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EFFECT OF POLYSTYRENE/FULLERENE COMPOSITES ON THE LIPID PEROXIDATION IN BLOOD SERUM

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Abstract. Pure polystyrene films and polystyrene films filled with fullerenes (C_{60}+C_{70} mix) were fabricated by the solution cast method. Effect of polystyrene/fullerene composite on free-radical processes in blood serum has been investigated *in vitro*. The parameters of lipid peroxidation in native serum after adding the composite films were determined by a chemiluminescent analysis and spectrophotometry. It was revealed that fullerene-containing nanocomposites can manifest antioxidant activity in blood serum.

Keywords: antioxidant activity, free-radical process, fullerene-containing nanocomposite, chemiluminescence, malonic dialdehyde.

1. Introduction

Nanocomposite materials are promising field of advanced material sciences with the scope of using in biology, medicine, pharmacology, *etc*. Nanomaterials are based on nanoparticles with unique characteristics resulting from their microscopic size [1].

The discovery of soccer-ball shaped Buckminster fullerene in 1985 [2] was an exciting and unexpected discovery that established an entirely new branch of chemistry. Fullerene is a molecular compound belonging to the class of carbon allotropes. Fullerene molecule is a closed convex polyhedron made of carbon atoms that arrange at the vertices of regular hexagons and pentagons.

Fullerene possesses the unusual physical and chemical properties. Based on these properties the new materials, such as superconducting "fullerenes/alkali metal atoms" compounds, thin films and solutions of fullerenes with nonlinear optical properties, new superhard materials based on fullerenes were obtained [3].

Currently, various derivatives of fullerenes exhibit a broad spectrum of biological activity: anticancer, antiviral, antibacterial, neuroprotective and antioxidant activities [4]. Biological abilities of fullerenes are due to lipophilic properties which facilitate cell penetration and lack of electrons, helping to react with free radicals and generate active oxygen species [5].

Interaction mechanisms of fullerenes with the cell are still poorly understood. To research these processes, various methods have been used, such as molecular dynamics simulations [6], radioactivity method [7], *etc*. It was demonstrated by S. Foley *et al*. [7] that fullerene derivative $C_{61}(CO_2H)_2$ could cross the external cellular membrane, and it localized preferentially to the mitochondria. Formation of oxygen free radicals occurs due to the electrons leakage in the mitochondrial electron transport chain. Therefore, the localization of fullerenes near derivatives mitochondrions may contribute to their antioxidant action.

The mechanism of biological role of fullerene is shown to depend on its aggregate form: crystalline, colloid or soluble organic complex [8, 9]. Soluble organic fullerene complex has the highest bioactivity [8]. L. Piotrovskiy *et al*. [8] explained this effect by low association of nanocarbonic particles.

A new direction of fullerene science has been developed, and the related studies in this direction have concerned the preparation of fullerene-containing polymers, which would combine the unique characteristics of fullerene with the useful properties of matrix polymers [10].

One of polymers able to complex with nanoparticles is polystyrene (PS), which is widely spread in industry. Also polystyrene is used, for example, for the manufacture of surgical instruments, as well as an arterial embolization to stop bleeding. Replacement of pure

polymer by fullerene-containing one will impart it antibacterial and antimicrobial properties. Therefore, polystyrene/fullerene composites are the subject of numerous studies [11-13]. It is considered that integration of fullerenes into polymer matrix can produce biocomposites which have medical potential as drug transporters, antiseptics, and antioxidants. Polystyrene/fullerene composite materials may be used to manufacture containers for blood storing too.

Early we reported on studies of the polystyrene/fullerene composites by a scanning electron microscopy, infrared spectroscopy, and X-ray diffraction [14, 15]. The above listed methods and mathematical modeling have been used to study the influence of the fullerene additives on the structure of polystyrene. We have concluded that during the solvent evaporation and film formation the individual polymer molecules collide and stick together into aggregates. In films without fullerenes, packing of straightened chains parallel to each other predominated. This shows that a polystyrene molecule, which had the shape of a coil in the solution, straightened and stretched itself along the aggregate surface when attached to it. In the polystyrene/fullerene composite, the intermolecular interactions between polystyrene and fullerene are appreciable, and under the influence of fullerene, polystyrene molecules straightened with the formation of ordering elements in the arrangement of chains.

So the fullerene additives effect on the structure of polystyrene. Such modification may result in occurrence of new properties of the polymer, for example, biological activity.

