OPTICAL METHOD FOR DETERMINING ANTIOXIDANT ACTIVITY

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Abstract: *The paper considers the main photophysical processes involving excited molecules of luminophores and oxygen and proposes a functional diagram of a device that implements an optical method for determining the antioxidant activity of products by the integrated intensity of luminescence in the presence and absence of oxidized oxygen, the achievement of the technical result of which is ensured by a special luminescent element sensitive to oxygen with a luminescence activator introduced into it, and also revealed ways to increase the useful signal in order to improve the ratio of the useful signal to noise and increase the susceptibility of low oxygen concentrations.*

Keywords: *antioxidant activity, luminophore and oxygen molecules, luminescence, sensitive element.*

Introduction

Antioxidants - as substances that prevent or slow down free radical oxidation processes in natural and synthetic objects, are widely used in the food, chemical, pharmaceutical and cosmetic industries. In addition, biologically active additives, creams, masks, extracts, solutions and other products produced by the cosmetic and pharmaceutical industries are enriched with antioxidants. The avalanche growth of this kind of products requires paying increased attention to the quality of products.

Today in the world there is no unified system for assessing the antioxidant activity of various products and preparations. In addition, laborious and time-consuming procedures and analyzes are used to study the material, the results of which cannot be compared with each other. In this regard, the development of new methods and devices that provide a reliable result in determining the antioxidant activity of products is an urgent problem [1].

Main part

To date, a huge number of devices and techniques have been developed for assessing antioxidant activity (AO) in natural and synthetic products. However, the literature data on this issue are fragmented, focused on different types of model systems, and rely on the existing requirements for the interaction of AOs with various radicals.

In addition, recently there has been a significant increase in interest in optical methods and photo physical processes involving excited molecules of sorbed organic phosphors and oxygen, as well as solid-state luminescent sensitive elements for determining the oxygen content in liquids.

The main photo physical processes involving excited molecules of phosphors and oxygen can be represented as:

$$S_1 + {}^3O_2 \xrightarrow{k_1} T_1 + {}^3O_2, \quad (1)$$

$$S_1 + {}^3O_2 \xrightarrow{k_2} T_1 + {}^1O_2, \quad (2)$$

$$T_1 + {}^3O_2 \xrightarrow{k_3} S_0 + {}^1O_2, \quad (3)$$

$$T_1 + {}^1O_2 \xrightarrow{k_4} S_1 + {}^3O_2, \quad (4)$$

$$T_1 + {}^3O_2 \xrightarrow{k_5} S_1 + {}^3O_2, \quad (5)$$

where S_0, S_1, T_1 – singlet, ground, first excited and triplet state of the phosphor molecule; ${}^3O_2$, ${}^1O_2$ – ground triplet and first excited singlet state of molecular oxygen; k_i – rate constants of the corresponding processes.

It was found that the processes of quenching of excited states of phosphors by molecular oxygen occur in phosphor-oxygen complexes by an exchange mechanism. The high efficiency of this interaction leads to the fact that the processes of quenching of excited states in liquid media are always limited by diffusion, and the rate constant of energy exchange is equal to the diffusion constant to within the spin factor.

The determination of the oxygen concentration can be carried out by quenching the singlet and triplet states of the phosphors, by measuring the energy (integral luminescence intensity) and kinetic characteristics of radiative deactivation of excited states. An analytical expression for the energy characteristics can be written as:

$$\frac{I_0}{I} = 1 + kPo_2, \tag{6}$$

where I_0, I – are, respectively, the integral luminescence intensities in the absence and in the presence of oxygen; k is the Stern-Volmer constant equal to $k = k_d$ to (here t_o is the lifetime of the excited state of the phosphor in the absence of oxygen); Po_2 - is the partial pressure of oxygen.

In the case of exponential decay of luminescence and diffusion quenching of excited molecules by oxygen, the lifetime of the excited state is related to the oxygen concentration as follows:

$$\frac{t_0}{t} = 1 + kPo_2, \tag{7}$$

where t_0 and t – are, respectively, the lifetimes of the excited state of the phosphor in the absence and in the presence of oxygen.

