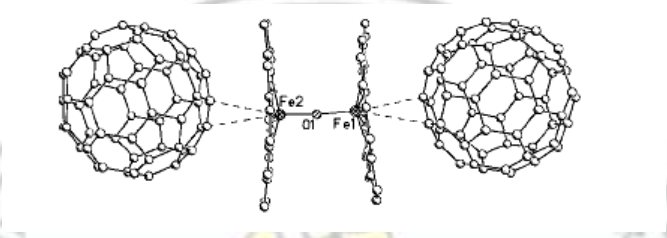

Fig. 1. The iron compound synthesized in the study

The purity of the synthesized systems was evaluated from the absorption spectra in different solvents:

- in Py, the monomer reacts leading to TPP-MeCl-Py, while the μ-oxo-dimer is inert to Py;

- in triethanolamine, the monomer reaches the μ-oxo-dimer form by polymerization.

In figure 2 are presented the UV-VIS spectra of TPP-MnCl and $(TPP\text{-}Mn)_2\text{-}O$

In figure 3 are presented the UV-VIS spectra of TPP-FeCl and $(TPP\text{-}Fe)_2\text{-}O$.

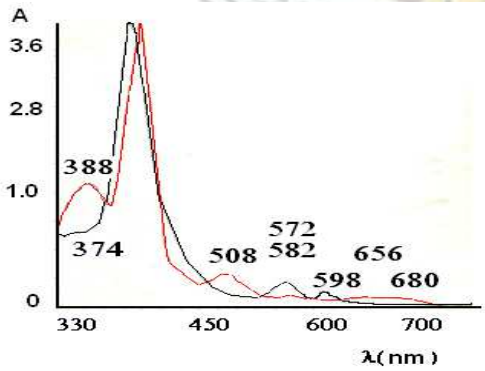

Fig. 2. The UV VIS spectra of $(TPP\text{-}Mn)_2\text{-}O$ and TPP-MnCl (insert)

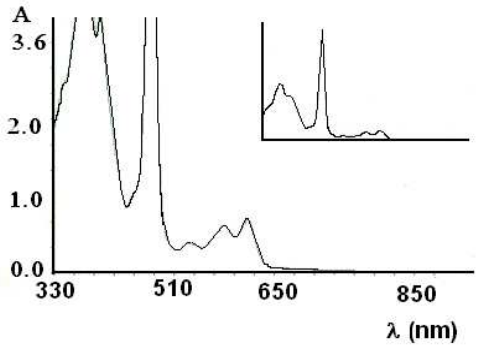

Fig. 3. Comparative UV-VIS spectra of $(TPP\text{-}Fe)_2\text{-}O$ (continuous line) and TPP-FeCl (interrupted line)

The IR spectra identifies the Metal-Oxygen-Metal by it's vibration, corresponding to the band at 850 cm^{-1} (Mn-O-Mn) and 773 cm^{-1} (Fe-O-Fe); the δ values were found for the Metal-Oxygen bounds (525 cm^{-1} for Mn-O and 435 cm^{-1} for Fe-O) and Metal-N (449 cm^{-1} for Mn-N and 395 cm^{-1} for Fe-N).

The IR spectra of TPP-FeCl in CCl_4 is presented in figure 4.

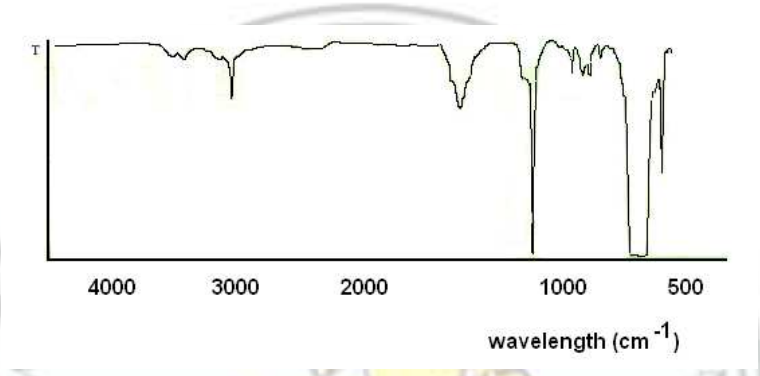

Fig. 4. IR spectra of TPP-FeCl

In order to study the biomedical applications, the systems was solubilized with poly (vinylpyrrolidone) (PVP) in DMSO: water, and applied to K562 cells system. On incubation of C60 with various concentrations of PVP, cell differentiation and proliferation were potently inhibited, although weaker than the vehicle controls.

