# Effect of Hydrated Fullerene $C_{60}$ in a Wide Concentration Range on Low-Level Photon Emission from Undiluted Human Blood

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**Abstract.** Hydrated fullerene $C_{60}$ ($HyFnC_{60}$) is a symmetrical molecule of $C_{60}$ Buckminster fullerene surrounded by a water shell obtained by an original technique. It can act as an antioxidant or a pro-oxidant in various biological objects, depending on the conditions. In this study we added $HyFnC_{60}$ at a range of concentrations obtained by a method of serial dilutions with vigorous shaking at each step to whole undiluted blood of healthy donors and hospital patients with COPD and examined lucigenin-enhanced blood chemiluminescence. We have found that $HyFnC_{60}$ at concentrations of $2.5 \times 10^{-6}$ M, $2.5 \times 10^{-7}$ M, $2.5 \times 10^{-17}$ M, and $2.5 \times 10^{-19}$ M increased lucigenin-dependent chemiluminescence in heathy donors' blood while in blood of patients with COPD it had an opposite effect. This can be interpreted as in healthy donors' blood reactive oxygen species (ROS) generation is enhanced by $HyFnC_{60}$ while in patients with a chronic inflammatory disease with already increased ROS generation it is attenuated. This indicates that $HyFnC_{60}$ preparations even in ultra-high dilutions may play the role of a tuner of the processes with ROS participation. Probable reasons of such action of $HyFnC_{60}$ on human blood even in ultra-high dilutions are discussed. 

**Keywords:** undiluted blood; hydrated fullerene; chemiluminescence; reactive oxygen species; photon emission.

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## 1 Introduction

Undiluted human blood is a multi-component self-regulating system able to keep many of the features of living systems even being withdrawn from the organism. It has been discovered long ago by A. G. Gurvich that freshly obtained undiluted human blood emits photons in the ultraviolet region more intensively than many other tissues [1]. According to him, ultra-weak photon emission from living systems reflects the intensity of metabolic processes proceeding in them, in particular of the processes in which reactive oxygen species participate [2]. Taking into consideration that Gurvich studied photon emission from non-diluted blood we studied earlier if non-diluted human blood may be the source of photon emission in the visible range of the spectrum. We found that undiluted human blood is a continuous source of low-level photon emission [3, 4]. Its intensity may be strongly amplified due to addition of the enhancers of chemiluminescence (CL) – luminol and lucigenin (LC) to blood. These substances are known as probes for reactive oxygen species (ROS). In particular, lucigenin is a common probe for superoxide anion radical, while luminol is a probe for wider range of ROS [5].

The phenomenon of self-luminescence of cells of various tissues enables us to evaluate the level of metabolism by the method of CL measurement in the presence of CL enhancers. The emission might be caused by lipid peroxidation and formation of singlet oxygen [6] and by relaxation of electronically excited products originating in the course of the processes with ROS participation [7, 8]. In the last decades it became clear that the production of ROS is widespread in blood and all other tissues in normal physiological state [8]. It has been demonstrated that ROS play key role in the normal physiological regulation [8–11]. Pathology occurs when uncontrolled production of ROS such as superoxide anion radical, hydroxyl radical, hydrogen peroxide and other very active compounds leads to oxidative damage in particular of biomacromolecules. Several mechanisms of regulatory action of ROS on a variety of cellular functions are suggested [12]. We suppose that one of the mechanisms may be biophysical one – the pumping of living matter with electron excitation energy released in the course of the processes with ROS participation [10]. In any case, the intensity of radiation can reflect the intensity of physiological processes occurring in living cells, and the factors affecting this intensity modulate the metabolic processes to one degree or another.

In the present study we investigated if the so-called hydrated fullerene $C_{60}$ ($HyFnC_{60}$) affects CL from whole undiluted human blood in a wide range of concentrations including low and ultra-low ones. Pristine $C_{60}$ fullerene molecules are water insoluble and several procedures were designed to make them water soluble. One of the most efficient method to introduce fullerene $C_{60}$ molecules to water in a monomolecule and not chemically modified form was designed by G. Andrievsky etal. [13]. Hydrated fullerene $C_{60}$ molecules ($HyFnC_{60}$) produced by his method represent $C_{60}$ molecules covered with structured multilayer water shells. $HyFnC_{60}$ possess both antioxidant and prooxidant activity, modulating the processes of ROS generation and elimination in aqueous systems [14, 15].

