Possibility of determination of the level of antioxidants in human body using spectroscopic methods

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Abstract. In this work, the processes of antioxidant defence against aggressive free radicals in human body were investigated theoretically; and the existing methods of diagnosis of oxidative stress and disturbance of antioxidant activity were reviewed. Also, the kinetics of free radical reactions in the oxidation of luminol and interaction antioxidants (such as chlorophyll in the multicomponent system of plant’s leaves and ubiquinone) with the UV radiation were investigated experimentally by spectroscopic method. The results showed that this method is effective for recording the luminescence of antioxidants, free radicals, chemiluminescent reactions and fluorescence. In addition these results reveal new opportunities for the study of the antioxidant activity and antioxidant balance in a multicomponent system by allocating features of the individual components in spectral composition. A creation of quality control method for drugs, that are required for oxidative stress diagnosis, is a promising direction in the development of given work.

1. Introduction
Evidently, an oxidative damage in cell structures is a basis of most mechanisms that lead to health diseases and senescence of human body. The main factor for such cell damage is oxygen. Oxygen permeates into the human body and, before turning into water, oxygen molecule becomes an aggressive oxidizing agent in the form of hydrogen peroxide or oxygen radical. This oxidizing agent can react with molecules, which are susceptible to oxidants and free radicals, under the influence of various interfering factors and deviations from the normal functioning of the body. Aggressive forms of oxygen molecules are triggers for a chain reaction of lipid oxidation. That leads to damages of the cell membranes and destruction of the cell. Antioxidants fully or partially suppress the activity of free radicals, while maintaining the integrity of cells and tissues, but prevent from the process of phagocytosis, in which a greater concentration of toxic oxygen is required [1]. In the process of phagocytosis (which was discovered in the 19th century by Ilya Mechnikov) specialized white blood cells (phagocytes) destroy morbific bacteria using a high concentration of oxygen free radicals.
It can easily be said that direct analysis of antioxidant activity allows diagnostics of diseases connected with the damage of tissues and organs structure, as well as genetic material, and is a topical issue of clinical diagnosis. Besides medical intervention is required in the presence of excess of normal antioxidants concentration in human body because of probable loss in defence mechanisms.

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against pathogenic bacteria (due to the lack of free radicals which were neutralized by antioxidants). Therefore, the problem of antioxidant studying requires to develop several ways of research to analyse directly the balance of antioxidants and free radicals, to control drugs for the treatment of oxidative stress, to analyse the mechanisms of antioxidants interaction with free radicals by spectroscopic method and to find promising antioxidants on the basis of knowledge of their interactions with radiation of a special spectral composition.

2. Problem description
The imbalance of antioxidants diagnosis in modern clinical diagnostics can be based only on the testimony that has an indirect relation to the antioxidant status. Therefore direct indicators of antioxidant status of the patient and high accuracy of diagnostic methods are required (taking into account the direct impact of the diagnosis on the action of physician). Immunoassay methods provide data on the reaction products of antioxidants or free radicals in the blood plasma. However, cell damage by free radicals most takes place directly in the tissues and organs. So body’s tissue samples (that may be under adversely oxidative influences) are to be selected as analysis objects. Moreover the work with biological objects has to be considered as an analysis of multi-component system, which is a determining factor in choosing a method of diagnosis in the absence of a preliminary chemical intervention.

3. Current methods
There are many methods for the study of antioxidants:

- Volumetric – the study of the antioxidant activity by volumetric absorption of oxygen;
- Photometric – the determination of the optical density for colorimetric analysis;
- Biological marking – the implementation of fluorescent markers in a living organism;
- Electrochemical – measurement of voltammetric characteristics of cathode reduced oxygen in a solvent, as it is known that the electrochemical activity correlates with the ability to inhibit lipid peroxidation;
- Radio spectroscopy or EPR-spectroscopy is the most direct method for the study of free radicals, but it is not applicable to dynamic systems;
- Chemiluminescent – is currently the most promising because it has significant advantages over the other: high accuracy and safety for the patient and the laboratory assistant [2].

4. Materials and methods

4.1. Investigation the kinetics of free radical reactions
First of all it was decided to study the free radical reaction with the help of reaction of luminol transformation to 3-aminophthalate, because it is well-studied by chemiluminescent method (Figure 1). This test is a part of the laboratory practical training program in the education of specialists in the medical field. This reaction is chosen deliberately with the aim of the study the kinetic of free radical reaction not by chemiluminometer but using spectroscopic method. It was necessary to compare the accuracy of the spectroscopic method for determining the concentration of the reactants by the energy from luminescence of radical reaction.

![Figure 1. Reaction scheme of luminol transformation to 3-aminophthalate](image-url)
The luminol chemiluminescent reaction with kalio heksacianoferatas (III) was selected as an object for studying the kinetics of the oxidation process. The luminescence of reaction was registered with the help of spectrometer USB4000 of Ocean Optics firm. Software for recording and processing the results of experiments were developed in LabVIEW environment. During the experiment, the obtained data was compared with the known model of the chemical reaction [3] and a match with the model of the behaviour of chemiluminescence reaction was found. Also, luminescence wavelength of the chemiluminescent reaction was defined.