Considering that the long range purpose of the study is the biomedical application of the compound, the *in vitro* cytotoxicity profile is obviously an important start point. In the 0.05 – 50 μM TPP-C60 concentration range, the LDH release from metabolically active K562 cells incubated for 1-6-18h displayed the same pattern (Figure 5). We have obtained at higher than 5 μM an increase of the LDH release compared to controls while concentrations above 25 μM kill over 50% of the cells. For studying the loading efficiency we have detailed the lower than 5 microM concentration namely 0.25-1 μM range is using 1-18h incubation time.

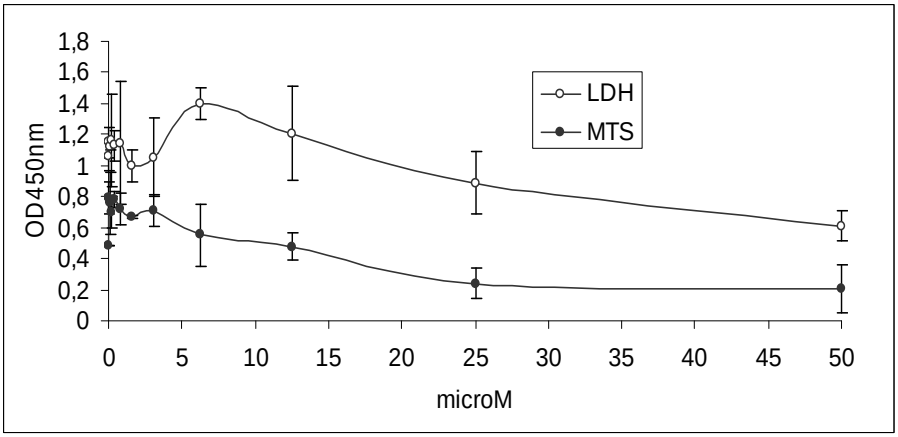

Fig.5. LDH release (LDH) from metabolically active K562 cell line (MTS) in presence TPP-PVP-C60 in non-irradiated protocols represented as OD450nm (mean±SD of 6 individual experiments with triplicates/sample).

Cellular loading efficiency was studied indirectly by registering the supernatants and cell lysates spectra. Cells were efficiently loaded with the 0.5 μM concentration, thus for irradiation we have chosen the mentioned concentration.

During irradiation of K562 cells loaded with 0.5 μM cells after time incubation are actively destroyed (Figure 6). The actual cell counting showed that after irradiation we recover a mean of 20% when cells were loaded for 18h compared to an 80% in unloaded and irradiated controls.

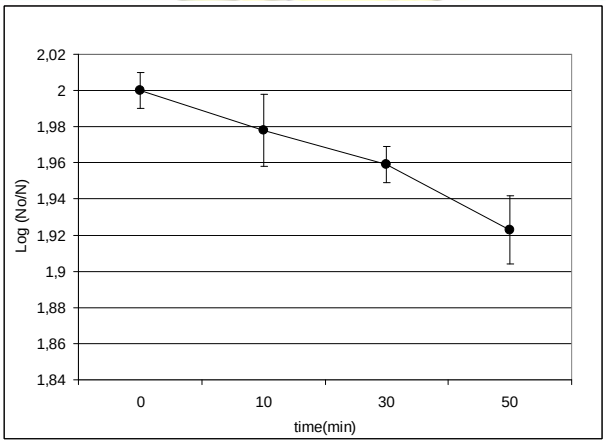

Fig. 6. Photodegradation kinetics of K562 cellular suspension during the lamp irradiation

Investigated in TEM, irradiated cells both, control and TPP-C60 loaded K562 cells, showed an increased number of modified mitochondria. Mitochondria were slender and longer than in control cells and showed an electron-dense appearance, whereas they were poorly defined and less dense in the K562 cells (Figure 7). TPP-C60 loaded cells showed an increased number of mitochondria and large liposomes (Figure 7) independent of the compound concentration. TPP-C60 seems to be located free in cytoplasm, in the nuclear matrix and in mitochondria. Electron-dense 10-20 nm round isolated particles could be seen in treated K562 cells with an uneven distribution.