Besides, in this study, we compared intensity of lucigenin-dependent CL (LC–CL) of blood of healthy donors and patients with chronic obstructive pulmonary disease (COPD), on which we previously studied the action of $HyFnC_{60}$ [16]. COPD is characterized by partial restriction of the airflow in the airways and chronic inflammation. This leads to pronounce changes in the ratio of ROS generation and ROS absorption by the antioxidant system of the body relative to the norm. Similar results were obtained in the study of CL of blood of patients with coronary heart disease (CHD) before and after the laser therapy, where the peculiar changes of CL can be used as a method of determining the effectiveness of the laser exposure [17]. In the present study, we show that $HyFnC_{60}$ in a wide range of concentrations including ultra-low ones enhances LC–CL of undiluted blood of healthy donors indicating elevation of ROS throughput. We also observed a consistently elevated intensity of LC–CL from blood of most patients with COPD in comparison of that from blood of healthy donors. In the presence of $HyFnC_{60}$ intensity of LC–CL was generally reduced in patients' blood.

## 2 Materials and Methods

### *2.1 Preparation of $HyFnC_{60}$ Dilutions*

Aqueous solutions of $HyFnC_{60}$ with concentration of 144 mg / l ($2\times10^{-4}$ M) were purchased from the Institute of Physiologically Active Compounds, Kharkov, Ukraine, the developer and exclusive manufacturer of preparations of hydrated pristine fullerene $C_{60}$ ($HyFnC_{60}$) (http://www.ipacom.com/index.php/en/product/concentrated-solution-of-hydrated-fullerene).

Serial dilutions of $HyFnC_{60}$ for $1 \times 10^{-9}$ M $HyFnC_{60}$ saline solution preparation solutions with concentrations of $1 \times 10^{-6}$ M and $1 \times 10^{-7}$ M were first prepared out of $2 \times 10^{-4}$ M stock solution. A portion of the stock solution was first diluted twice to obtain a solution with a concentration of $1 \times 10^{-4}$ M $HyFnC_{60}$. Further 30 μl of a $1 \times 10^{-4}$ M solution were added to 2970 μl of saline for preparation of $1\times10^{-6}$ M $HyFnC_{60}$ solution. The solution was shaken vigorously for 1 min with a vortex shaker, and then it was placed in the dark for 30 min. Then 300 μl of $1 \times 10^{-6}$ M solution was added to 2700 μl of saline for the preparation of $1 \times 10^{-7}$ M $HyFnC_{60}$ solution that was shaken vigorously. For $HyFnC_{60}$ solution with a concentration of $1 \times 10^{-9}$ M a centesimal dilution of $1 \times 10^{-7}$ M solution was prepared likewise. After each dilution solutions were vortexed and placed in the dark for 30 min. This procedure was repeated for all dilutions of $HyFnC_{60}$ solution. Dilutions were prepared by mechanical dispensers BIOHIT Proline 1–10 μl, 50–200 μl, 200–1000, and 1000–5000 μl. Also, a control sample free of fullerenes was prepared.

### *2.2 Manipulations with Blood*

The study involved healthy donors and patients with COPD. Healthy donors were aged from 32 to 53 years. All patients with COPD were older than 40 years, all had the forced expiratory volume in one second (FEV1) reduced to less than 50%, the volume of forced vital capacity (FVC) of the lungs reduced to less than 70% of the expected. All patients had stable COPD without acute exacerbations. Patients with COPD are given basic therapy, which included: Ventolin Nebula® – inhalation solution containing 2.5 mg of salbutamol sulphate in 2.5 ml (Glaxo Wellcome, UK) or Berodual® – solution for inhalation therapy, containing 500 μg fenoterol hydrobromide (0.5 mg), 250 μg of ipratropium bromide (0.25 mg) in 1 ml (Boehringer Ingelheim, Germany).

