Carbon nanotubes and conducting polymers in biohybrids

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Our work aimed to design two kinds of biohybrids based on dipalmitoyl phosphatidylcholine biomimetic membranes and carbon nanotubes or polyaniline. These composites were marked with chlorophyll *a* as an optical sensor, and subjected to thermal and oxidative stress conditions. Biohybrids were spectral characterized (Dynamic Light Scattering, Vis absorption and emission spectroscopy) and the antioxidant properties of the biohybrids were assessed by chemiluminescence technique.

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1. Introduction

The development of biohybrid systems gained a large interest in nanosciences due to their unusual properties leading to a wide range of applications of these materials (optics, electronics, and biomedical field).

In our work, biomimetic membranes (liposomes) were used to design two types of biohybrid systems: liposomes-single walled carbon nanotubes and liposomes-polyaniline (emeraldine-salt).

Liposomes are self-assembling lipid vesicular entities consisting of an aqueous compartment surrounded by a phospholipidic double layer, structure very similar with that of biomembranes. The biological matrix is very useful to enhance the biocompatibility to carbon nanotubes (CNTs) and polyaniline (emeraldine-salt) (PANI-ES).

In this research, the artificial lipid membranes were marked with chlorophyll *a* (Chl*a*), an antioxidant bio-porphyrin, for spectral monitoring the formation of biocomposites and for optical detection of the changes of the biomimetic membranes.

As *materials of the future* – carbon nanotubes have a wide variety of applications in optoelectronics [1-4], environmental protection [5] or biomedical field [6-8]. Their biofunctionalization with artificial lipid membranes results in biomaterials with interesting properties [9-12].

Polyaniline (PANI) finds applications in many areas: electronics [13-15], biomedical field [16], or in food industry to remove unwanted substances from food matrices [17]. PANIs are cheap and stable polymers, presenting low cytotoxicity and good biocompatibility [18] so that they are not metabolized by common microorganisms [19].

The biohybrids prepared in our work, were characterized under thermal and oxidative stress conditions using spectral methods (Vis absorption and emission, DLS) and chemiluminescence assay.

2. Experimental part

2.1. Reagents

Dipalmitoyl phosphatidylcholine (DPPC), PANI-ES and single walled carbon nanotubes (SWCNTs) were supplied from Sigma Aldrich (Germany). Potasium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), *tris*(Hydroxymethyl) aminomethane (Tris) and the analytical grade solvents (ethanol, methanol, n-propanol, petroleum ether, acetone, ethyl ether, chloroform) were purchased from Merck (Germany).

Chl*a* was prepared in our laboratory, from fresh spinach leaves according to [20].

2.2. Liposome and biohybrid preparation procedures

Preparation of biomimetic membranes

Small unilamellar lipid vesicles (SUVs) were prepared by ultrasonic irradiation of milky suspensions of multilamellar lipid vesicles obtained through the hydration of a thin film of Chl*a*/DPPC (1:100 molar ratio), as previously described [12, 21].

Preparation of bio-based hybrids

Two types of hybrids based on biomimetic membranes were obtained as follows:

SUV-CNT: specific aliquot from a SWCNT stock suspension was added to a SUV suspension (in a final concentration of 0.9 μg/μL) and further sonicated by using a titanium probe sonicator (15 min with breaks; Hielser,

UP 100 H).
SUV-PANI: specific amount of polyaniline was added to a liposomal suspension (in a final mass ratio of 1:175); this mixture was further sonicated (15 min with breaks; Hielser, UP 100 H Ti probe sonicator).

2.3. Characterization methods

DLS technique

The size of the samples was estimated by DLS technique on a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.), as hydrodynamic diameters of particles suspended in phosphate buffer KH_2PO_4 - Na_2HPO_4 pH 7.4.

The particle size analysis data was performed in the diameter range of 0.6 nm-6 μm, at a scattering angle of 90° and 25°C temperature, by using the intensity distribution. The average diameters (calculated from Stokes-Einstein equation) and polydispersity index were measured in triplicate.

Visible (Vis) absorption spectroscopy analysis

The Vis absorption spectra of liposomes and biohybrids were recorded on a Lambda 2S Perkin Elmer double beam spectrophotometer in the wavelength range of 400-800 nm.

Fluorescence analysis

The fluorescence emission spectra of Chl*a* in liposomes and biohybrids were performed on a Perkin-Elmer, LS55 fluorescence spectrometer, by using 430 nm excitation light.

Fluorescence anisotropy measurements were carried out on the same spectrophotofluorometer as previously described [9].

Chemiluminescence assay

The *in vitro* antioxidant activity of the samples has been determined by chemiluminescence method (CL) on a Chemiluminometer Turner Design TD 20/20, USA, by using a free radicals' generator system consisting of luminol, H_2O_2 in TRIS-HCl buffer solution (pH 8.6).