Instrumental implementation of the method for determining the oxygen concentration is fraught with difficulties. First, measuring the lifetime of the singlet state ($t \approx 10 –9$ s) (reactions (1) and (2)) is associated not only with the need to use rather bulky lasers, but also with significant difficulties in creating high-speed high-frequency measuring equipment for signal processing ... Second, in the case of quenching of triplet states by molecular oxygen (reactions (3) - (5)), the problem arises that the decay of long-lived luminescence is not exponential due to the appearance of highly efficient singlet-triplet annihilation (reactions (4)).

At the same time, it should be noted that the method of measuring the kinetic characteristics can be useful for determining sufficiently high oxygen concentrations in the presence of phosphors with a time t_0 comparable to the value of k_d. On the other hand, the implementation of the method based on the measurement of the energy characteristics of the luminescence can be brought to an instrument implementation by using a relatively simple measuring technique.

Expression (7) can be reduced to the following form:

$$\% = 100\left(1 - \frac{I}{I_0}\right) = 100\left(1 - \frac{1}{1+X}\right), \tag{3}$$

where $X = t0 * k_d * Po2$.

From (8) it can be seen that the luminescence will be determined by the oxygen concentration regardless of which values k_d or t are chosen as an argument. And the quenching curve will have the same form, determined by expression (8).

In addition, it can be concluded that the integrated luminescence intensities in the absence and presence of oxygen can be used to judge the concentration of oxygen.

The paper proposes a device for quantitative determination of antioxidant activity by oxygen concentration in a sample, the structural diagram of which is shown in Figure 1 [2-3]. It works as follows.

After power is supplied from the switching power supply 1 to the radiation source 2, the light falls on the light filter 3, and from it on a special cell-cell 4 with the sample and the reaction mixture, in which the oxygen-sensitive element 5 is located, made in the form of a plate made of germanium absorbate, where acriflavin dye is used as a luminescence activator, which is optically coupled through a light filter 6 to a photodetector 7, a direct current amplifier 8. The amplified signal of a photodetector 7 from amplifier 8 is fed to the input of the first ADC 9 and a comparison circuit 10, where signals are superimposed and a sequence of pulses or an error ... Next, the signal from the amplifier 8 is fed to the first information input of the microprocessor 13, to the second input of which the error signal amplified by the amplifier 11 and converted by the second ADC 12 is fed at the output of the comparison circuit 10. In the microprocessor 13 there is a quantitative assessment of the ratio and between the integrated luminescence intensities in the absence and in the presence of oxidized oxygen according to the Stern-

Volmer formula. Further, information from the microprocessor 13 is transmitted to the input of the information display device 14, where a quantitative assessment of the antioxidant activity (ability) of the sample is displayed [4-9].