Under indications they were treated with antibiotics and steroids – orally methylprednisolone 4 mg tabs (Orion Pharma) at a daily dose of not more than 24 mg/day or prednisolone 5 mg tabs (Gedeon Richter) at a daily dose rate of 0.5 mg/kg, but not more than 60 mg/day during a short course (10–14 days). If necessary, oxygen therapy was provided.

The study was approved by the Ethics Committee of Sechenov Moscow State Medical University and Gorodskaya Clinical Hospital No 23 named after "Medsantrud". All the volunteers agreed to participate in the experiment.

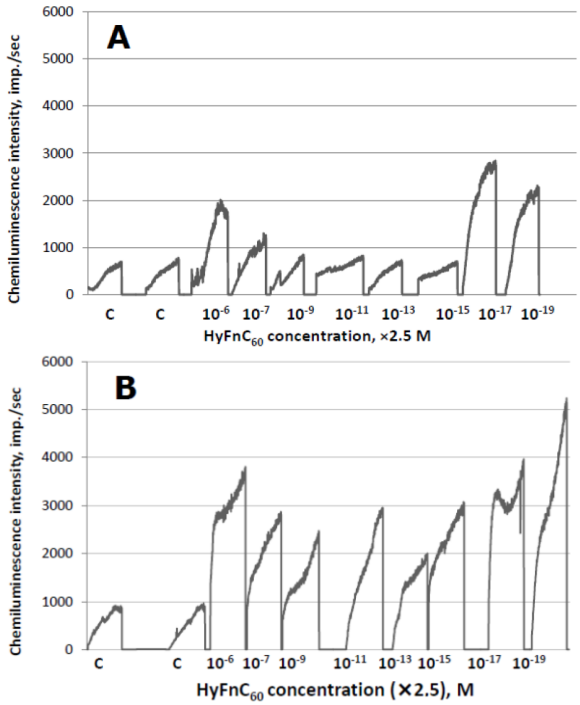


Fig. 1 Effect of $HyFnC_{60}$ at concentrations ranging from $2.5 \times 10^{-23}$ to $2.5 \times 10^{-6}$ M on LC–CL of undiluted blood of a healthy donor. (A) LC–CL measurement 3 min after adding $HyFnC_{60}$; (B) LC–CL measurement in the same sample of blood a day after adding $HyFnC_{60}$. "C" means Control.

In patients with COPD, forced expiratory volume (FEV1) per second was $1.32 \pm 0.53$ l, FEV1 / FVC (volume of forced vital capacity) at the level of $53.9 \pm 10.85\%$ as expected. FEV1 and FVC were measured using a spirometer (Erich Jaeger GmbH, Germany). The level of oxygen saturation ($SaO_2$) was $94.8 \pm 1.29\%$. $SaO_2$ was measured by pulse oximeter Onix®Nanin.

Blood from healthy donors and patients with COPD was obtained by venous puncture within 9.00 to 10.00 a.m. and stabilized by heparin. Subsequently, blood was used in measurements within 3 h. During this time, it was stored in plastic tubes at room temperature.

