

UDC 519.9

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MOLECULAR FULLEROL C₆₀ AND BIOTIN WATER SUSPENSIONS : MODELLING AGGREGATION AND OPTICAL ABSORPTION SPECTROSCOPY TESTING

The peculiarities in the absorbance spectra – the absorption bands with maxima near 266, 340 and 522 nm – for C₆₀(OH)_n(O)_m aggregates in water, which are controlled by the addition of biomolecules (biotin with 1mM content) were revealed. With aim to explain these peculiarities the proposed model for deaggregation of hydroxylated C₆₀ with C-O⁻ by biotin molecules due to the interaction between its C-OH and biotin (-CO)⁻ groups with the self-organization C₆₀ quasicrystals with observed absorbance peak at 400nm confirming of this model.

Key words: C₆₀ aggregates, C₆₀ derivatives, suspension, biotin

Introduction. Chemical and physical features of fullerene C₆₀, together with its spherical shape, have raised the hope of successful use in many different areas either in biological or material chemistry (6, 1). The condensed aromatic rings present in the compound lead to an extended p(π-π) conjugation of molecular orbitals causing significant absorption of visible light. The facile electron acceptability of up to six electrons makes them good candidates as electron acceptors (10, 11). Also, fullerene compounds have avid reactivity with free radicals. Potential biological activities of fullerenes have been investigated with the aim of using it in the sphere of medicine (5–7). An important hindrance for this application is the low solubility of fullerenes in polar solvents and the consequent formation of aggregates in aqueous suspensions (4). However, the development of covalent chemistry of C₆₀ has revealed the possibility to attach these spherical structures with several groups, which allow increment in the biological activity (1, 3, 8). Therefore, for future performance in biomedical application, fullerenes have to be functionalized by dint of hydrophilic substituents. Many substituents have been bound attached to C₆₀ to produce water solubility, such as:

1) negatively charged carboxylic acids (10), 2) positively charged quaternary ammonium (4), 3) neutral poly (ethylene glycol) (4). Within this category of water-soluble fullerenes, the hydroxylated C₆₀ – C₆₀(OH)_n holds a special place and has already been studied in very various fields (5).

Variety biologically important biospecies such as enzymes proteins and antibodies can be used as recognition elements for biosensors (7). However, because most of these biologically important biospecies are water soluble, they cannot be employed as recognition elements of biosensors. In addition, these water-soluble biospecies are difficult to recover and reuse after analysis by the biosensors. Therefore, water-insoluble and biospecies were prepared and applied as reusable recognition elements of biosensor (6) There are immobilized C₆₀-biospecies, e.g. C₆₀-catalase, C₆₀-anti IgG and C₆₀-anti-hemoglobin were also applied as coating.

The fullerene C₆₀ molecule has 30 double bonds and can be considered as an olefin molecule. Like olefin molecules, fullerene C₆₀ can be electrophilically attacked by electron-releasing molecules such as amines (8). The enzyme, protein and antibody molecules like amines contain the NH group, so they can be expected to chemically materials for biosensors to detect various biospecies, e.g. hydrogen peroxide, IgG, anti-gliadin and hemoglobin in suspensions.

We have found that nano – C₆₀ can form over a wide range of mixing conditions and pH and is quite stable at ionic strengths, at or below 0.05 I, for months. Andrievsky and al. suggested that pH was an important parameter for these colloids because of the stability of the surface charge. This is founded that for pH values between 3.75 and 10.25, nano-C₆₀ is formed and that, as the pH of the water is varied, a change in the average particle size is

observed. Higher pH values result in smaller nano-C₆₀ populations and lower pH values give rise to larger particle populations. In addition, as the pH is increased and the average particle size is smaller, a blue shift in the UV/Vis spectrum is observed in the 330–350 nm range (7).

With this information about the physical structure, chemical properties, and stability in hand, we examined the biological effects of C₆₀ using biotin molecules (9).