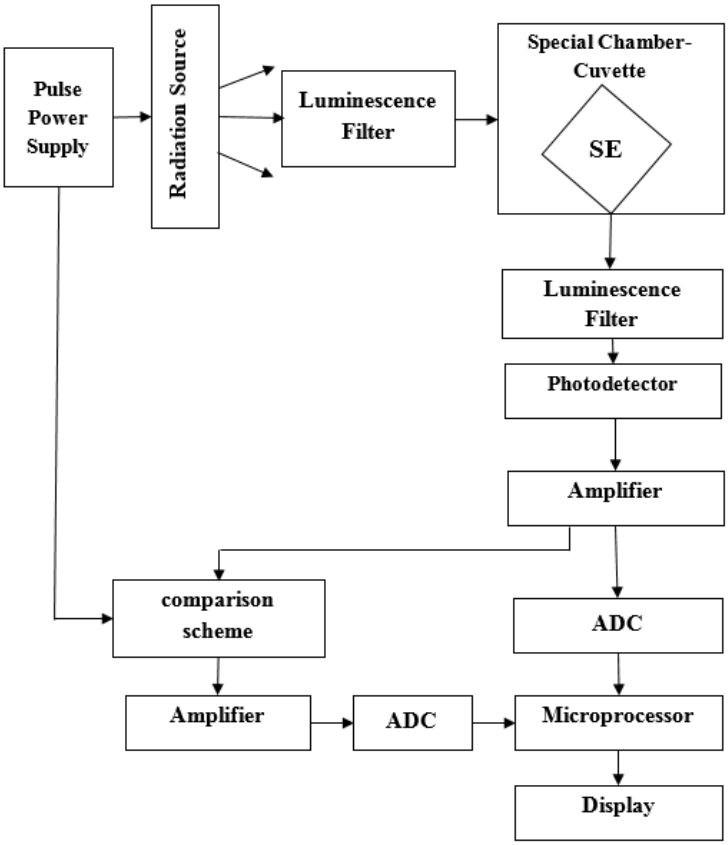

Figure 1. ***Block diagram of a device for the quantitative determination of antioxidant activity by the oxygen concentration of the sample.***

In addition, to reveal the antioxidant properties of a sample in a special cell-cell, a reaction mixture is used — a solution of hydrogen peroxide and an iron catalyst, which is used to oxidize a wide range of reactions [3]. In this case, the reaction of oxygen oxidation is observed in the presence of antioxidants in the sample. It generates both peroxyl and hydroxyl radicals, so they can be added together.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \quad (9)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + H^{+} \quad (10)$$

As you can see, ferrous and ferric ions can be used to catalyze the reaction. When combined with dihydrophthalazinedione, such as luminol, a luminescence is observed, the intensity of which characterizes the antioxidant activity of the sample, determined by the Stern-Volmer formula.

The essence of the invention is illustrated in Figure 1, which shows a block diagram of a device for determining the antioxidant activity of a sample by oxygen concentration using a chemiluminescent reaction. The hardware part contains a pulsed power supply 1, a radiation source 2, two light filters 3 and 6, a sensitive element 5, a special cell-cell 4 with a reaction mixture, a photodetector 7, two DC amplifiers 8 and 11, two analog-to-digital converters 9 and 12 , a comparison circuit 10, a microprocessor 13 and an information display device 14.

As a result, the specified combination of means through the use of a reaction mixture and an oxygen-sensitive luminescent element made in the form of a plate of germanium absorbate, and

acriflavine dye as a luminescence activator, the versatility of the device is achieved due to the provision of high accuracy of quantitative determination of the antioxidant activity of the sample.

Figure 2 shows timing diagrams: $U_{бпи}$ - are pulses from the output of the power supply of a pulsed radiation source, $U_{чэ}$ is the luminescence signal of an oxygen-sensitive element, Uc are pulses from the output of the comparison circuit corresponding to the luminescence signal.

When the power supply unit of the pulsed radiation source 1 is started, a power signal $U_{бпи}$ of the pulsed radiation source with a duration of t_0 is generated, which excites the luminescence signal $U_{чэ}$ when the oxygen-sensitive luminescent element 5 comes into contact in a special cell-cell 4 with the medium being measured, in which it is necessary to determine the antioxidant activity of the mixture by concentration oxygen and reagent. The signals from the switching power supply 1 and the first amplifier 8 are fed to the comparison circuit 10, where they are superimposed on each other and divided into a time sequence, for example, t_1, t_2, t_3, t_4, etc., where U_c appears at the output of the comparison circuit a sequence of pulses with amplitudes u_1, u_2, u_3, u_4. These pulses, describing the excitation signal (u_1), the luminescence decay signals (u_2 and u_3), and the signal characterizing the circuit noise in the absence of luminescence (u_4) from the output of the comparison circuit 10 through the second amplifier 11 are fed to the second ADC 12, where the signal is converted into digital form, and then fed to the microprocessor 13 and the information display device 14.

The device for quantitative determination of the antioxidant activity (ability) of the sample operates as follows.