As a result, the conclusion about the possibilities of studying the kinetics of the chemiluminescent reaction by spectroscopic method was made.

4.2. Identification of obstacles at this point

Following problems for direct analysis were detected during the research:

- Study of multi-component system;
- At the moment, there is no opportunity to avoid a chemical treatment to the sample;
- Instability of free radicals for their study by spectroscopic method.

The review of proposed solutions of this situation and a selection of samples for testing were made. So it was decided to study directly antioxidants (that are presented in the human body) to isolate their features from the rest of the components of blood plasma or tissue samples. Free radicals cannot be studied directly in this method of analysis due to their short lifetime, so the individual study plan will be developed for them.

The realization of the experimental setup as a whole depends on the subject and the research goals: working with chemicals or biological materials; register the luminescence or the fluorescence caused by ultraviolet light. These facts suggest that the evaluation of the total antioxidant status in human body requires a complex analysis of biological samples of patients. Nevertheless, the work is done in stages for following methodology development and design of the device for the complex analysis of biological samples and the diagnosis of oxidative stress.

4.3. Investigation of plants as sources of antioxidants

The experimental study of UV radiation influence to the plant leaves were carried out along with the analysis of the chemiluminescent reaction.

Despite the fact that the leaves of the plants are multi-component system, it was decided to carry out experiments to study how UV radiation effects on the leaves of plants as sources of antioxidants for humans [4].

The optical properties of chlorophyll in the leaves of plants were investigated in the theory before the experiment. The special equipment (Figure 2) for the experiment was prepared.
The radiation source DT-Mini-2-GT contains the Halogen and the Deuterium lamps. Preliminary experiments have shown that for the study of fluorescence should be used the Deuterium lamp. Figure 3 shows the one of results of fluorescence study of plant leaves.

![Fluorescence emission spectrum of a plant leaves under radiation of Deuterium lamp](image)

**Figure 3.** Fluorescence emission spectrum of a plant leaves under radiation of Deuterium lamp.

The results of experiments on plant leaves irradiation by Deuterium lamp showed that the spectroscopic method allows the registration of chlorophyll fluorescence and photosynthesis process of plants. A comparison of different plant species and plants growing in different conditions was made and the match of the spectral composition of fluorescence response was found. As a result, it may be said that the spectral method allow us to divide the signals from the various components during the analysis and processing of the plant leaves fluorescence under known fluorescent responses from the each individual component of the plants leaves.

4.4. *Investigation of the optical parameters of antioxidants used to treat patients*

The ubiquinone was selected for the investigation as a substance insufficiently explored by optical methods. Ubiquinone is known in the pharmaceutical industry as a coenzyme q10. It is currently widespread in the dietary supplement form, which is called "anti-aging". And it was first used for the treatment of cardiovascular diseases in 1965. This antioxidant actually inhibits cell senescence. But an excess of antioxidants can slow down the normal metabolism and lead to disease, oxidative stress feedback, in which process the leukocytes are not provided with enough free radicals to fight against pathogenic bacteria. Therefore it is necessary to study not only the quantity of products of chemiluminescent reaction, but antioxidants themselves.

Experiments with ubiquinone were based on the phenomenon of fluorescence coenzyme solutions in oil. The registration of the fluorescence was carried out by the spectrometer. UV LEDs with a narrow spectral composition of the maxims at 280 and 355 nm were chosen as fluorescence excitation sources. Figures 4 and 5 represent the effects of UV irradiation of different wavelengths (280 and 325 nm) to ubiquinone solution and solvent.

The results of studying the optical properties of the ubiquinone showed that the spectroscopic method can be applied to the fluorescence investigation of this antioxidant. Also the data obtained are dependent on the luminescence excitation source and on the solvent, if the solution is irradiated.
5. Results and discussion
The results of these studies show the possibility of antioxidant research by spectroscopic methods. Also the prospects of this method in clinical diagnosis of possible antioxidant imbalances (on the basis of the known normalized antioxidant activity and in the early stages of the disease) were proved. Spectroscopic method allows us to separate the fluorescent response of the various components, has a sufficiently high sensitivity and allows analysis of the multicomponent system, highlighting the characteristics of each component on the background of the whole system.

6. Conclusions
The research results at this stage allowed us to make a choice between the directions of future research and to identify entirely new research problems:
- The study of the relation between optical density of ubiquinone solutions and the ubiquinone concentrations in different solvents;
- The research of colour characteristics of ubiquinone solutions (for possible use in quality control of drugs, which are based on this coenzyme);
- The study of antioxidants in association with their pro-oxidant counterparts (ubiquinone-ubiquinol);
- Organization of parallel researches with the existing biochemical analysis of antioxidants.

7. Acknowledgments
This work was financially supported by Ministry of Science and Education of the Russian Federation (GOSZADANIE 2014/190).

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