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Automated chemiluminescent analysis of the antioxidant and prooxidant activity of natural and synthetic substrates having perspective for bioengineering

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Abstract. Automated CL-control has a lot of advantages, such as rapidity, information value, objectivity, reproducibility, low-price. As a rule, the method is used for detecting antioxidant capacity of natural substrates. The results described in the paper show that this method has wide opportunities for deciding actual tasks in nutritional industry, preventive and restorative medicine, bioengineering in future. First, the results demonstrated that the method allows to determine optimal extraction conditions to obtain phytopreparates which include nonstability antioxidant and (or) prooxidant compounds. This is shown using phytopreparates (leuzea, rhodiola, rheum, plantago) obtained using local plant materials. Second, results are obtained to determine the presence of real, non-virtual antioxidant or prooxidant properties of commercial micronutrients complexes. In addition, the method allows to provide an individual selection of nutrients in accordance with the characteristics of the ROS balance of the internal environment of the consumer's body. This way allows to get the reproducible results within a relevant experimental model in short time, which is very important looking for dynamic development of the functional nutrients market. Finally, using this method one get more effective and economic navigation of chemical synthesis. As a result, the efficiency of the subsequent functionalization of synthetic compounds is increased.

1. Introduction

An important criterion of the biological value of exogenous substances is the ability in small doses to effectively regulate metabolic processes. The most important process which has to be regulated is the rate of production of reactive oxygen species (free radicals) in the tissues of the body [1]. Reactive oxygen species (ROS) are constantly forming in the course of all oxygen-dependent reactions, including biological processes. These metabolites are able to participate in both constructive and destructive processes, depending on their concentration in solution. The regulation of the rate of formation of ROS is a necessary condition for homeostasis and ensuring a high adaptive capacity of the organism [2]. Therefore, one of the central problems of modern bioengineering is to create functional food, feed and drugs with antioxidant or prooxidant functions [3].

It has been shown that homeostasis in practically healthy people and animals is often disturbed under the influence of a variety of exogenous and endogenous factors, mainly due to excessive production of ROS [4]. It forms the metabolic basis for the onset or exacerbation of chronic diseases in the future [5]. At the same time, functional states of the body are known, associated with a low rate of ROS generation. In this case, the body needs to obtain exogenous prooxidants.

Therefore, both antioxidants and prooxidants are highly in demand in preventive and restorative medicine. Metabolism regulators can be not only natural, but also artificial and synthetic products of bioengineering [6]. For the design and engineering of such compounds, reliable control of the radical-oriented ability of biologically active compounds is necessary.

The current level of development of bioengineering in Russia is not high enough, it is lagging behind the world [7]. The range of biologically active complexes is replenished mainly with imported products, not domestic ones. A deficiency of local raw materials is not the reason for this situation. On the contrary, there are many well known different types of local raw materials. The key reason is insufficient methodological and technical support of measurements.

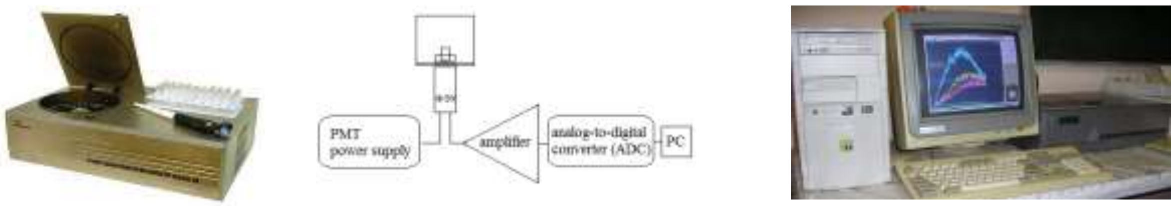
The study of the kinetics of free radical processes is most often associated with the determination of antioxidant activity, not prooxidant. It isn't quite right, becouse prooxidants may be important biology regulators too. For the evaluation of antioxidant activity, as a rule, methods of volumetry, photometry, fluorimetry, potentiometry and others are used [8]. Under this the result are unforeseeable and often depends on the type of oxidation substrate, the nature of the oxidizing agent, the type of oxidized product and the method of measurement. These methods used expensive equipment and are time-consuming. Moreover, the results obtained are usually difficult to interpret and extrapolate in vivo.