The antioxidant activity (*AA*) was calculated for each sample as percentage of free radical scavenging according to the relation:

$$AA = \frac{I_0 - I}{I_0} \cdot 100\% \qquad (1)$$

where I_0 is the maximum CL intensity for *standard* (the reaction mixture without the sample) at t = 5 s and *I* is the maximum CL intensity for sample at t = 5 s. All the tests were performed in triplicate.

High value of *AA*% indicates high percentage of free radical scavenging, so strong antioxidant properties of a sample.

3. Results

Spectral characterization of the Chl*a* - based liposomes and biocomposites

The size of the samples was measured by DLS technique. The hydrodynamic diameters, Z_{av} (meaning the particle diameter plus the double layer thickness) and polydispersity index, PdI, are depicted in Fig. 1. PdI represents a measure of the width of the size distribution of the particle population, having a value between 0 and 1, so that high values for PdI indicate a large size distribution with multiple populations of particles.

Liposomes alone presented a mean diameter of 203 nm and PdI = 0.31, while biohybrids are larger, with Z_{av} of 281 and 277 nm for SUV-CNT and SUV-PANI, respectively, with lower values of PdI of 0.25 and 0.23, respectively (Fig. 1). The polydispersity index Pdl < 0.3 shows a narrow distribution of the biohybrid population.

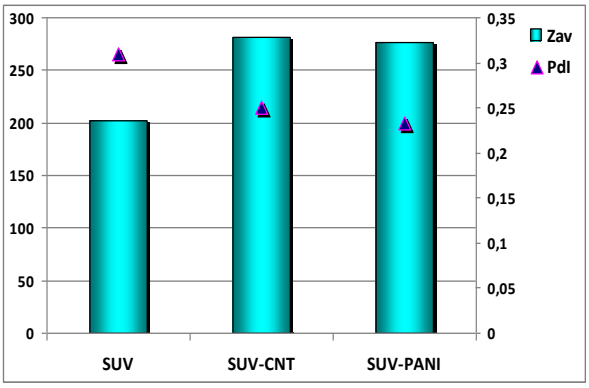

Fig. 1. Size, Z_{av}, and polydispersity index, PdI, of the samples

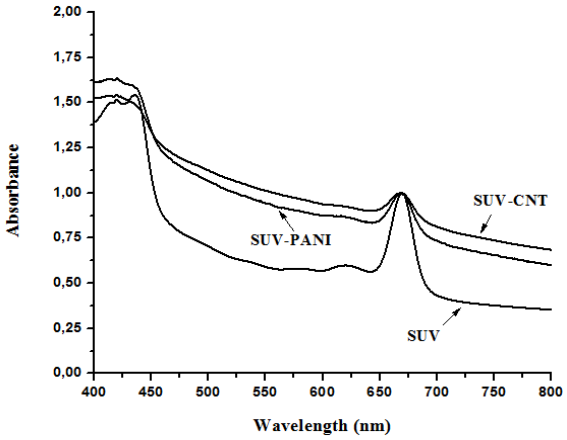

Fig. 2. The Vis absorption spectra of Chla in SUV liposomes and in biocomposites (SUV-CNT and SUV-PANI)

Chl*a* inserted into artificial lipid bilayers was used as a spectral sensor to monitor the events occurred within the Chl*a* - based samples, at molecular level. The absorption spectra of the obtained samples (Fig. 2) show the spectral

fingerprint of Chl*a* consisting of two characteristic bands: the Soret band in the blue region, and a sharp peak in the red region (at 669 nm) of the electromagnetic spectrum. A pronounced light scattering was observed in the case of biohybrids due to their large size as compared to liposomes alone. The Vis absorption spectra were normalized against the maximum in red region. It was observed a decrease in the main red peak area in the order: SUV > SUV-PANI > SUV-CNT.

The emission spectra (Fig. 3) of Chl*a* embedded in liposomes (SUV) revealed a sharp peak at 678 nm (for SUV), 679 nm (for SUV-PANI), and 677.5 nm (for SUV-CNT). The emission fluorescence was quenched in the case of biocomposites.

