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Research Article

Effect of Polystyrene/Fullerene Composites on Free-Radical Processes in Biologic Fluid

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Abstract

Polystyrene films and polystyrene films filled with fullerenes were fabricated by the solution cast method. The effect of fullerene-containing polystyrene nanocomposites on free-radical processes in blood serum has been researched in vitro. The parameters of lipid peroxidation in native serum after adding nanocomposites were determined by chemiluminescent analysis and spectrophotometry. It was revealed that polystyrene/fullerene composites can manifest antioxidant properties in blood serum.

Keywords: polystyrene/fullerene composite, chemiluminescence, lipid peroxidation.

Introduction

The development of polymeric nanocomposites with controllable structure and properties is one of the promising fields of advanced material sciences with scope of using in biology, medicine and pharmacology. Special attention is paid to fullerene-containing polymers which have unique features of both fullerenes and polymers. Andreev et al (2008) mentioned that nowadays a wide specter of fullerene derivates is synthesized, having anticancer, antiviral, antibacterial, neuroprotective and antioxidant activities. According to Da Ros (2008) 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. Piotrovskiy et al (2007) and Lyon et al (2006) discussed

the mechanism of fullerene biological role, and concluded it depends on its aggregate form: crystalline, colloid or soluble organic complex. According to Piotrovskiy et al (2007) soluble organic fullerene complex has highest bioactivity. The authors explain this by low association of molecules in nanocarbonic particles.

One of polymers able to complex with nanoparticles is polystyrene (PS) which is widely spread in industry. Therefore, polystyrene/fullerene composites are the subject of numerous studies by Alekseeva et al (2009), Badamshina and Gafurova (2008), Weng et al (1999). It is considered that the integration of fullerenes into polymer matrix can produce biocomposites which have medical potential as drug transporters, antiseptics and antioxidants. Okovitiy (2003) notes the regulation of free-radical

processes is adjusted by both natural and synthetic pharmaceutical compositions. As any other medicine some antioxidants may produce adverse events. So, the finding of safe preparations with high antioxidant activity is still actual.

The goal of this research was to investigate the influence polystyrene/fullerene nanocomposites on free-radical processes in biologic fluid (blood serum) in vitro.

Materials and Methods

We chose polystyrene (Aldrich, Germany, $M_n=1.4\cdot10^5$, $M_w/M_n=1.64$) as a matrix for fabrication of fullerene-containing nanocomposites, because it has high solubility in aromatic hydrocarbons like fullerene itself. Fullerenes C_{60}

("NeoTechProduct", Russia) were preliminary purified by methods reported by Evlampieva et al (2007). Batches of polymer and C_{60} were solved separately in aromatic solvent (o-xylene or toluene) and then mixed together in necessary proportion to prepare PS/C_{60} composites. The mass fraction of fullerenes in film, Φ, varied from 0 to 0.01. Composite films were prepared by the casting of solution on glass carrier and following slow evaporation over several days. The thickness of the film was equal to 60÷80 μm.

The subject of research was native blood serum mixture of 10 patients managed in V.N. Gorodkov Research Institute of Maternity and Childhood (Ivanovo, Russia). Film specimen (size 1.5 cm^2, weight 5 mg) with curtain fullerene concentration (Φ=0,

0.0001, 0.0003, 0.001, 0.005 or 0.01) was put into blood serum (1 ml). The system was incubated for 1 hour at 4^{o}C.

The parameters of lipid peroxidation in serum after the exposure of the film nanomaterials were determined by chemiluminescent analysis and spectrophotometry.

The induced chemiluminescence (ChL) tests were performed on BChL-07 (Medozons, Russia). We 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 0.01M ferric sulfate and 0.2 ml of 2 % hydrogen peroxide were put into cuvette. Luminescence was registered for 40 s.

To estimate the intensity of lipid peroxidation, we used the following parameters:

J_{max} is the maximum intensity of ChL during the experiment. The value of J_{max} quantifies the level of free radicals, i.e. gives an idea of the potential ability of the blood serum to free radical lipid peroxidation;

$tan\alpha$ is the tangent of the maximum slope angle of ChL curve towards time axis. This value characterizes the decay rate of free radical oxidation, i.e. quantifies an effectiveness of the antioxidant system;

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

$Z=AJ_{max}^{-1}$ is normalized light sum.