Biotin – dependent carboxylases catalyze a variety of carboxyl transfer reactions in a number of metabolic pathways and are found in all free-living organisms. They are large molecules which can comprise a single polypeptide chain with three domains or up to three subunits, each of which performs a particular part of the overall reaction. Biotin plays a central role in the action of enzymes (2, 3).

Results and discussion. The aim of the investigation is to reveal peculiarities in absorbance spectra – electronic structure – of the hydroxylated C₆₀ in water suspensions (Fig.1a) with biotin molecules. Biotin is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring, which is an organic compound consisting of a five-membered ring containing four carbon atoms and a sulfur atom.(3) A valeric acid substituent–straight chain carboxylic acid with the chemical formula CH₃(CH₂)₃COOH – is attached to one of the carbon atoms of the tetrahydrothiophene ring.(Fig.1b).

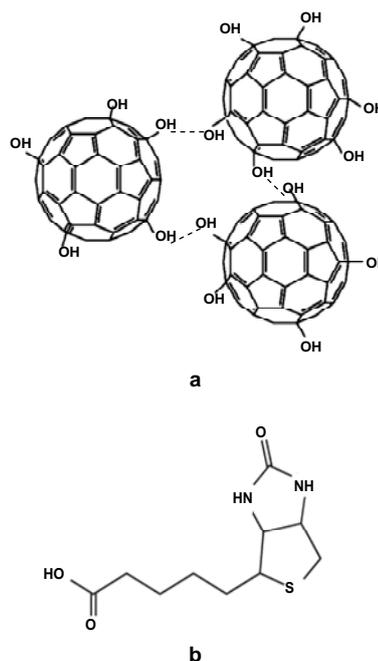


Fig. 1. Schematic images of: a – C₆₀(OH)_n(O)_m aggregate in aqueous as a network of intermolecular located inside hydrogen bonds and with negatively charged O⁻ which are located outside the aggregate's core; b- atomic, structural model of biotin molecule C₁₀H₁₆N₂O₃S

We build our model on the hypothesis that biotin has "+" charge on the chemical tails like as $(-\text{CO})^+$ or "-" charge like $(\text{COO})^-$ groups, in water suspensions.(13) Then, hydroxylated C_{60} in water suspension will have model $\text{C}_{60}(\text{OH})_x(\text{O})_y$ aggregate is presented in Fig.1,a (11, 12)

For the experimental verification of C_{60} aggregates model in water suspension (Fig.1a) and ones changing with added biotin molecules having "+", "-" charges, which induce self-organization groups from C_{60} ($\text{C}-\text{O}^-$) biotin with $(-\text{CO})^+$ group were preparing water suspension with controlled pH =10.2 to 2.2. controllability of the consist by added biomolecules in water suspension were determined from absorbance spectra at 200–300 nm range for biotin (D- Biotin, $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$, Sigma-Aldrich). We started with fullerene C_{60} , >99% pure, MER Corporation Fullerenes.

UV-vis absorption spectroscopy was performed using a UV-vis-NIR spectrometer Jacobs32 and carry out experiment at 300K. The suspensions prepared with ultra pure water ($18.2 \text{ M}\Omega\text{cm}^{-1}$); Milli-Q purification systems Millipore.

The typical absorption spectra for biotin molecules in aqueous suspensions are presented in the Fig.2. The intensity decreasing for absorption bands for biotin molecules incubation during 6 days and maxima are centered at 214nm and 258 nm(the curves 1), and the shifting of these maximum positions after incubation period (the curves 2, respectively) correspondingly to 219 and 262nm are revealed. These peaks confirm our model for building active pair biotin molecules/ C_{60} derivatives, as biotin have absorption maximum in region 210–300 nm, which corresponds to absorption of C_{60} derivatives.