So, the search for more adequate methods to control the radical-directed activity of nutrients and other biosubstrates is needed. Automated chemiluminescence analysis can be one of such methods. To confirm, it is necessary to use this method to assess the activity of dissimilar compounds in their interactions with free radicals.

The aim of the work was to show the versatility and informality of the automated chemiluminescent control when radical directed activity of dissimilar compounds was estimated.

2. Materials and methods

An automated PC-controlled biochemiluminometer, BCLM-3606M-01/3607 was used in the work (figure 1). The device is designed and constructed in Special Design and Technology Bureau «Nauka» ("Science") of the Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk).



a b c

Figure 1. A general view and scheme of automated system for chemiluminescence qualimetry
a – luminometer BL-3606M-01, b – device scheme, c – general view of the measuring system.

The device operates in the mode of light quanta counting. Luminescence occurs in the conversion of chemical energy of free radicals produced in the incubation space. To obtain a baseline CP in the incubation medium, the Fenton model was used in two versions. ROS were produced during the Fe2+ initiated decomposition of 0.1 mM hydroperoxide (chemical model) or during latex-induced chemiluminescence (CL) of human capillary blood (biological model).

The latex was opsonized with antibodies from the blood plasma of several healthy donors. To enhance the signal, 1 mM luminol was used. All reagents were taken from "Sigma", Novosibirsk. The volume of the tested samples was 100 μl, the total sample volume was 0.8 ml. Only blood phagocytic leukocytes (phagocytes) of the blood have the ability to form free radicals during the "respiratory burst" [8]. The use of a biogenic model increases the possibility of extrapolating test results to the level of physiological processes in the body. The results obtained in both models predict the possibility not only alimentary, but also pharmacological using of the synthesized compounds [9]. The recording and storage of results, graphic and statistical processing of the kinetic curves (kinetograms) were performed using the BLM 07 software package (instrument operation control using a PC) and BLM 07PR software (processing of measurement results).

The antioxidant or prooxidant activity of the compounds was evaluated by the direction of the change in the peak of the CL or light sum regarding control under the influence of the samples.

The sensitivity of the CL-control was assessed by the frequency of increase or decrease in the light sum relative to the control in the presence of samples. The measurement time in the chemical model was 10 minutes and in the biogenic one – 90 minutes. The check material included phytoextracts, commercial micronutrients and original chemical synthesis products.

Four types of sources for phytoextracts were used: Rhaponticum carthamoides Iljin, Rhodiola rosea, Rheum officinale, Plantago major. Plant material was collected and processed in accordance with the pharmacopoeia rules. Phytoextracts were obtained by varying the temperature and time of extraction. Commercial biology active micronutrients (n=70) were in various pharmaceutical forms (granules, capsules, solutions). Samples for analysis suspended in water.

The original products of chemical synthesis (aminoanthraquinones, 12 compounds, 0.1% solutions of preparations in dimethyl sulfoxide, DMSO) were obtained at the Department of Chemistry, KSPU (Krasnoyarsk). The method of analysis is described in detail [10]. The kinetic parameters were automatically recorded and archived as a database. Each sample was analyzed at least three times. Statistical processing was carried out using Student's criterion (the distribution of sample data was normal, the variances were comparable) at a confidence level of 0.95.

3. Control of chemiluminescence under the influence of compounds of different nature

3.1. Radical-directed activity of natural substances

Siberian flora is a rich source of new therapeutic and prophylactic nutrients and medicaments. Many of them are able to replace many imported drugs. The use of natural compounds is an economical and safe way to correct prenosological disorders of homeostasis. An objective sign of such disorders is hyperproduction of ROS in the blood [11].