Method of registration of whole blood chemiluminescence was described in our previous publications [3, 4]. Five µl of $HyFnC_{60}$ solution in saline was added to "Eppendorf" (Vitlab, Germany) 1.5 ml plastic tubes with 200 µl of blood to a desired concentration and kept in a dark place for 3 min. The final concentrations of $HyFnC_{60}$ in test tubes were $2.5 \times 10^{-6}$ M, $2.5 \times 10^{-7}$ M, $2.5 \times 10^{-9}$ M, $2.5 \times 10^{-11}$ M, $2.5 \times 10^{-13}$ M, $2.5 \times 10^{-15}$ M, $2.5 \times 10^{-17}$ M, and $2.5 \times 10^{-19}$ M. These are defined as low and ultra-low concentrations (LC and ULC) [18]. Five µl of saline were added to control samples. Then 10 µl of LC (Sigma, USA) were added to a final concentration of $1 \times 10^{-4}$ M LC in blood. Stock solution of LC was prepared by dissolving the LC in saline to a concentration of $2 \times 10^{-3}$ M. Chemiluminescence was measured immediately after the addition of LC by using single photon counter "Biotoks-7A" (Engineering Center "Ecology", Russia), equipped with a photomultiplier tube 9750QB/1 (EMI Electronics, USA) with a 5 cm diameter of a photocathode (dark current of about 25 pulses/s, the spectral sensitivity is in the range of 380–710 nm and the maximum sensitivity of 450 nm). The emission was usually recorded for 300 sec. On some figures "light sum" – cumulative counts for 300 sec are presented. In some cases, the same blood samples were stored in tubes for one day in a refrigerator at 4 ºC for re-measurement of LC–CL [16].

The relationship between data groups was tested using a two-sample t-test. Data processing was performed using a PC with a processor Pentium (R) DualCore processor with installed Windows 7 operating system and software package Microsoft ® (USA) – Microsoft Office 2007, Excel 2007, Visio 2007 and statistical software Statistica V.8.0.

## 3 Results

We have shown that $HyFnC_{60}$ in ULC stimulates the LC–CL of undiluted whole blood from healthy donors (Fig. 1). The greatest influence on the intensity of blood LC–CL was registered at $HyFnC_{60}$ concentrations of $2.5 \times 10^{-6}$ M, $2.5 \times 10^{-7}$ M, $2.5 \times 10^{-17}$ M and $2.5 \times 10^{-19}$ M. In the presence of $HyFnC_{60}$ at these concentrations blood LC–CL increases several-fold over that from control samples to which the saline was added. As shown in Fig. 1 (a, b) this effect is maintained and enhanced within days after addition of $HyFnC_{60}$. Twenty-four hours after the addition of $HyFnC_{60}$ blood LC–CL increases in the presence of $HyFnC_{60}$ in concentrations $2.5 \times 10^{-6}$ M, $2.5 \times 10^{-7}$ M, $2.5 \times 10^{-17}$ M, and $2.5 \times 10^{-19}$ M compared to the data of the first day. Moreover, on the second day LC–CL starts to increase in the presence of $HyFnC_{60}$ at the concentrations in which no increase was observed on the first day ($2.5 \times 10^{-9}$ M, $2.5 \times 10^{-11}$ M, $2.5 \times 10^{-13}$ M, and $2.5 \times 10^{-15}$ M).

In the experiment which results are shown in Fig. 2, saline was added to a control sample of blood of a healthy donor. At the same time $HyFnC_{60}$ was added to another the portion of blood from the same donor obtain a concentration of $2.5 \times 10^{-19}$ M. Then LC was added to both samples, and measuring the LC–CL simultaneously on different devices was begun. A correction to different sensitivities of the two luminometers is considered. Fig. 2 shows that both in the sample containing $HyFnC_{60}$ and control sample light emission increases for several hours. However, in the sample to which $HyFnC_{60}$ preparation was added the maximum value of LC–CL during the observation period is several times greater than in the control.

Amplification by certain dilutions of $HyFnC_{60}$ of intensity of blood LC–CL was demonstrated not only on blood taken from different healthy donors, but even on blood of a single donor taken at an interval of 58 days (Fig. 3). This reaction of undiluted blood is individually specific, and the ratio of the intensity of LC–CL in the presence of various concentrations of $HyFnC_{60}$ vary in the blood of different donors in different conditions.

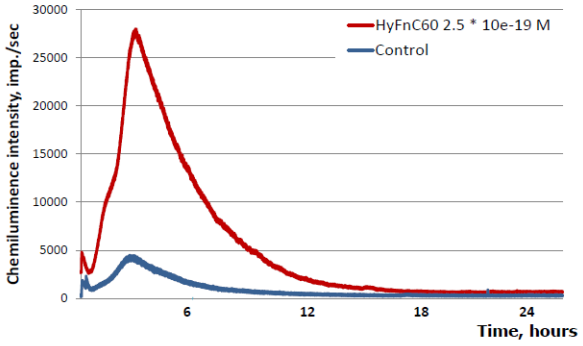


Fig. 2 Change of LC–CL of undiluted human blood during the day-long observation. LC–CL is more intense in the presence of $HyFnC_{60}$, than in controls. Maximum luminescence is observed 6 h after the beginning of the measurement.