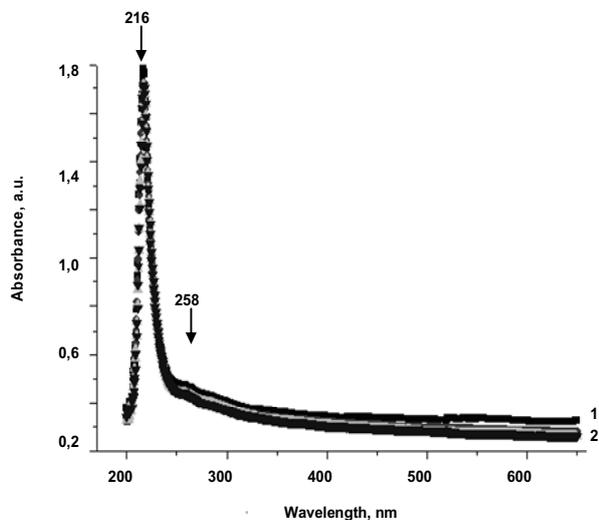


Fig. 2. Recognition of absorption bands initiated by incubation of biotin molecules in aqueous suspension during one day after preparing (were recorded several times) and during three days (were recorded several times)

As was shown in investigations of particle stability at relevant ionic strengths that fullerene aggregates will not remain in solutions simulating seawater or even brackish waters with ionic strengths at or above 0.1 I.

However, at ionic strengths below this (0.05 I and below) an appreciable percentage (0.05 I) if not all (0.01 and 0.001 I) of aggregates remain stable for 15 weeks. These results are important as potential long-term stability is limited to aqueous systems at below 0.05 I, which includes most freshwater environments such as typical groundwaters and surface waters (8). But this study did not investigate other coagulating factors, such as protein, humic acids, or sorption onto or within solid matrixes such as organic matter and soil fractions, which may influence stability.

Two intense broad absorption bands with maxima at 265, 351 nm (Fig. 3) and 270, 347 nm (Fig. 5) dominate in the range 190–410 nm for the fullerene aqueous solutions without and with biotin molecules. The energy positions of these maxima correspond to allowed electron transitions $h_u \rightarrow h_{2g}$ (264, 284 nm) and $h_g, g_g \rightarrow t_{1u}$ (340, 336 nm) (12).

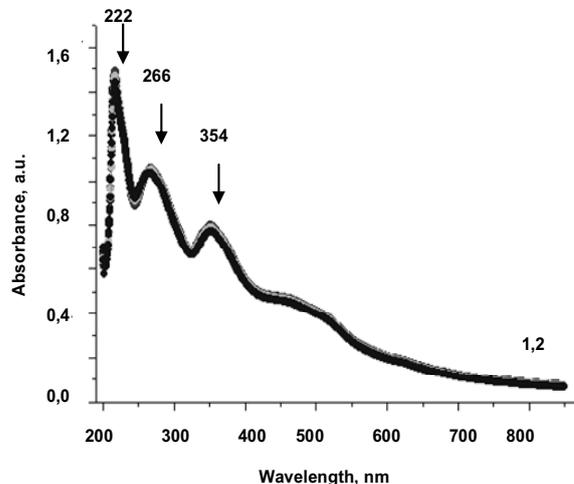


Fig. 3. Recognition of absorption bands initiated by incubation C_{60} derivative molecules in aqueous suspension: the comparison of the absorption spectra of C_{60} derivatives after preparation and after period two days (recorded several times)

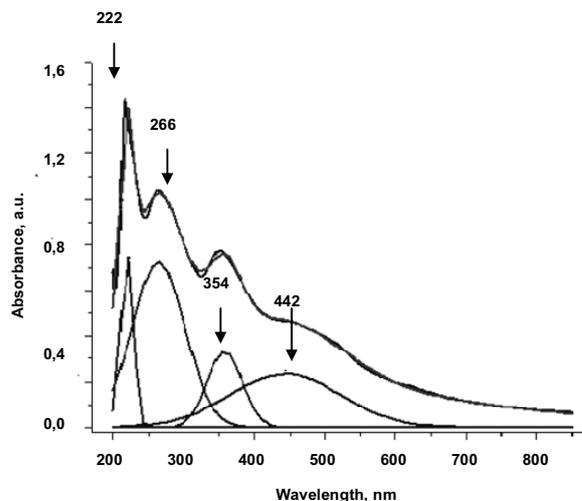


Fig. 4. Deconvolution of C_{60} absorption spectrum shows positions of four bands, residuals are scarcely evident. The positions of Ib, IIb, IIIb, IVb bands are at 222, 266, 354 nm and 442 nm, respectively

The shifts between the maxima in C_{60} and C_{60} /biotin suspension are 2 and 4 nm. With aim to detect the changes in the a marked absorption band for C_{60} fullerol under biotin fictionalization we analyzed the spectra in 200–400 nm region (Fig.5) because, as it can see from Fig.4, the fullerol have the position of the absorption max at 266 nm (12).

