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Chemiluminescent hydrogen peroxide sensor based on luminol and a colloidal solution of metal nanoparticles

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The effect of gold and silver nanoparticles with plasmon resonance on the chemiluminescence of luminol in an oxidizing environment was studied. It has been shown that when a colloidal solution of metal nanoparticles is added to luminol, the intensity of chemiluminescence caused by the presence of hydrogen peroxide and sodium hypochlorite increases and can be easily recorded at a pH level characteristic of biological media close to neutral, at which chemiluminescence is weak in the absence of metal nanoparticles.

Keywords: chemiluminescence, plasmon resonance, silver nanoparticles, gold nanoparticles, pH value.

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Introduction

Detection and determination of the level of hydrogen peroxide (H_2O_2) and hypochlorites (HClO salts) in a biological system can play a vital role in monitoring various pathological changes. High levels of these reactive oxygen species can harm the body, causing cell damage, inflammatory diseases and cancer [1–12]. Although most of the used methods for monitoring H_2O_2 and OCl- [13–18] are based on electrochemical processes, optical methods for detecting ROS have recently been developed, which have such advantages as high sensitivity, selectivity, speed and portability [19]. Particularly attractive is the opportunity of using chemiluminescence (CL) of chemiluminophore molecules, since its excitation does not require a light source. The energy source for CL is an exothermic chemical reaction accompanied by the emission of photons, while the operation of optical sensors based on photoluminescence requires an external light source [20]. The advantages of the detection method based on CL also include the comparative simplicity of preparing the substances under study for analysis [21–24].

Along with the mentioned advantages, CL-based sensor systems have a significant drawback, which is the low intensity of the CL signal, leading to the need to use sensitive optical radiation detectors and additional amplification of weak detected signals [25,26]. In changed, the choice of chemiluminophores suitable for practical use is small. The most effective are luminol and lucigenin. The chemiluminescence of the selected chemiluminophore can be enhanced in the presence of additional activator substances in the solution, in particular metal ions, as well as some enzymes [27–29].

An alternative approach to enhancing CL is to increase the rate of radiation-induced transitions of the excited chemiluminophore molecule, which allows to reduce the probability of nonradiative deactivation of the excited molecule. The implementation of this approach became possible with the development of methods for creating metal nanoparticles with plasmonic resonance. To effectively accelerate radiative transitions, two conditions should be met: the surface plasmonic resonance (SPR) absorption band of a metal nanoparticle should be spectrally close and overlap with the emission band of the chemiluminophore, and the chemiluminophore molecule itself must be located near the surface of the nanoparticle at distances on the order of the nanoparticle size [30]. In this case, the rate of radiation-induced transitions in the phosphor molecule can be increased due to the Purcell effect. To implement this approach, various types of metal nanostructures have been used, including nanoparticles, nanocrystals, nanoclusters and nanofilms [30–37]. The position of the SPR bands of noble metal nanoparticles is favorable for enhancing the CL of luminol. The SPR band of silver nanoparticles (AgNP) in water with a maximum at a wavelength of 400 nm significantly overlaps with the CL band of luminol, the maximum of which is at 425 nm. The SPR band of gold nanoparticles (AuNPs) in water with a maximum at a wavelength of 520 nm also overlaps with the CL band of luminol, although a little worse.

We observed chemiluminescence of luminol, enhanced in the presence of silver nanoparticles in the form of a colloidal solution and granular film, previously [34,38]. This work reports the dependence of the CL intensity of luminol on the concentration of hydrogen peroxide in the presence of silver and gold nanoparticles. Due to plasmonic enhancement,

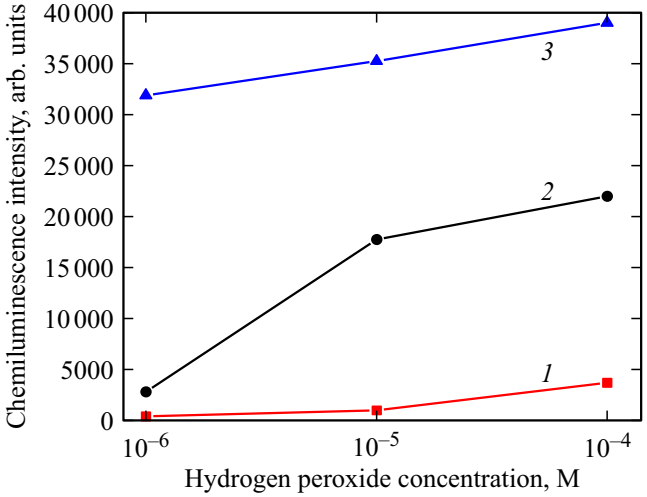

Figure 1. Luminol CL intensity depending on the concentration of hydrogen peroxide at different concentrations of silver nanoparticles: *1* — without nanoparticles, *2* — with a concentration of silver nanoparticles 10^9 ml^{-1}, *3* with concentrations of silver nanoparticles 10^{10} ml^{-1}.

lower concentrations of hydrogen peroxide become available for observation and recording.