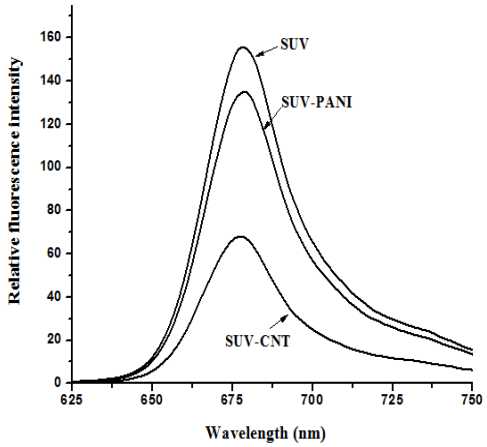

Fig. 3. Fluorescence emission spectra of Chla in DPPC (0.5 mM) liposomes and in biocomposites: Chla-DPPC (0.5 mM) liposomes/SWCNTs & Chla-DPPC (0.5 mM) liposomes/PANI (excitation wavelength: 430 nm)

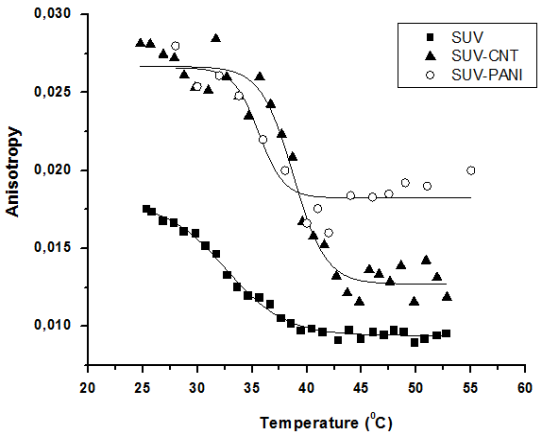

Fig. 4. Thermal behaviour of Chla in the samples

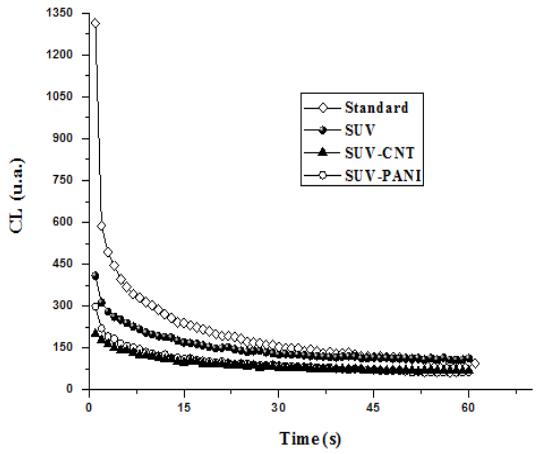

Fig. 5. The profiles of CL signals of the samples as compared to standard system (the reaction mixture without samples)

Thermal behaviour of bionanocomposites based on biomimetic membranes, SWCNTs and PANI was evaluated in the temperature range of 25-55 °C (Fig. 4). Steady-state fluorescence anisotropy of Chl*a* inserted in the DPPC bilayers was measured when the temperature increased from 25 to 55°C using the following wavelengths: λ_{ex} = 430 nm (for excitation) and λ_{em}= 678 nm (for emission). The biohybrids exhibited different thermal behaviour as compared to the artificial lipid bilayers.

The antioxidant capacity of the bio-based samples

It is well known that the *oxidative stress* leads to many diseases [22], so the finding of new antioxidant systems is still a new challenge. In this work, the samples were subjected to an oxidative stress simulated *in vitro*, using a free radicals' generator system based on H_2O_2 and luminol. Fig. 5 shows the profiles of CL signals of the samples as compared to the standard system (the reaction mixture without samples).

As one can observe, all the samples exhibited antioxidant properties. Liposomes alone showed moderate antioxidant capacity (*AA* = 68.95%), due to the presence of chlorophyll in their structure. Bio-based composites presented the highest antioxidant activities: 84.79% for SUV-CNT and 77.32% for SUV-PANI.

4. Discussion

Spectral characterization of the samples revealed a strong quenching of fluorescence intensity in the case of SUV-CNT as compared to SUV-PANI. This fact is explained by an efficientization of energy transfer between the Chl*a* molecules embedded in the lipid bilayers of the biomimetic membranes deposed along the carbon nanotube surfaces, being in accordance with our previous studies [9, 10, 12]. In the case of Chl*a*-DPPC (0.5 mM) liposomes/PANI biocomposites, the fluorescence quenching was lower.

Under thermal stress conditions, the behaviour of Chl*a* fluorescence anisotropy in liposomes was different from those of biocomposites. Chl*a* is sensing a more rigid microenvironment in the presence of SWCNTs and of

PANI, the fluorophore motion being more restricted in these cases. The PANI-based hybrids proved to be more stable under thermal stress.

Both biohybrids exhibited good antioxidant capacities. The kinetics of the oxidative reaction showed a first period of time when the ability of CNT-based hybrids to remove free radicals in system was significant as compared to SUV-PANI. After that, PANI biohybrids became more effective than SUV-CNT.

The antioxidant properties of CNTs could be attributed to their high electron affinity. The free radicals may be "grafted" at the CNT surface *via* radical addition to the nanotube framework [9].

The free radical scavenging mechanism of polyaniline could be explained by the fact that a single unit of the emeraldine salt of polyaniline (which has two benzenoid units) is capable of donating one hydrogen atom [18, 23, 24], thereby eliminating free radicals derived from luminol.