Water phytoextracts are of great importance for consumers. They are easy to obtain and inexpensive. At the same time, these are valuable compositions of natural prooxidants and antioxidants. The mix composition is high sensitive to the conditions of extraction. So, minimal changes in temperature and time of extraction can lead to a change, disappearance or inversion of the effect. It is necessary to accurately determine the conditions for the preparation of phytoex tracts that have a reliable and stable radical-directed effect. Automated CL analysis is the way to verify such conditions (figure 2).

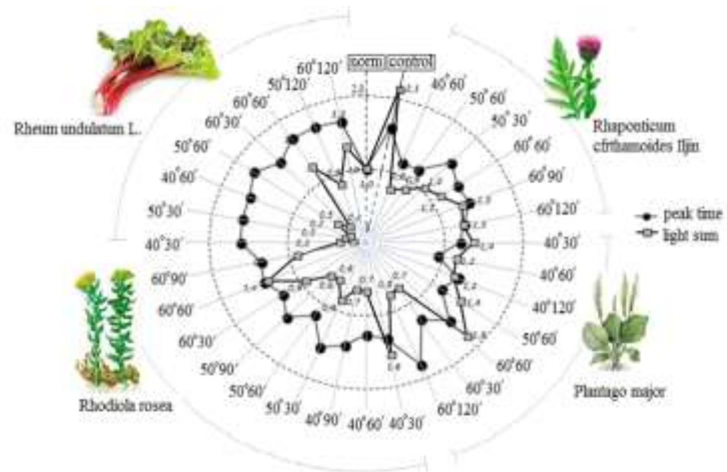


Figure 2. CL reduction ratio in the biogenic Fenton model under the influence of water phytoextracts under various temperature and time of extraction.

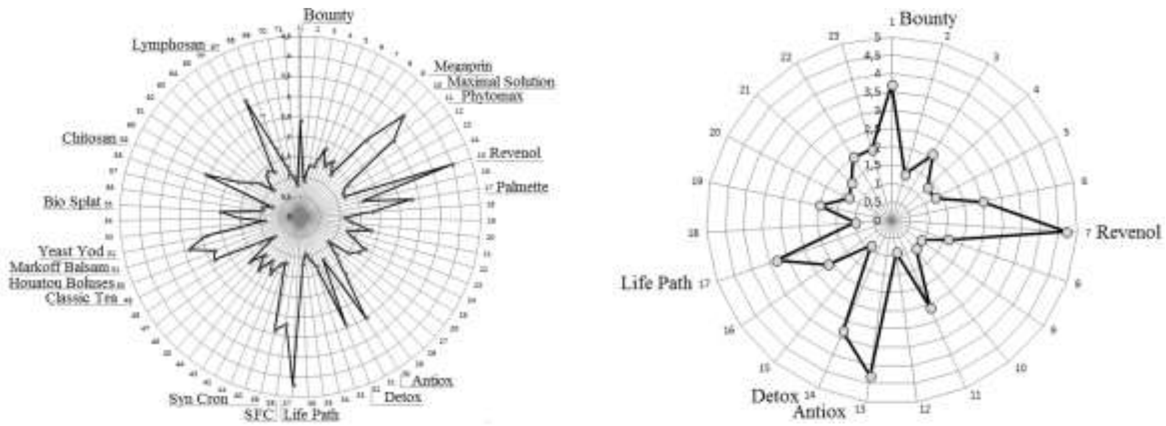
IOP Conf. Series: Materials Science and Engineering **537** (2019) 062008 doi:10.1088/1757-899X/537/6/062008

The results show that under biogenic conditions, the antioxidant properties of water phytoextracts are more depend on the duration of extraction than on temperature in the range of 40o...60o. The strongest antioxidants were the rheum extracts obtained under a shortest extract. Under the influence of these samples, the production of ROS was almost blocked. This corresponds to the available data on the high content of anthraquinone derivatives and tannins in rheum [12]. These substances provide a high anti-radical effect of the extract. So, such nutrients have not to be everyday alimentaries.