Bioactivity of the material may be evaluated by its effect on the free radical processes in a biologic fluid. Currently regulation of free-radical processes is adjusted by both natural and synthetic pharmaceutical compositions [16]. As any other medicine some antioxidants may produce adverse events. Thus, finding the safe preparations with high antioxidant activity is still actual.

We have analyzed the literature on studies of the polystyrene/fullerene composites, and have concluded that the antioxidant activity of these materials was not researched enough, although for fullerenes it was known. In our opinion, it is necessary to fill this gap. Therefore the goal of present research was to investigate the influence of polystyrene/fullerene nanocomposites on free-radical processes in the biologic fluid (blood serum) *in vitro*.

2. Experimental

2.1. Materials and Subjects

We have chosen polystyrene (Aldrich, Germany, $M_n = 1.4 \cdot 10^5$, $M_w/M_n = 1.64$) as a matrix for fabrication of fullerene-containing nanocomposites, because it has high solubility in aromatic hydrocarbons like fullerene itself. $C_{60}+C_{70}$ fullerene mix (Fullerene Technologies Ltd., Russia) was preliminary purified [17].

Fullerene-polystyrene composition films were produced as follows. Batches of polymer and $C_{60}+C_{70}$ were solved separately in *o*-xylene (or toluene). Concentrations of fullerenes in *o*-xylene solutions were equal to 0.018, 0.054, 0.18, and 1.8 g/l. Concentrations of fullerenes in toluene solutions were equal to 0.18, and 0.9 g/l. Polystyrene batches were dissolved in respective solvents (179.38 g/l of PS) too. Then, fullerene/*o*-xylene solutions and polystyrene/*o*-xylene solutions were mixed together, so that weight fractions of fullerene were equal to 0.01, 0.03, 0.1 and 1 wt %. The mixed solutions were stirred for about 1 day before being cast into thin films. Similar actions were carried out with toluene solutions. After casting the solvent was slowly evaporated over several days to produce the polystyrene/fullerene composite films.

Subject of research was native blood serum of 10 patients managed in V.N. Gorodkov Research Institute of Maternity and Childhood (Ivanovo, Russia). Blood sampling has been carried out from non-pregnant women admitted to hospital with menstrual dysfunction of endocrine genesis. Specimen of pure PS or composite film (size 1.5 cm^2, weight 5 mg) was put into blood serum (1 ml). The system has been incubated for 1 h at 277 K to reduce the effect of microbiological agents.

2.2. Methods

After contact of blood with pure polystyrene films or fullerene-containing polystyrene films, we have studied the free radical processes in blood. Also we have studied the free radical processes in native blood serum without preliminary contact, chosen as a control one. The parameters of lipid peroxidation have been determined by chemiluminescent analysis and spectrophotometry.

Chemiluminescence. Induction of chemiluminescence (ChL) by hydrogen peroxide and iron sulfate is based on Fenton reaction: at mixing the components the catalytic decomposition of hydrogen peroxide by divalent iron ions takes place. Thus formed free radicals oxidize the lipoproteins of blood serum in the test samples, leading to the formation of new free radicals. At the recombination of radicals, unstable products are formed and decomposed with the release of photons.

For the process, the ChL curve dips because of antioxidant agents. The decay rate constant of free radicals k is defined by the dip rate of ChL curve. Therefore, the main indicator of the antioxidant activity of the system is tangent of maximum slope angle of ChL curve towards time axis, $\tan\alpha$.

Also, we used the following parameters:

I_m is maximum intensity of ChL during the experiment. Value of I_m quantifies the level of free radicals, ***i.e.*** gives an idea of the potential ability of the blood serum to free radical lipid peroxidation;

S is an area covered by intensity curve or total light sum. Value of S is inversely proportional to the antioxidant activity of the sample;

$S_n = S/I_m$ is a normalized light sum. The value of S_n evaluates antioxidant activity more correctly than the value of S, because the total area covered by ChL curve depends on the value of I_m.

The induced chemiluminescence tests have been performed by BChL-07 luminometer (Medozons, Russia). We have used hydrogen peroxide and ferric sulfate as inductors of ChL. 0.1 ml of serum, 0.4 ml of phosphate buffer (pH 7.5), 0.4 ml of 0.01 M ferric sulfate and 0.2 ml of 2 % hydrogen peroxide have been put into cuvette. Luminescence has been registered for 40 s.