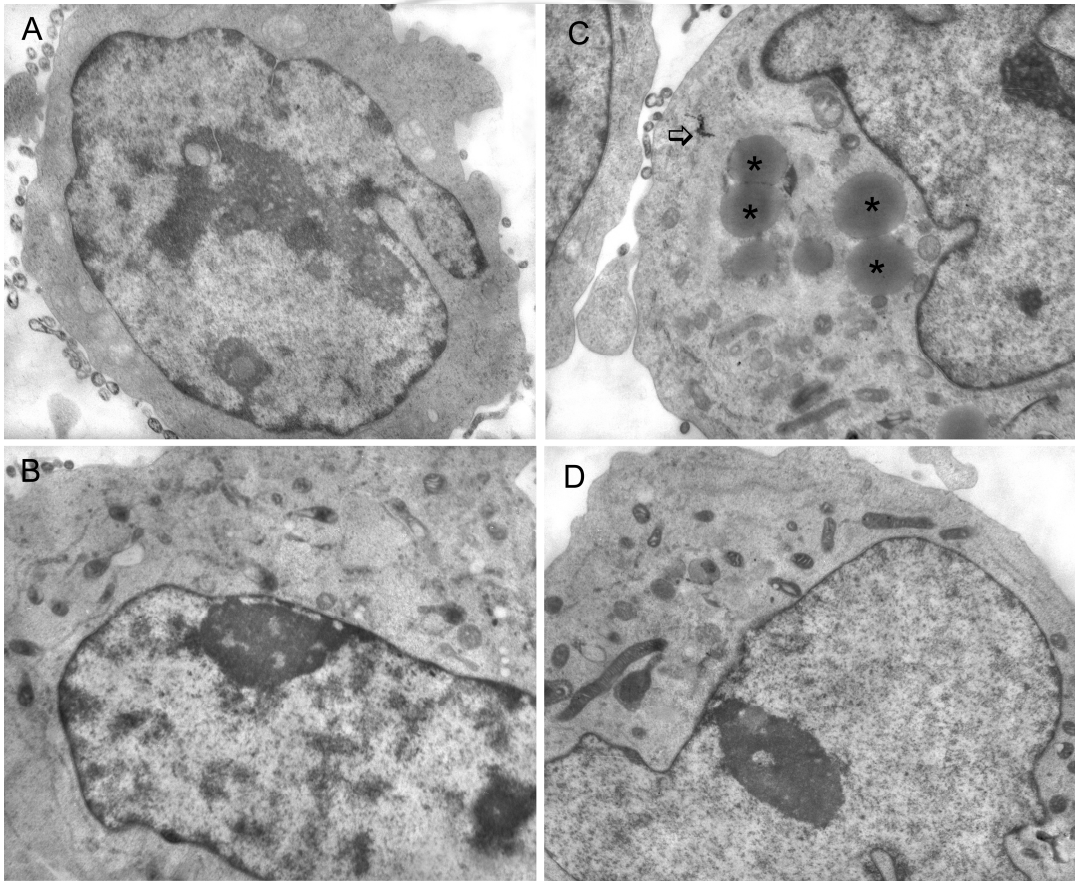

Fig. 7. Irradiated cells investigated in TEM

As the intracellular localization is, among other organelle, mitochondria we have investigated the mitochondrial membrane potential. We found that the higher rate of depolarization was found when cells were irradiated after 18h of incubation with the compound and that irradiation of unloaded cells induce a good mitocondrial depolarization. The lack of a caspase-3 activation correlated with the mitochondrial depolarisation could be explained by the fact that active caspase-3 localizes in the mitochondria after translocation from the cytosol [6] and PDT protocol hindered the translocation. Moreover, we cannot exclude the findings that outer mitochondrial membrane permeabilization during apoptosis triggers caspase-independent mitochondrial potential depolarization [7 - 9].

Conclusions

Even if a series of porphyrinic compounds or nanostructures are already in different study stages, from pre-clinic to clinic, the mechanisms at cellular and molecular level, which are the base of the effects on biological systems and of their use in photodynamic therapy (PDT) are still the subject of scientifical controversy. Because most of the photosensitizers are amphyphilic and are partitioned in the lipid membrane layer, the activation by laser irradiation induces the destruction of the protein from the membrane level, and the singlet oxygen generated is diffused by the lipid membrane phase to the cytosolic phase. There are some proves that the mitochondria is the central coordinator of the mechanisms by which PDT induces apoptosis in the targeted cells. Recent studies demonstrate that the permeability of the external mitochondrial membrane favors the release of some pro-apoptotic factors in cytosol and the activation of the caspaze. The regulatory mechanism activated during PDT is dependent of the photosensitizer type, its dosage and of the type of the target cell. In laboratory research, nano-oncology offers certain promises in cancer treatment. As an example, nano-vectors can contribute to antitumoral drug-delivery and at the local destruction of the cancerous and pre-cancerous cells. Before those nano-instruments could be clinical applied, some preliminary studies are necessary, including toxicological evaluation. The possible toxicological problems associated to those nano-particles are to be investigated, right now being a deficit of data in this problem.

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