After power is supplied from the switching power supply 1 to the radiation source 2, the light falls on the light filter 3, and from it on a special cell-cell 4 with the sample and the reaction mixture, in which the oxygen-sensitive element 5 is located, made in the form of a plate made of germanium absorbate, where acriflavin dye is used as a luminescence activator, which is optically coupled through a light filter 6 to a photodetector 7, a direct current amplifier 8. The amplified signal of a photodetector 7 from amplifier 8 is fed to the input of the first ADC 9 and a comparison circuit 10, where signals are superimposed and a sequence of pulses or an error. Next, the signal from the amplifier 8 is fed to the first information input of the microprocessor 13, to the second input of which the error signal amplified by the amplifier 11 and converted by the second ADC 12 is fed at the output of the comparison circuit 10. In the microprocessor 13 there is a quantitative assessment of the ratio and between the integrated luminescence intensities in the absence and in the presence of oxidized oxygen according to the Stern-Volmer formula. Further, information from the microprocessor 13 is transmitted to the input of the information display device 14, where a quantitative assessment of the antioxidant activity (ability) of the sample is displayed.

Achievement of the technical result is ensured by the fact that the oxygen-sensitive luminescent element is made in the form of a plate made of germanium adsorbates with a luminescence activator (phosphor) introduced into it, where acriflavin is used as a dye, which has a high luminescence quantum yield. The thickness of the plate is 1 mm, which is many times higher than the parameters of the prototype and, therefore, allows the use of a larger volume of phosphor. Superimposing time diagrams on top of each other and dividing into a time sequence t1-t4 makes it possible to measure the luminescence amplitude in different time intervals, which in turn provokes a weakening or complete elimination of such external disturbing factors as noise in the operation of the comparison circuit, changes in the parameters of the radiation source, and allows you to determine the decay time of luminescence and the change in its intensity at different time intervals, and through the intensity to reveal the concentration of oxidized oxygen, which makes it possible to judge the antioxidant activity of the sample. All this gives rise to an increase in the useful luminescence signal, which greatly improves the ratio of the useful signal to noise and increases the susceptibility of low oxygen concentrations. Revealing the decay time of the luminescent signal and changes in its intensity is based on the values of the intensity of the luminescent signal, which leads to an increase in the stability of measurements.