In our investigation we founded: Position I peak for 1 spectrum – 3,63; 2,3 spectrum – 3,62; 4 spectrum – 3,63(eV); Position II peak for 1 spectrum – 4,65; 2,3 spectrum – 4,63; 4 spectra – 4,65(eV); Position III peak for 1 spectrum – 5,64; 2,3 spectrum – 5,7; 4 spectrum – 5,72 (eV) (Fig.6). We can conclude that after adding biotin changes in position evaluated near 0, 01 and 0,2 eV. We suggested this molecular surface modification of the fullerene molecules is promising to the biological activity.

When we added biotin molecules we observed shift in longest wavelength region for biotin suspension and it is confirm the changes of pH solution, it is mean that – OH group in fullerol core added in biotin molecules. The comparison of spectra for fullerene solutions with biotin molecules demonstrates the decreasing of the absorbance bands intensity for fullerol C₆₀ with maxima, centered at 266nm and their maximum position shifting to 270nm, after addition biotin in this solution. We can predict that all – OH group are reacting with single biotin molecule.

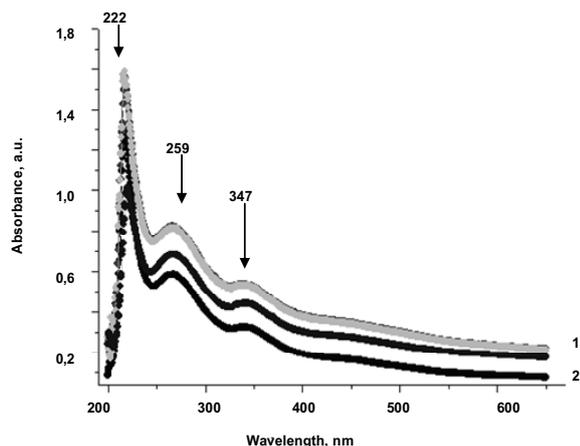


Fig. 5. Recognition of absorption bands initiated by biotin molecules at interface with C₆₀ derivatives in suspension: the comparison of the absorption spectra of C₆₀ derivatives with biotin molecules recorded several times 1 – after preparation, 2 – after 6 days

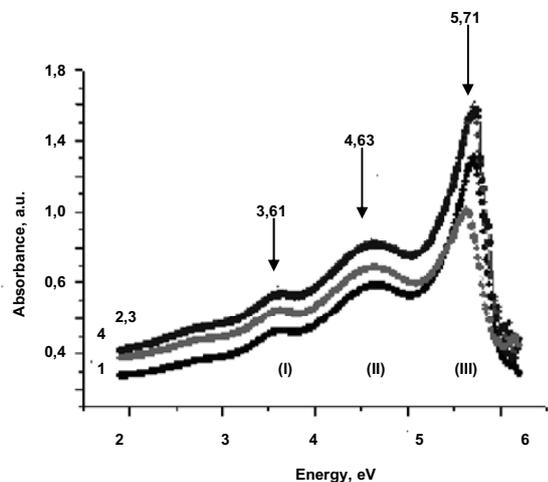


Fig. 6. Recognition of absorption bands initiated by biotin molecules at interface with C₆₀ derivatives in suspension: the comparison of the absorption spectra of C₆₀ derivatives with biotin molecules recorded several times 1 – after preparation (#1, 2 curves), 2 – after 6 days (#3, 4 curves)