Free radical processes in serum have been studied after contact with original polystyrene films and fullerene-containing polystyrene films. The mean values of ChL parameters in native serum without addition of the film have been used as controls. The results have been expressed as percentages relative to controls and have been given as mean values ± standard deviations. Level of significance p was 0.05.

Spectrophotometry. Also we have defined the malonic dialdehyde (MDA) concentration in blood samples after contact with PS and PS/C_{60}+C_{70} films. This indicator reflects the amount of the lipid peroxidation products. MDA concentration has been determined using the SF-46 spectrophotometer (Russia) at wave length of 532 nm. This method based on the formation of complex MDA with 2-thiobarbituric acid [18].

Value of total antioxidant reactivity, TAR, has been evaluated by measuring the absorbance before and after incubation of samples, and tacking into account the same quantities for solution of linolenic acid which has been chosen as a standard [19].

3. Results and Discussion

Using above described technique and *o*-xylene as a solvent, we have prepared one sample of polystyrene film and four samples of polystyrene/fullerene composite films with various C_{60}+C_{70} percentages. Unmodified polystyrene samples were colorless, whereas the polystyrene/fullerene composite films were light purple. The intensity of color depended on the content of C_{60}+C_{70} in the composite.

Figure shows kinetics of chemiluminescence in blood serum after contact with original polystyrene and nanocomposite films. Peak of chemiluminescence due to free radical production was in 2 s of the reaction. This can be explained by production of active oxygen species (HO_2^*, O_2^*, O_2^-, OH^-). The highest intensity I_m was registered in case of nanocomposites with 0.01 and 0.03 wt % of fullerenes.

In Table 1 we have represented the main ChL parameters for films prepared by casting of *o*-xylene solution. It can be seen in case of original polystyrene film the ChL parameters were approximate to controls. Value of I_m for the PS/C_{60}+C_{70} composites is higher than for control serum samples. A light sum S was significantly increased only for films with 0.01 and 0.03 wt % of fullerenes ($p < 0.05$). For nanocomposite containing 1 wt % of C_{60}+C_{70}, no significant change in value of S was revealed. In addition we found both significant growth in value of tanα and reduction in value of S_n for all fullerene-containing films (Table 1). So regardless of the fullerenes content, the antioxidant activity of PS/C_{60}+C_{70} composites is higher than for original polystyrene. It seems nanocomposites containing fullerenes were easy to react with oxygen species, preventing lipid peroxidation.

Also intensity of lipid peroxidation has been estimated by malonic dialdehyde concentration and total antioxidant reactivity assessed by spectrophotometry (Table 2).

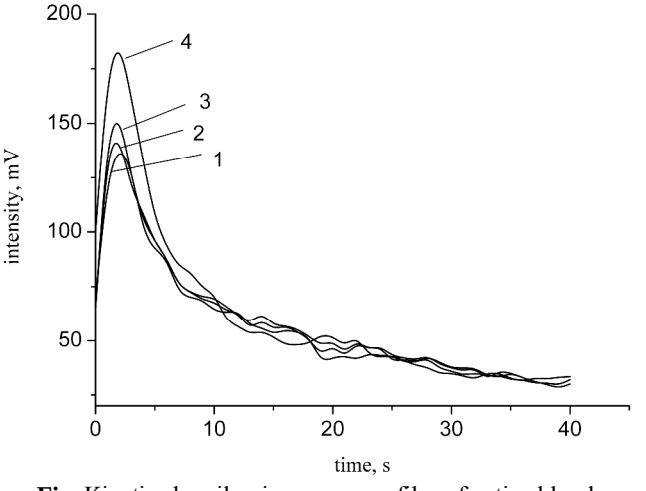

Fig. Kinetic chemiluminescence profiles of native blood serum (1) and after its contact with studied materials: PS (2); PS/C_{60}+C_{70} (1.0 wt %) (3) and PS/C_{60}+C_{70} (0.03 wt %) (4). Solvent: *o*-xylene

We have obtained for serum samples after contact with both pure polystyrene film and composite films, the MDA level was approximate to controls ($p > 0.05$). The exception was the composite film with 0.03 wt % of C_{60}+C_{70}. Currently the reason for this anomaly is unknown, and we plan to continue this study.