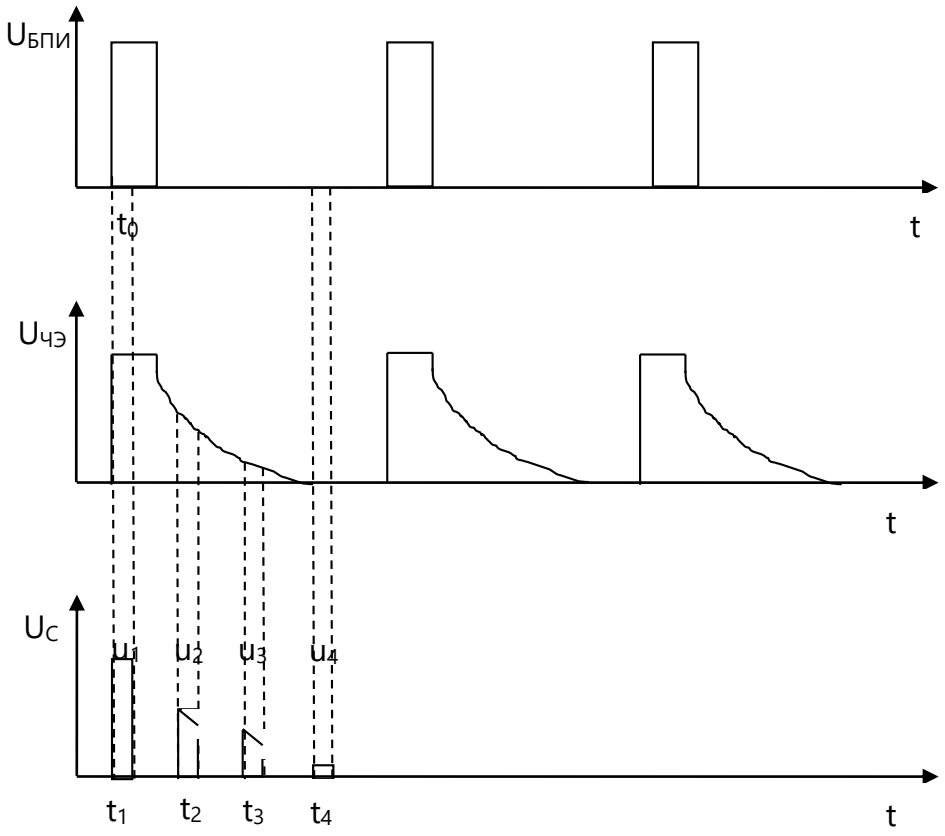

Figure 2. ***Timing diagrams:***
$U_{бти}$ - pulses from the output of the power supply of a pulsed radiation source, $U_{чэ}$ - luminescence signal of an oxygen-sensitive element, Uc - pulses from the output of the comparison circuit corresponding to the luminescence signal.

In addition, the use of the reaction mixture in a special chamber-cuvette makes it possible to reduce the time of redox reactions from half an hour to several minutes due to its catalytic action, which in turn also contributes to the speed of measurements.

Conclusion

Thus, the paper considers the implementation of an optical method for determining the antioxidant activity of products from the integral intensity of luminescence in the presence and absence of oxidized oxygen, and the achievement of the technical result is ensured by the fact that the oxygen-sensitive luminescent element is made in the form of a plate of germanium adsorbates with an activator introduced into it luminescence (phosphor), where acriflavine with a high quantum yield of luminescence is used as a dye. The ways of increasing the useful signal in order to improve the ratio of the useful signal to noise and increase the susceptibility of low oxygen concentrations have been identified. The identification of the decay time of the measuring signal and the change in its intensity, based on the values of the intensity of the luminescent signal, are determined, which leads to an increase in the stability of measurements. It was also shown that the use of the reaction mixture in a special chamber-cuvette allows reducing the reaction time from half an hour to 1-2 minutes due to its catalytic action, which contributes to the speed of measurements.

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NON-DEPENDENT CUBIC SPLINE FUNCTION AND ITS USE IN DIGITAL PROCESSING OF SIGNS

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Abstract. *This paper proposes a method of constructing cubic spline functions of a local character with equal distances between nodes. Previous studies have shown precise models for the digital processing of signals using interpolated cubic splines, with accurate examples showing the high degree of accuracy of the approximation of interpolated functions. In this study, the size of the calculations required to find the parameters to be determined during the construction of the spline function does not depend on the number of node points. Local-based splines are used to build such spline functions. Digital processing of the gastroenterological signal was performed on the basis of the spline-function model discussed in the article.*

Keywords. *Gastroentrological signal, spline-function, interpolation cubic spline, interpolation, cubic spline independent of node points.*

Introduction

Today, spline-function models are widely used by researchers in the field of digital signal processing [1, 2]. Because the accuracy of spline functions is higher than that of classical polynomials, and the algorithms based on them require less computation. Existing classical interpolation models, their application in signal recovery and digital processing algorithms are performed depending on the node points. In this case, a large number of node points are required for these models to have a high degree of convergence. In cases where the nodes are point-dependent, the order of the system of equations formed in the construction of the model under consideration increases. And this complicates the process of solving a system of equations and does not ensure a high level of accuracy of the model [3, 4, 14-20].

The process of digital processing of signals of the spline model discussed in this article was carried out independently of the node points. In this case, the increase in the degree of convergence of the model under consideration does not depend on the increase in the order of the system of equations. Methods for determining nonlinear spline functions, their derivatives, and estimating errors are performed in the same way as for simple interpolation splines [5, 9,10].