In the range of 410–650 nm absorption spectrum of fullerol suspensions there is a wide area of absorption available between 425 and 570 nm, which is a characteristic feature of the absorption spectra of crystals of C₆₀ molecules in the film. The appearance of the absorption band associated with close electronic interaction between neighboring C₆₀ molecules in the crystals, and its location within the 450 and 600 nm depending on the shape and density of the location of these crystals (12). The weak bands near 442 nm appeared in these region (Fig.4). It is known that in the range 490–640 nm (1.9–2.5 eV) (Fig.6) the weak

absorption takes place associated with electric dipole-forbidden transitions between the one-electron HOMO level with h_u symmetry and one-electron t_{1u} LUMO level.

Conclusion. In optical absorbance spectra for C₆₀ aggregates (model in Fig.1a) in prepared water suspensions with and without added biotin presented in Fig. 3 and Fig.5, correspondently, we observed: 1) in compare with theoretical calculations for optical transition in C₆₀ molecule (the peaks in the absorbance spectrum are at 220, 263, 345, 450 nm) for C₆₀ aggregates is the two main peaks at 266 nm and 340 nm (Fig.3), which correspond of the absorbance on (–OH)_n groups and O[–] (groups), respectively. We evaluated that C₆₀ have components 11 for C–OH groups and 7 for C–O[–] groups (5, 9); 2) added biotin molecules determined the shifts of the position of the absorbance peaks from 266 and 354 to 271 and 258, 337 nm, respectively. The intensity of absorbance for C₆₀ aggregates in suspension with and without biotin molecules coincides from 450–650 nm. We assume that the pairs from C–O[–] /one in bases with "+" charge self – organized and than added electron transition at this interface were revealed. 3) due to biotin molecules addition in C₆₀ suspension the absorbance peaks, corresponding electron transition in C₆₀ aggregates disappear. It is the base for our hypothesis about deaggregation of hydroxylated C₆₀ due to the interaction between its C–OH[–] and biotin (–CO)⁺ groups. Finally, model of organization by biotin molecules of C₆₀ quasicrystals confirmed by changes in 410–650 nm region.

Acknowledgments. I would like to thanks my advisors, during measurement at Institute of chemical and biotechnology, TU Ilmenau, Prof. Uwe Ritter, Eugenia Buzaneva for bringing me this amazing filed and endless support of my research.

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Submitted on 01.05.13

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МОЛЕКУЛЯРНІ ВОДНІ СУСПЕНЗІЇ ФУЛЛЕРОЛУ C_{60} ТА БІОТИНУ : МОДЕЛЮВАННЯ АГРЕГАЦІЇ ТА ПІДТВЕРДЖЕННЯ ОПТИЧНИМИ СПЕКТРАМИ ПОГЛИНАННЯ

Проаналізовано особливості спектрів поглинання з максимуми біля 266, 340 та 522 нм для $C_{60}(OH)_n(O)_m$ агрегатів у воді, які керуються додаванням біомолекул (біотин з концентрацією 1 μM). З метою обґрунтування даних особливостей запропоновані моделі для дезагрегації гідроксилваного C_{60} з С-О біотин молекулами за рахунок взаємодії між С-ОН та (-CO)* групами з самоорганізацією квазікристалів з піком поглинання при 400 нм, який спостерігали, та підтверджує дану модель.

Ключові слова: C_{60} агрегати, C_{60} похідні, суспензія, біотин

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МОЛЕКУЛЯРНЫЕ СУСПЕНЗИИ ФУЛЛЕРОЛА C_{60} И БИОТИНА: МОДЕЛИРОВАНИЕ АГРЕГИРОВАНИЯ И ПОДТВЕРЖДЕНИЕ ОПТИЧЕСКИМИ СПЕКТРАМИ ПОГЛОЩЕНИЯ

Проанализированы особенности спектров поглощения с максимумами вблизи 266, 340 и 520 нм для $C_{60}(OH)_n(O)_m$ агрегатов в воде, которые управляются добавлением биомолекул (биотин с концентрацией 1 μM). С целью обоснования этих особенностей предложены модели для дезагрегирования гидроксильованного C_{60} молекулами биотина С-О* за счет взаимодействия между С-ОН и (-CO)* группами и самоорганизацией квазикристаллов с пиком поглощения при 400 нм, который наблюдался, и подтверждает данную модель.