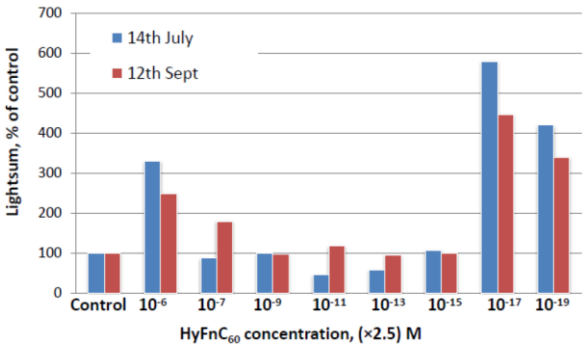


Fig. 3 Effect of $HyFnC_{60}$ at concentrations ranging from $2.5 \times 10^{-6}$ to $2.5 \times 10^{-23}$ M on LC–CL of undiluted blood of a healthy donor. The data were normalized to the intensity of LC–CL in the absence $HyFnC_{60}$ that is taken as 100%.

Interestingly, the degree of influence of each $HyFnC_{60}$ concentration is not in linear relationship with concentration of $HyFnC_{60}$. In other words, the dependence of the effect on the $HyFnC_{60}$ concentration is multimodal. It is important to note that the changes in LC–CL were observed after prolonged incubation of blood with $HyFnC_{60}$ in all examined concentrations compared to controls.

It has been found that $HyFnC_{60}$ differently affect LC–CL of blood of healthy donors and blood of patients with COPD.

Figs. 4 and 5 show that in the presence of $2.5 \times 10^{-6}$ M, $2.5 \times 10^{-7}$ M, $2.5 \times 10^{-17}$ M, and $2.5 \times 10^{-19}$ M $HyFnC_{60}$ LC–CL increases in blood of healthy donors. This effect was maintained when $HyFnC_{60}$ was added to the blood samples which then were stored in refrigerator, and the LC was added at 24, 48 h, etc. (Fig. 5). On the second day of observations in the samples containing $2.5 \times 10^{-13}$ M $HyFnC_{60}$ LC–CL also increased and continued to rise for the third, fourth and fifth day. On the sixth and seventh days of the experiment LC–CL decreased in these samples.

The absolute values of blood LC–CL of COPD patients is several times higher than the LC–CL of blood of healthy donors without $HyFnC_{60}$ addition [16]. This can be attributed to uncoordinated work of prooxidant and antioxidant systems of blood in patients with COPD. Fig. 6 shows that immediately after the addition of $HyFnC_{60}$ to the concentrations of $2.5 \times 10^{-19}$ M intensity of the LC–CL of blood of patients with COPD decreases. In samples to which $HyFnC_{60}$ was added to concentrations of $2.5 \times 10^{-6}$ M and $2.5 \times 10^{-19}$ M and stored in a refrigerator prior to the addition of the LC for 24 h, a decrease of LC–CL in comparison to control was also observed.

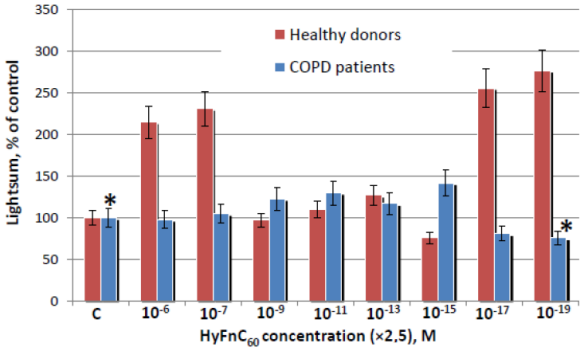


Fig. 4 Comparison of $HyFnC_{60}$ effect in various concentrations on the intensity of the LC–CL of blood of healthy donors and patients with COPD. Data are based on the study of blood from 8 healthy donors and 7 patients with COPD. Mean values with standard deviations are presented, Mann-Whitney U-test used. * – Denotes statistically significant ($p < 0.05$) decrease of the mean LC–CL emission in the presence of $2.5 \times 10^{-19}$ M $HyFnC_{60}$ compared to control in the blood of COPD patients.