Free radical processes in serum have been studied after the exposure of original polystyrene films and fullerene-containing polystyrene films. The mean values of ChL parameters in native serum without the addition of film were used as controls. 8-10 measurements required for each film were carried out on the same day. The results have been expressed as percentages relative to controls and were given as mean values ± standard deviations. A p-value of 0.05 was chosen as the significance limit.

Also, the lipid peroxidation reaction was identified by SF-46 spectrophotometer (Russia) (λ=532 nm). According to Ishihara (1978), a malonic dialdehyde (MDA) was estimated as peroxidation derivate by its complexing with 2-thiobarbituric

acid. Total antioxidant reactivity (TAR) was evaluated by measuring the MDA concentration before and after the incubation of samples using the method reported by Promyslov and Demchuk (1990).

Results and Discussion

The figure shows kinetics of chemiluminescence in serum after the exposure of original polystyrene and nanocomposite films. The peak of chemiluminescence due to free radical production was in 2 s of reaction. This can be explained by the production of active oxygen species (HO_2^*, O_2^*, O_2^-, OH^-). The highest intensity, J_{max}, was registered when the value of Φ was equal to 0.0001 and 0.0003.

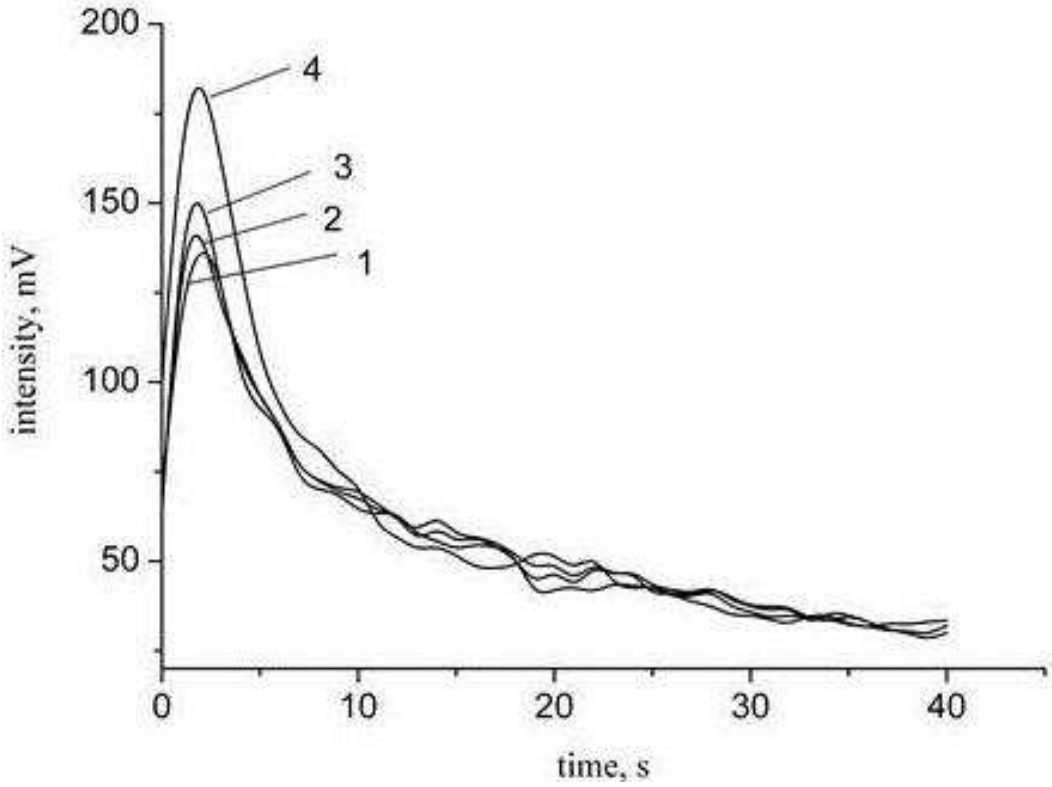

Figure1: Kinetic Chemiluminescence Profiles of Native Blood Serum and after Exposure of Studied Materials: *1*- Native Serum; *2* - PS Film (Φ=0); *3* – PS/C_{60} (Φ=0.01); *4* - PS/C_{60} (Φ=0.0003). (Solvent: o-xylene)