Materials and methods of experimental study

To carry out CL studies, a solution of luminol (Lenreaktiv) was prepared with a concentration of 2×10^{-4} M (an aqueous solution of luminol had a pH of 6, pH of 7 was achieved by adding NaOH). The concentrations of aqueous solutions of NaOCl and H_2O_2 were determined spectrophotometrically before use ($\varepsilon_{290} = 350\,M^{-1}cm^{-1}$ at pH 12 and $\varepsilon_{230} = 74\,M^{-1}cm^{-1}$ for NaOCl and H_2O_2, respectively [39]). To carry out the reaction, mixtures of a solution of luminol (1 ml) with H_2O_2 of the required concentration were prepared. NaOCl solution (400 μl) was injected using a syringe (1 ml) through a thin plastic tube. The concentration of NaOCl in the working volume of the mixture was 10^{-4} M, the concentration of H_2O_2 varied from 10^{-4} to 10^{-6} M.

An aqueous solution of NaOCl with a concentration of 10^{-4} M has a pH 8.5. When it is added to a luminol solution, the pH of the working mixture is approximately 7.5.

To study the influence of plasmonic effects on the CL intensity, silver and gold nanoparticles were used. A colloidal solution of gold nanoparticles was prepared as follows. Precisely 240 ml of deionized water was poured into the Erlenmeyer flask; the water was brought to a boil with a reflux water cooler on a magnetic stirrer with electric heating; added 2.5 ml of 1% solution $HAuCl_4$; added 7.5 ml of 1% sodium citrate solution; continued boiling for another 30 min; The formation of a bright red sol was observed.

The diameter of the synthesized gold nanoparticles (15 nm) was determined using a NanoDrop 2000c UV-Vis spectrophotometer (Thermo Scientific) and a particle size analyzer. MilliQ deionized water, freshly prepared filtered sodium citrate solutions (Sigma), and $HAuCl_4$ (Sigma, Aldrich) were used in all experiments. The concentration of this solution was $1.6 \cdot 10^{12}$ ml^{-1}. The concentration of nanoparticles in the obtained colloidal solution was evaluated using the extinction coefficient. In the working volume, the concentration was 10^{10} ml^{-1} and 10^{11} ml^{-1}.

Silver nanoparticles were synthesized using laser ablation. The silver target was placed at the bottom of a quartz cuvette filled with distilled water. Radiation from a pulsed Nd:YAG laser (Solar LS) was focused on the target surface. The wavelength of the second harmonic was 532 nm, the pulse energy was 50 mJ, and the pulse duration was 7 ns. Irradiation was carried out for 15 min.

The concentration of silver nanoparticles in the obtained colloidal solution was theoretically estimated taking into account the particle size distribution (from images obtained in a scanning electron microscope) by comparing optical absorption spectra with the Mie scattering theory. The concentration was $6 \cdot 10^{10}$ ml^{-1}. In the working volume, the concentration of silver nanoparticles was 10^9 and 10^{10} ml^{-1}.

To study the CL kinetics of luminol at different concentrations of hydrogen peroxide, a photomultiplier tube (PMT) with an H9305-04 ADC (Hamamatsu) placed in a dark container with a cell holder was used. The exposure time was 100 ms. A mixture of luminol with hydrogen peroxide of the required concentration in a volume of 1 ml and with the addition of silver or gold nanoparticles was placed in a plastic cuvette (1 cm) and installed in the cuvette holder in front of the PMT window. The introduction of a NaOCl solution initiated the oxidation reaction of luminol molecules. The CL of luminol arising as a result of the reaction was recorded by a PMT and transmitted to a computer using software written in Python. With the onset of the oxidation reaction, the CL intensity of luminol rapidly increased, reached a maximum and began to decrease due to the consumption of reagents. Its peak value was taken as a measure of CL intensity.