In turn, the CL level decreased under the influence of other extracts too, but these changes were not so strong. Thus, under the influence of extracts of leuzei and rhodiola with a short exposure (30 minutes, 60° C), and plantain with a long exposure (120 minutes, 60° C) there was a decrease in ROS production to the physiological norm. Peak time (T_{max}) varied insignificantly. This means that the antioxidant effects of this phytoextracts are due to the direct interaction of their active molecular groups with free radicals [13]. Therefore, they can be recommended as substrates for bioengineering as precursors or ingredients.

3.2. Radical-directed activity of artificial substances

Many of producers of biologically active complexes and functional food declare the obligatory presence of antioxidant properties of all products they manufactured. Using these drugs, consumers often not only pay a high price, but also risk their health. Automated CL analysis is a tool for quickly screen the actual antioxidant activity of biologically active complexes. This was shown in an experiment where 70 commercial micronutrients were tested (figure 3). The petal length of the diagram corresponds to the reduction ratio of base CL under the influence of the sample.



a b

Figure 3. The reduction ratio of CL as antiox activity of commercial mycronutrients under stimulated (a) or unstimulated (b) production of free radicals in the biogenic model.

Most of the samples did not significantly changed the production of biogenic ROS in either the stimulated CL model (by latex, figure 1a), or the basal CL model (in the absence of latex, figure 2b). Less than ¼ of all objects which was tasted had an antioxidant effect. In the figures, these objects are indicated by the longest petals of the diagram with sample names. An important result was the identification of the ability of some micronutrients to reduce hyper production of ROS by phagocytes in unstimulated model. As was shown earlier [4], the phenomenon of basal hyperactivity of blood phagocytes is observed nearly in 80% of practically healthy people. In this case, the metabolic reserve of the body is exhausted and the pathogenic basis is formed. To prevent this, alimentary correction is need using food with antioxidant or prooxidant properties. In this case it is very important to have confidence that the nutrient is real, not a virtual radical-directed activity. Automated CL-control allows you to verify this fact. In addition, it is possible to provide an individual selection of the most adequate nutrient in accordance with the ROS-balance of the consumer body internal environment.

IOP Conf. Series: Materials Science and Engineering **537** (2019) 062008 doi:10.1088/1757-899X/537/6/062008

3.3. Radical-directed activity of synthetic substances

Production of synthetic compounds occurs at a rate much higher than information on their biological activity accumulates. Automated CL analysis allows get results about the antioxidant or prooxidant capacity of substances in short time. This allows you to select the most promising substrates and avoid unnecessary costs of materials, time and labor. As an example, the screening results for anthraquinone derivatives will be discussed here. Anthraquinones are not synthesized in body of humans and animals. For a long time, they had no practical use. However, at present, many of them are considered as important intermediates in the synthesis of anticancer drugs [14].

It is well known that the anticancer activity of compounds is largely due to their antioxidant properties [15]. Therefore, for screening and directed chemical modification, objective information about the antioxidant activity of anthraquinones is required. First of all, information on which functional groups of molecules correlate the appearance or disappearance of antioxidant properties is actual.

In particular, the chemical properties and routes of synthesis of aminoanthraquinones and their thiol derivatives are well studied. At the same time, it is not known how the structure of sulfur-containing anthraquinones is related to their redox properties. Earlier (2008), a number of original aminoanthraquinones derivatives (phenothiazines and isoxazoles) were synthesized at the Chemistry Department of the Krasnoyarsk State Pedagogical University (Krasnoyarsk). It was necessary to evaluate the radical-directed properties of these derivatives. It is impossible to obtain this information by calculation way. The empirical path is the only possible one. Using conventional experimental methods is very time consuming and costly. In contrast, automated CL analysis provides reproducible empirical results using adequate experimental model. The test results of aminoanthraquinone derivatives (n=12) are shown in figure 4.