Ключевые слова: C_{60} агрегаты, C_{60} производные, суспензия, биотин.

UDC 537.52, 537.528

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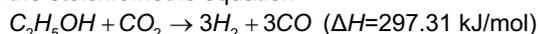
THE INFLUENCE OF CO_2 CONCENTRATION ON THE EFFECTIVITY OF SYN-GAS GENERATION FROM THE ETHANOL IN THE NON-EQUILIBRIUM PLASMA

The results of study of the influence of the CO_2 concentration on the syn-gas compound during the plasma assisted conversion of ethanol in tornado-type electrical discharge are presented. The comparison between both the experimental and numerical simulation results showed the good agreement. It was obtained that the concentrations of the main components of the syn-gas on the reactor outlet do not depend significantly on the rate of CO_2 pumping through the discharge.

Key words: Plasma, numerical simulation, syn-gas, electrical discharge, plasma assisted conversion.

Introduction. Syn-gas (the mixture of carbon monoxide and molecular hydrogen) could act either a fuel or an important component for the synthesis of different organic materials [5]. Depending on the type and compound of organic material one needs different ratios between concentrations of CO and H_2 in syn-gas.

The several methods of syn-gas producing are existed today. There are the treatment of the coal by the overheated water vapors, the partial oxidation of the natural gas, plasma chemical conversion of different hydrocarbons, etc [7]. The latter method is very attractive because it allows one to control the ratio H_2/CO . Usage of ethanol as the raw material for plasma assisted conversion was proposed in [2]. The ethanol was chosen since it is nontoxic and renewable fuel. It can be obtained from the agricultural products and wastes. However, the generation of syn-gas from the pure ethanol has one disadvantage; namely, it does not allow one to control the ratio H_2/CO . In order to avoid it, the add of CO_2 into the ethanol during the plasma assisted conversion is proposed. One can see from the stoichiometric equation



that the admixture of CO_2 allows one to decrease in two times the ethanol consumption, since the carbon atoms present in CO_2 . In addition, the syn-gas with different ratios H_2/CO could be obtained varying the ratio between concentrations of C_2H_5OH and CO_2 .

In the present paper we propose to carry out the plasma assisted conversion of ethanol in tornado-type electrical discharge [1, 3–4, 6].

Experimental setup. Fig. 1 shows the scheme of experimental setup. The main camera 1 is made from quartz. The camera is closed from the upper and the bottom sides by the metal flanges 2 and 3. The level of the fuel 4 is kept constant by the pump through the orifice 5. The metal electrode 6 cooling by the water could act as the cathode or the anode.

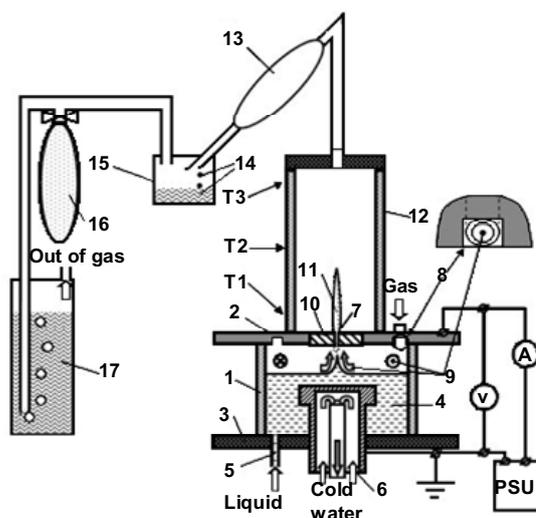


Fig. 1. Experimental setup

The upper flange 2 acts as the second electrode. In the middle of this flange the copper hop 11 with the nozzle 7 is placed. The mixture of CO_2 and air is injected into the vessel through the orifice 8 in the upper flange 2