Table 1

Chemiluminescence parameters in blood serum after contact with original polystyrene film and fullerene-containing nanocomposites (solvent: *o*-xylene)

Fullerene content, wt %	S, %	I_m, %	tanα, %	S_n, %
Controls	100.0	100.0	100.0	100.0
0	96.0±9.0	97.0±7.0	97.5±10.5	99.0±11.5
0.01	121.5±10.5*	131.5±11.5*	139.0±27.0*	92.0±6.5*
0.03	115.0±8.0*	125.0±11.0*	146.0±27.0*	92.0±7.0*
0.1	105.0±3.0	110.0±6.0*	110.0±8.0*	95.5±5.0
1.0	96.0±12.0	111.0±6.0*	121.0±7.0*	86.5±7.0*

Note: * significant differences compared to control ($p < 0.05$)

Table 2

Lipid peroxidation parameters (MDA, TAR) in blood serum after contact with original polystyrene film and fullerene-containing nanocomposites (solvent: *o*-xylene)

Fullerene content, wt %	MDA	TAR
Controls	100.0	100.0
0	103.0±23.0	108.0±4.0
0.03	113.0±5.0*	116.0±5.0*
0.1	94.0±8.0	110.0±4.5*
1.0	96.5±10.5	107.0±4.0*

Note: * significant differences compared to control ($p < 0.05$)

Table 3

Chemiluminescence parameters in blood serum after contact with original polystyrene film and fullerene-containing nanocomposites (solvent: toluene)

Fullerene content, wt %	S, %	I_m, %	tanα, %	S_n, %
Controls	100.0	100.0	100.0	100.0
0	98.0±7.5	99.0±7.0	98.5±7.5	100.0±7.0
0.1	101.5±10.5	120.5±14.0*	100.5±10.5	84.0±8.5*
0.5	103.0±7.5	101.0±7.5	130.0±14.0*	102.0±5.0

Note: * significant differences compared to control ($p < 0.05$)

It can be seen in Table 2 that total antioxidant reactivity was increased in serum samples after contact with nanocomposites containing 0.03, 0.10, and 1.00 wt % of fullerenes ($p < 0.05$). This proved antioxidant effect of experimental materials. It appears that the amount of active centers, capable to effectively capture and inactivate the free radicals, increases with concentrations of fullerenes in a composite material.

It is interesting to reveal the effect of the medium in which the films have been fabricated. For this we have performed experiments for films prepared by casting of other aromatic compound – toluene.

The main ChL parameters for "toluene" films are given in Table 3. It can be seen that in case of original polystyrene film all values were approximate to controls. But using nanocomposites (0.5 wt % of C_{60}+C_{70}) we have found significant increase in value of tanα. It demonstrates the antioxidant activity of researched PS/C_{60}+C_{70} composites.

In addition, it can be seen in Tables 1 and 3 that at the same concentration of fullerene (0.1 wt %), the value of tanα is higher for the film formed of *o*-xylene than for the film formed of toluene. This can be explained by the fact that the solubility of fullerenes in *o*-xylene is higher than in toluene [20]. It appears that in toluene solution the fullerene molecules are in the form of clusters, which do not ensure uniform distribution of the nanoparticles in the composite film during its formation of the solution.

Also we have performed the preliminary experiments with PS films containing fullerene that have been fabricated by casting of aliphatic compound – chloroform. The results of chemiluminescent analysis and

spectrophotometry for serum samples after contact with both original polystyrene film and composite films regardless of the fullerenes content were approximate to controls. This again emphasizes the significance of the medium in which the films were fabricated.

4. Conclusions

We proved that polystyrene/fullerene nanocomposites have ability to inhibit the lipid peroxidation in blood serum. Furthermore, possibility of such inhibition depends on the fullerene concentration and conditions for forming composites.

Acknowledgments

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ВПЛИВ ПОЛІСТИРОЛ/ФУЛЕРЕНОВИХ КОМПОЗИТІВ НА ПЕРОКСИДНЕ ОКИСНЕННЯ ЛІПІДІВ У СИРОВАТЦІ КРОВІ

Анотація. *Методом лиття з розчину виготовлено плівки чистого полістиролу та полістиролу, наповненого фулеренами (C60 + C70 Mix). Проведено дослідження in vitro стосовно впливу полістирол/фулеренового композиту на перебіг вільно-радикальних процесів у сироватці крові. За допомогою хемілюмінесцентного аналізу і спектрофотометрії визначено параметри пероксидного окиснення ліпідів у нативній сироватці після додавання композиційних плівок. Встановлено, що фулерен-вмісні нанокомпозити можуть проявляти антиоксидаційну активність у сироватці крові.*

Ключові слова: *антиоксидаційна активність, вільно-радикальний процес, фулерен-вмісний нанокомпозит, хемілюмінесценція, малоновий диальдегід.*