In Table 1, we represent the main ChL parameters for films prepared by the casting of o-xylene solution. It can be seen in case of original polystyrene film the ChL parameters were approximate to controls. The value of J_{max} in case of PS/C_{60} composites is higher than for control serum samples. A light sum, *A*, was significantly increased only for films with Φ=0.0001 and 0.0003 ($p<0.05$). When the value of Φ was equal to 0.01, no significant change in the value of *A* was revealed. In addition, we found both significant increase in the value of tanα and reduction in the value of *Z* for of all fullerene-containing films researched (Table 1). So, regardless of the fullerenes content the antioxidant activity of PS/C_{60} composites is higher than for original polystyrene. It seemed nanocomposites containing fullerenes were easy to react with oxygen species, preventing lipid peroxidation.

Table 1: Chemiluminescence Parameters in Blood Serum after Exposure of Original Polystyrene Film and Fullerene-Containing Nanocomposites (Solvent: o-xylene)

Φ	J_{max}, %	*A*, %	tanα, %	*Z*, %
Controls	100.0	100.0	100.0	100.0
0	97.0±7.0	96.0±9.0	97.5±10.5	99.0±11.5
0.0001	131.5±11.5 *	121.5±10.5 *	139.0±27.0 *	92.0±6.5 *
0.0003	125.0±11.0 *	115.0±8.0 *	146.0±27.0 *	92.0±7.0 *
0.001	110.0±6.0 *	105.0±3.0	110.0±8.0 *	95.5±5.0
0.01	111.0±6.0 *	96.0±12.0	121.0±7.0 *	86.5±7.0 *

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

Table 2: Lipid Peroxidation Parameters (MDA, TAR) in Blood Serum after Exposure of Original Polystyrene Film and Fullerene-Containing Nanocomposites (Solvent: o-xylene)

Φ	**MDA**	**TAR**
Controls	100.0	100.0
0	103.0±23.0	108.0±4.0
0.0003	113.0±5.0 *	116.0±5.0 *
0.001	94.0±8.0	110.0±4.5 *
0.01	96.5±10.5	107.0±4.0 *

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

The intensity of lipid peroxidation was also estimated by malodic dialdehyde concentration and total antioxidant reactivity assessed by spectrophotometry (Table 2). We revealed that nanocomposite with Φ=0.0003 increased MDA level in blood serum ($p<0.05$). Films with higher fullerene content decreased this parameter. Total antioxidant reactivity was increased in serum samples after the exposure of nanocomposites when the value of Φ was equal to 0.0003, 0.001 or 0.01 ($p<0.05$). This proved the antioxidant effect of experimental materials. It appears that the amount of active centers, which is able to effectively capture and inactivate the free radicals, increases with concentrations of fullerenes in composite material.

It is interesting to reveal the effect of the medium in which the films were fabricated. For this we performed experiments for

films prepared by the casting of other aromatic compound – toluene.

The main ChL parameters for “toluene” films are given in Table 3. It can be seen that in the case of original polystyrene film, all values were approximate to controls. But using nanocomposites (Φ=0.005), we found significant increase in the value of tanα. It indicates the antioxidant activity of the researched PS/C_{60} composites.

Table 3: Chemiluminescence Parameters in Blood Serum after Exposure of Original Polystyrene Film and Fullerene-Containing Nanocomposites (Solvent: Toluene)

Φ	J_{max}, %	*A*, %	tanα, %	*Z*, %
Controls	100.0	100.0	100.0	100.0
0	99.0±7.0	98.0±7.5	98.5±7.5	100.0±7.0
0.001	120.5±14.0 *	101.5±10.5	100.5±10.5	84.0±8.5 *
0.005	101.0±7.5 *	103.0±7.5	130.0±14.0 *	102.0±5.0

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

In addition, it can be seen in Tables 1 and 3 that at the same concentration of fullerene (Φ=0.001) 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, what correlates with the findings of Zhou et al (1997). It appears that in toluene solution the fullerene molecules are in the form of clusters, which does not ensure uniform distribution of the nanoparticles in the composite film during its formation of solution.

Note also, we had preliminary experiments with films containing fullerene that were fabricated by the casting of aliphatic compound – chloroform. The results of chemiluminescent analysis and spectrophotometry for serum samples after exposure of 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.

In conclusion, our investigation proved that polystyrene/fullerene nanocomposites have the ability to activate lipid peroxidation in blood serum. Moreover, the possibility of such activation depends on the composite forming conditions.

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