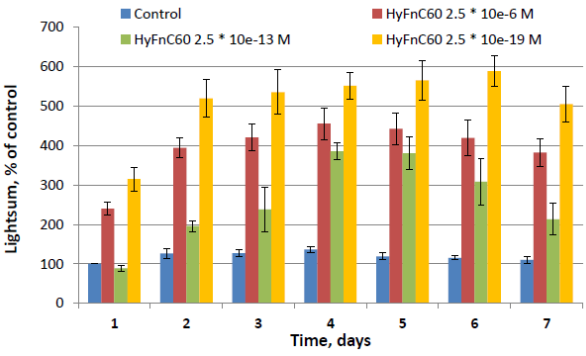


Fig. 5 Changes in LC–CL of undiluted blood of healthy donors after $HyFnC_{60}$ addition and 7- days storage. Data are based on studies of blood 8 healthy donors. The data are normalized to the intensity of LC–CL in the absence of $HyFnC_{60}$ on the first day of the experiment taken as 100%. Mean values with standard deviations are presented, Mann-Whitney U-test used.

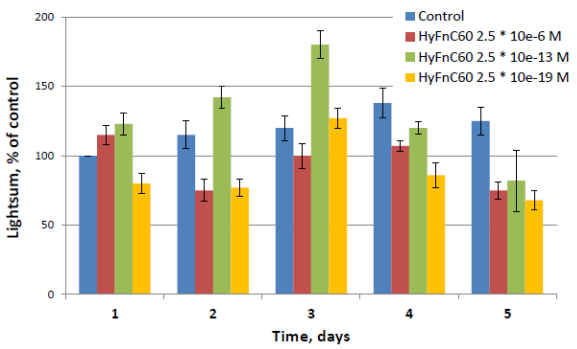


Fig. 6 Change of LC–CL of undiluted blood of patients with COPD in the presence of $HyFnC_{60}$ during 5 days. Data are presented on the basis of blood tests of 7 patients. The data are normalized to the intensity of LC–CL of blood of patients in the absence of $HyFnC_{60}$ on the first day of the experiment taken as 100%. Data analyzed using Mann-Whitney U-test, mean values with standard deviations are presented.

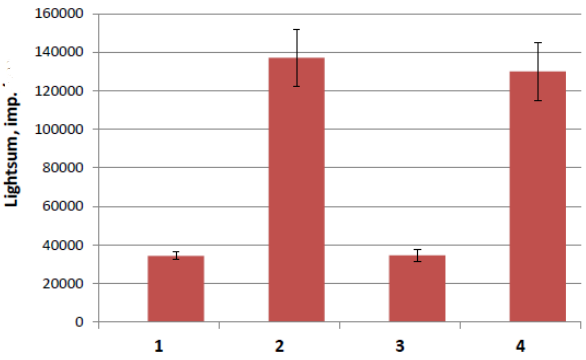


Fig. 7 Comparison of LC–CL of whole blood of one donor in the presence of native $HyFnC_{60}$ or $HyFnC_{60}$ subjected to autoclaving: 1 – control blood samples to which saline was added; 2 – blood samples to which $HyFnC_{60}$ solution in saline to a final calculated concentration of $2.5 \times 10^{-19}$ M was added; 3 – control blood samples to which autoclaved saline was added; 4 – blood samples to which $HyFnC_{60}$ solution in saline (prepared from autoclaved $1 \times 10^{-7}$ M $HyFnC_{60}$ stock solution) to a final calculated concentration of $HyFnC_{60}$ $2.5 \times 10^{-19}$ M was added. Data analyzed using Student's t-test, mean values with standard deviations are presented.