Results

Figure 1 shows the dependence of the luminol CL intensity on the concentration of hydrogen peroxide at various concentrations of silver nanoparticles and in their absence. An increase in the CL intensity of luminol with increasing concentration of hydrogen peroxide is also recorded in the absence of silver nanoparticles, however, the signal is small and at minimal concentrations of hydrogen peroxide 10^{-6}–10^{-5} M cannot be reliably measured. When silver nanoparticles are introduced into the reaction mixture, the CL intensity increases tens of times, and its dependence on the concentration of hydrogen peroxide becomes more pronounced. With an increase in the concentration of silver

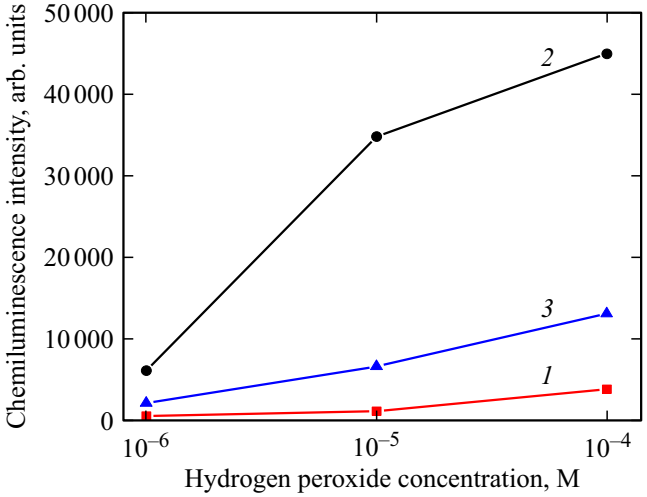

Figure 2. Luminol CL intensity depending on the concentration of hydrogen peroxide at different concentrations of gold nanoparticles: *1* — without nanoparticles, *2* — with a concentration of silver nanoparticles 10^{10} ml^{-1}, *3* with concentrations of silver nanoparticles 10^{11} ml^{-1}.

nanoparticles, the CL intensity increases, but its dependence on the concentration of hydrogen peroxide in the region of low concentrations becomes less sharp.

Figure 2 shows the dependence of the luminol CL intensity on the concentration of hydrogen peroxide at various concentrations of gold nanoparticles. For comparison, the dependence of the CL intensity of luminol in the absence of metal nanoparticles was also reproduced.

The introduction of gold nanoparticles into the reaction mixture at a concentration of 10^{10} ml^{-1} leads to an increase in CL intensity comparable to the increase with the introduction of silver nanoparticles. However, when the concentration of gold nanoparticles increases to 10^{11} ml^{-1}, the enhancement effect does not increase, as in the presence of silver nanoparticles, but decreases.

Results and discussion

In this work, the main attention was paid to the opportunity of detecting and determining the concentration of hydrogen peroxide using the CL of luminol. In connection with this, the oxidation reaction was started by adding a fixed amount of sodium hypochlorite to a previously prepared reaction mixture with a variable concentration of hydrogen peroxide. Since, to simulate conditions favorable for biochemical studies, the pH of the reaction mixture was maintained at a neutral level (pH 7.5), the CL intensity without the introduction of metal nanoparticles was low. Due to this, comparison of CL intensities caused by the presence of hydrogen peroxide in different concentrations was difficult. The introduction of metal nanoparticles, both silver and gold, into the reaction mixture leads to a significant enhancement of luminol CL in the presence of oxidizing agents, which facilitates its registration and makes it possible to compare CL intensities at different concentrations of hydrogen peroxide.

The results of the experiments are insufficient for unambiguous determination of the mechanism of CL enhancement by gold and silver nanoparticles and its connection with the Purcell effect. The change in CL intensity over time is determined by the kinetics of the chemical oxidation reaction, which precludes the use of this dependence to determine the rate of radiative transitions in the excited chemiluminophore molecule. Another characteristic feature of the plasmonic enhancement mechanism is associated with the degree of overlap of the plasmonic resonance bands and luminol CL. In this regard, silver nanoparticles are superior to gold nanoparticles, since the SPR band of gold nanoparticles with a maximum at a wavelength of 520 nm overlaps with the CL band of luminol with a maximum at a wavelength of 425 nm, which is significantly worse than the SPR band of silver nanoparticles with a maximum at a wavelength 400 nm. In accordance with this, the maximum CL enhancement by silver nanoparticles exceeded 30 times, and by gold nanoparticles only — 10 times. Additional evidence in favor of the plasmonic mechanism of CL enhancement by silver nanoparticles was obtained in [38] by isolating the surface of nanoparticles from direct contact with chemiluminophore molecules. Since this only resulted in a weakening of the enhancement effect, but not to its complete disappearance, the plasmonic mechanism of CL enhancement seems much more likely than chemical catalysis, which requires direct contact of the phosphor molecule with the metal surface.

Conclusion

Application of colloidal solutions of gold and silver nanoparticles allows to increase the CL intensity of luminol in an oxidizing medium tens of times. What is especially important is that this enhancement is in conditions favorable for the study of biological samples, close to neutral (pH 7.5), in which the CL intensity of luminol without enhancement by metal nanoparticles is low, which complicates its use for detecting and determining the concentration of hydrogen peroxide.

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Conflict of interest

The authors declare that they have no conflict of interest.

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