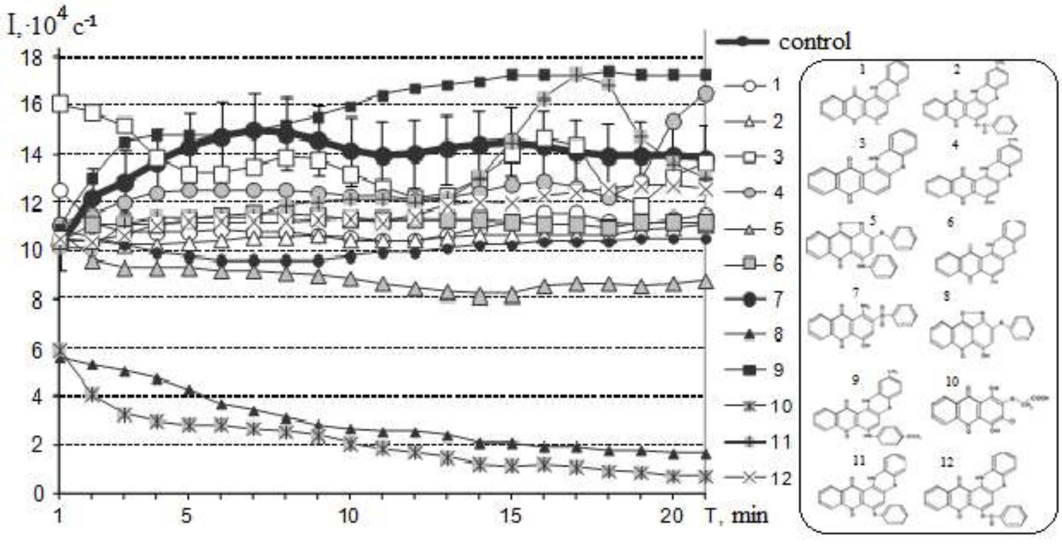


Figure 4. Chemiluminescence kinetics in the biogenic Fenton model under the influence of aminoanthraquinone derivatives.

It can be seen from figure 4 that most of the kinetograms did not differ from the control. This fact means that the relevant substances are not of interest for further modification. At the same time, curves corresponding to phenothiazines 9 and 11 were located above the control. This fact indicated that the samples had prooxidant properties. Three curves (5, 8 and 10) were located below the control. This fact indicated that the samples had antioxidant properties. The antioxidant properties of reduced anthraquinones (preparation 10) are well known. At the same time, the strong antioxidant effect of sulfur-containing isoxazoles was unexpected. The effect was well reproduced in the experiment.

Thus, using CL analysis it was shown that the appearance of antioxidant properties in aminoanthraquinones is associated with the formation of the isoxazole fragment of the molecule.

IOP Conf. Series: Materials Science and Engineering **537** (2019) 062008 doi:10.1088/1757-899X/537/6/062008

Phenothiazines have the oppositely directed effect on free radicals. The next studies have shown that Se-derivatives anthraquinones are stronger antioxidants than S-derivatives [16].

4. Conclusion

Thus, automated CL-control of the antioxidant or pro-oxidant activity of compounds has great prospects for use in bioengineering. The method allows to evaluate the antioxidant capacity of phytopreparations when extraction conditions are high variability and analitic propeties are not stability. The method makes it possible to simultaneously compare the activity of a large number of samples under the conditions of a oneness dynamic experimental model. As a result, extraction conditions can be determined to obtain a reproducible antioxidant effect of the preparations. The method allows to determine the presence of real, non-virtual antioxidant or prooxidant properties of commercial nutrients. In addition, it will be possible to provide an individual selection of nutrients in accordance with the characteristics of the CP-balance of the internal environment of the consumer's body. This way allows you to get the reproducible results within a relevant experimental model in short time, which is very important looking for dynamic development of the functional nutrients market. Using this method one get more effective and economic navigation of chemical synthesis. More oriented synthesis will be carried out. It will be possible to reduce the amount of unnecessary synthetic substances that are usually took away into the environment as waste. As a result, the efficiency of the subsequent functionalization of synthetic compounds is increased. In general, it can be seen that the range of application of automated CL analysis can and should be significantly expanded in order to improve the quality of life.

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