On the 5$^{th}$ day intensity of LC–CL in all samples of blood to which $HyFnC_{60}$ was added in all tested concentrations ($2.5\times10^{-6}$ M, $2.5\times10^{-13}$ M, and $2.5\times10^{-19}$ M) decreased in comparison to control. This is contrary to the intensification of LC–CL of blood from the 2$^{nd}$ to the 7$^{th}$ day after $HyFnC_{60}$ addition in the same concentrations (compare Fig. 5 and Fig. 6).

These findings point out the different nature of the processes with ROS participation in blood of healthy people and patients with COPD. The character of $HyFnC_{60}$ influence on the blood of patients with COPD is largely dependent on the degree of severity of symptoms [16]. For this study, blood was taken from patients not in acute phase with a mild disease course before a course of therapy.

For cytological and microbiological studies all reagents used should be sterilized in an autoclave. If we assume that $HyFnC_{60}$ will be used in such study, a stock $1\times10^{-7}$ M $HyFnC_{60}$ solution will need to be sterilized. To find out whether autoclaving of $HyFnC_{60}$ stock solution influences $HyFnC_{60}$ activity in ultra-low concentrations (ULC), we used whole blood of a healthy donor as a biological test system as it is very sensitive to the addition of $HyFnC_{60}$.

As seen in Fig. 7, LC–CL of blood increases in response to the addition of $HyFnC_{60}$ in ULC at the same rate whether or not the stock solution for preparation of the dilutions was subjected to autoclaving. Data presented as lightsums in the form of summarized impulses (counts) of CL peaks. Thus, autoclaving of the stock solution does not affect the biological activity of $HyFnC_{60}$ in ULC.

## 4 Discussion

Monitoring of LC–CL from non-diluted human blood under resting conditions revealed that blood is a continuous source of biophotons indicating that it persists in electronically excited state [19]. This state is pumped through continuous generation of electron excitation produced due to the ongoing generation of ROS by aerobic blood cells in the process of reduction of oxygen coming from the red blood cells [8]. As we demonstrated earlier [19], this non-equilibrium state of blood with the signs of electronic excitation is extremely sensitive to some subtle external factors but it is resistant to more intensive influences such as temperature variations. Blood retains these properties for long period of time due to its ability as of a complex holistic system to store energy of electron excitation that is produced in course of its own normal oxidative metabolism.

ROS are produced continuously in all tissues of healthy people and perform physiological regulatory functions [8–12]. Such regulation has a significant impact on growth, differentiation, aging of living cells, their death by apoptosis and even redifferentiation of cultured malignant cells [20, 21]. However, many authors for a long time believed that ROS only have a negative effect on the structure and biological processes [22, 23]. One of the reasons of harmful action of ROS to which much more attention has been paid previously then to their beneficial action is oxidation of bioorganic molecules when ROS metabolism is dysregulated. Under normal physiological processes, ROS are a delicate instrument of interaction and signaling in cells and tissues. Among other mechanisms [12] their regulatory role may be provided by their ability to generate electronically excited states (EES) and as a result, electromagnetic radiation, and the radiation in the UV, visible light and infrared regions [6, 8].

Elements of blood in non-equilibrium states with signs of electronic excitation are more sensitive to ultra-