5. Conclusions

In this work, two types of biocomposites have been designed: liposomes - carbon nanotubes and liposomes-polyaniline. The insertion of chlorophyll *a* in artificial lipid membranes was very helpful in monitoring the lipid bilayer dynamics and to check the biohybrid formation.

Both of the designed and tested biohybrids were able to remove the free radicals *in vitro*.

The results are promising and represent a starting point to design new generation of antioxidant biomaterials with multiple applications in biomedical field or in food packaging.

References

[1] V. C. Lokhande, A. C. Lokhande, C. D. Lokhande, J. H. Kim, T. Ji, Journal of Alloys and Compounds **682**, 381 (2016).
[2] G. Saeed, F. A. Khalid, T. S. Chani, M. U. Farooq, I. H. Abidi, T. Sattar, Optoelectron. Adv. Mat. **10**(3-4), 212 (2016).
[3] I. Borazan, A. Bedeloglu, A. Demir, Optoelectron. Adv. Mat. **9**(3-4), 347 (2015).
[4] M. Z. Razali, H. Abdullah, S. Shaari, M. R. Taha, Optoelectron. Adv. Mat. **8**(5-6), 451 (2014).
[5] M. Miculescu, A. Muhulet, A. Nedelcu, S. I. Voicu, Optoelectron. Adv. Mat. **8**(11-12), 1072 (2014).
[6] Z. Liu, S. Tabakman, K. Welsher, H. Dai, Nano Res. **2**, 85 (2009).
[7] A. Lorinczi, M. Popescu, F. Sava, A. Anghel, J. Optoelectron. Adv. M. **6**(1), 349 (2004).
[8] M. Ionita, V. Ciupina, E. Vasile, J. Optoelectron. Adv. M. **13**(7), 769 (2011).
[9] M. E. Barbinta-Patrascu, S. M. Iordache, A. M. Iordache, N. Badea, C. Ungureanu, Beilstein J. Nanotechnol. **5**, 2316 (2014).
[10] M. E. Barbinta-Patrascu, C. Ungureanu, S. M. Iordache, I. R. Bunghez, N. Badea, I. Rau, J. Mater. Chem. B **2**, 3221 (2014).
[11] M. E. Barbinta Patrascu, C. Ungureanu, S. M. Iordache, A. M. Iordache, I. R. Bunghez, M. Ghiurea, N. Badea, R. C. Fierascu, I. Stamatin, Mat. Sci. Eng. C **39**, 177 (2014).
[12] M. E. Barbinta Patrascu, A. Cojocariu, L. Tugulea, N. M. Badea, I. Lacatusu, A. Meghea, J. Optoelectron. Adv. M. **13**(9), 1165 (2011).
[13] N. Hui, F. Chai, P. Lin, Z. Song, X. Sun, Y. Li, S. Niu, X. Luo, Electrochim. Acta **199**, 234 (2016).
[14] N. Parveen, N. Mahato, M. O. Ansari, M. H. Cho, Compos. Part B-Eng. **87**, 281 (2016).
[15] S. Bhadraa, D. Khastgir, N. K. Singha, J. H. Lee, Progr. Polym. Sci. **34**, 783 (2009).
[16] R. Balint, N. J. Cassidy, S. H. Cartmell, Acta Biomaterialia **10**, 2341 (2014).
[17] V. Carrasco-Sánchez, A. John, A. Marican, L. S. Santos, V. F. Laurie, Molecules **20**, 14312 (2015).
[18] M. Gizdavic-Nikolaidis, J. Travas-Sejdic, G. A. Bowmaker, R. P. Cooney, C. Thompson, P. A. Kilmartin, Current Appl. Phys. **4**, 347 (2004).
[19] P. C. Ashley, M. J. Joseph, P. V. Mohanan, Food Chem. **127**, 1808 (2011).
[20] H. H. Strain, W. A. Svec, The Chlorophyll (L. P. Vernon and G. R. Seely eds.), Academic Press, New York, USA, 21 (1966).
[21] M. E. Barbinta-Patrascu, L. Tugulea, A. Meghea, A. Popescu, Optoelectron. Adv. Mat. **2**(2), 113 (2008).
[22] H. Sies, Oxidative stress: introduction. In Oxidative Stress: Oxidants and Antioxidants, ed. H. Sies, Academic Press, London, UK (1991).
[23] J. C.-C. Wu, S. Ray, M. Gizdavic-Nikolaidis, J. Jin, R. P. Cooney, Synthetic Metals **217**, 202 (2016).
[24] A. V. Nand, S. Ray, M. Gizdavic-Nikolaidis, J. Travas-Sejdic, P. A. Kilmartin, Polym. Degrad. Stabil. **96**, 2159 (2011).

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