low intensity factors' impact, which include their own blood radiation, electromagnetic fields generated by the ROS and more [19]. Therefore, whole blood is an important test system for study of such states. Here we have shown that adding $HyFnC_{60}$ in ultralow concentrations in whole blood of healthy donors results in the growth of the LC-CL. Of the greatest influence on the blood was $HyFnC_{60}$ in concentrations of $2.5\times10^{-6}$, $2.5\times10^{-7}$, $2.5\times10^{-17}$, and $2.5\times10^{-19}$ M. This effect is maintained and enhanced within several days. In those samples to which $HyFnC_{60}$ was added to concentrations of $2.5\times10^{-9}$, $2.5\times10^{-11}$, $2.5\times10^{-13}$, and $2.5\times10^{-15}$ M no CL amplification was observed immediately after the addition of $HyFnC_{60}$. In these samples it occurred after one day long incubation in a refrigerator. We can assume that $HyFnC_{60}$ in different concentrations in blood starts to affect the CL at different times. Interestingly, the opposite effect is found in blood of patients with COPD: $HyFnC_{60}$ in ULC lowers blood CL intensity. The character of $HyFnC_{60}$ effect on CL blood of patients with COPD depends on the condition of patients and the severity of the disease, but in most cases the addition of $HyFnC_{60}$ lowers LC–CL in blood of patients who did not undergo the standard treatment at the moment of blood withdrawal [16]. We suggest that further studies of $HyFnC_{60}$ effect on blood CL will allow to offer a new diagnostic test.

Over the past three decades, the effects of low and ultra-low concentrations of various substances including hydrated fullerene $C_{60}$ on biological test systems (from isolated enzymes and enzyme systems to whole organisms) were found [18, 23–26]. Assumptions about the special role of water in the implementation of ULC effects were made long ago. In recent years, several discoveries of peculiar properties of water and aqueous solutions subjected to mechanical stress (potentiation) with successive dilutions of the starting substance (dynamization) has been made. It was found that in aqueous systems, which are strongly diluted solutions of various biologically active substances, nano-sized water clusters are present, ranging in size from a fraction of micrometer [27–30] to a few microns [31]. The clusters retained even at dilutions corresponding to "imaginary" concentrations of the diluted substances. These clusters carry a negative charge and remain in water for a long time. There is strong evidence that water clusters which are dynamically active structures that determine the effect of biologically active substances after their high and ultra-high dilutions [32]. Some authors base on the cluster hypothesis explanation of a surprising undulating dose dependence of the effect of successive dilutions an increase of the effect at some high dilutions with decrease and further increase of the effect with subsequent dilutions. For example, Demangeat draws attention to the fact that the sizes of nanoassociates or clusters during successive dilutions grow to a certain critical value, and then decrease, and then grow again [33]. If the effect depends on the size of such structures (and a similar correlation was observed in works by Konovalov [29, 30]), then this may explain the undulation of dose dependencies that we observed in our experiments (see, for example, Figs. 3 and 4). However, the mechanism of cluster formation is unclear and for its clarification a deep revision of the traditional notions of physical and chemical properties of water systems is required.

Interestingly, both enhancing and inhibitory effects are possible at the same concentrations of $HyFnC_{60}$ for different objects (the blood of healthy donors and patients with COPD). This phenomenon can be explained by taking into account complex structural properties of the aqueous medium. A lot of data appeared indicating that water is a heterogeneous substance containing both ordered and disordered regions [30, 34]. Ordered regions such as nanoassociates and clusters differ from bulk water in many respects. One of the most important of them is that they are practically always negatively charged, therefore they may be electron donors and may modulate reactions with ROS participation proceeding in aqueous systems. In fact, ROS may be spontaneously generated in all aqueous systems [10], but intense mechanical perturbation of water such as its dynamization in the course of preparation of high and ultra-high dilutions significantly intensifies the processes with ROS participation [34, 35]. On the other hand, when converted to $HyFnC_{60}$, molecules of $C_{60}$ strongly polarize water in their hydration shell, and as a result the shell becomes negatively charged. This shell can be regarded as a special case of dynamically structured "interfacial water", that may actively participate in red/ox reactions [32]. The lower the $HyFnC_{60}$ concentration, the more extensive water fullerene shell is. It is assumed that the contribution of the fullerene molecule itself in regulation of ROS content in the medium is minimal. $HyFnC_{60}$ changes the aquatic environment around them, and this "interfacial water" water either generates or eliminates ROS depending on the intensity of their production in an aqueous system to which a solution of $HyFnC_{60}$ is added [14, 15]. We suppose that as soon as the intensity of ROS generation in blood of healthy donors and patients with COPD differs significantly $HyFnC_{60}$ dilutions may exert on them opposite action.

## Disclosures

The authors declare no conflict of interest.

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