19

Metal-enhanced chemiluminescence of luminol in a microfluidic system with vacuum-deposited silver nanoparticles

© D.V. Kononov[1], A.V. Palekhova[1], N.A. Filatov[2], N.B. Leonov[1], A.S. Bukatin[2,3], D.R. Dadadzhanov[1], T.A. Vartanyan[1]¶

[1] ITMO University, St. Petersburg, Russia
[2] Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences, St. Petersburg, Russia
[3] Institute for Analytical Instrumentation of the Russian Academy of Sciences, St. Petersburg, Russia

¶ E-mail: Tigran.Vartanyan@mail.ru

Received November 28, 2024
Revised November 28, 2024
Accepted December 04, 2024

Enhancement of luminol chemiluminescence in the presence of silver nanoparticles was studied in a microfluidic chip specially developed for this purpose. The chip design provides intensive mixing of luminol with an oxidizer and delivery of the mixture to nanoparticles that have a plasmon resonance in the luminol chemiluminescence band. The dependence of the luminol chemiluminescence intensity on the sodium hypochlorite concentration was established. Coating the bottom of the microfluidic chip with silver nanoparticles led to an increase in the luminol chemiluminescence intensity in a neutral medium by an average of one and a half times.

Keywords: microfluidic chip, chemiluminescence, plasmon resonance, luminol.

DOI: 10.61011/EOS.2024.12.60453.7399-24

Introduction

Chemiluminescence — emission of light caused by chemical reaction, has multiple various applications, including for analysis of biological materials. Chemiluminescence may most directly be used to detect and determine the degree of body oxidative stress, which is a marker and sometimes a cause for some diseases. Luminol and lucigenin emitting when in contact with oxidants in the blue-green area of the spectrum, are successfully used to record singlet oxygen, hydrogen peroxide and other peroxides in alkaline environment. In neutral media used to study biological specimens, the intensity of chemiluminescence of both mentioned chemiluminophores drops drastically, which prevents from recording of low oxidant concentrations. Under these conditions the weakness of chemiluminescence is related to both low probability of the molecule transition to electron-excited state as such in process of reduction-oxidation reaction, and to small quantum yield of chemiluminescence due to competition of radiative and nonradiative channels of deactivation of electron-excited product of chemical reaction [1]. The last circumstance opens the possibility to increase the intensity of chemiluminescence by increasing its quantum yield due to the acceleration of the radiation transition.

Dependence of speed of radiation transitions on dielectric properties of the environment surrounding the emitter is known as Purcell effect [2]. The action of metal nanoparticles with localized plasmon resonance, in particular, gold and silver ones, may be especially strong. Metal nanoparticles made of silver and gold are widely used in such biomedical applications as targeted drug delivery [3], photothermal [4] and photodynamic [5] cancer therapy, optical coherence tomography, immunoassay [6,7] and biosensing [8–10]. The biological research already includes papers dedicated to using plasmon amplification, for example, to register mycotoxins [11]. The important feature of metal nanoparticles is dependence of their optical properties on the size and shape [12]. For the effective connection between the emitting molecule and metal nanostructure, it is necessary to provide for overlap of the chemiluminophore emission spectrum and plasmon resonance band. The first results for plasmon-amplified chemiluminescence were obtained [13] on chemiluminophores that are not suitable for biological use due to toxicity. The decisive role of electrodynamic effect of radiation transition acceleration compared to the potential catalytic action of silver at the chemiluminescence intensity of luminol, which is suitable for biological applications, was demonstrated in [9].

This paper reports use of the specially developed microfluid chip to study the amplification of luminol chemiluminescence in the oxidizing medium with neutral pH = 7 when in contact with silver nanoparticle. The assembly of silver nanoparticles was received by vacuum deposition of metal vapors on a glass substrate, which was integrated into a microfluid chip. On the second wafer of the microfluid chip made of polydimethyl siloxane (PDMS), microchannels and functional elements were formed, which are necessary to mix reagents and deliver them to the area of emission registration. Intensity of luminol chemiluminescence in presence of silver nanoparticles on average increased 1.5 times.

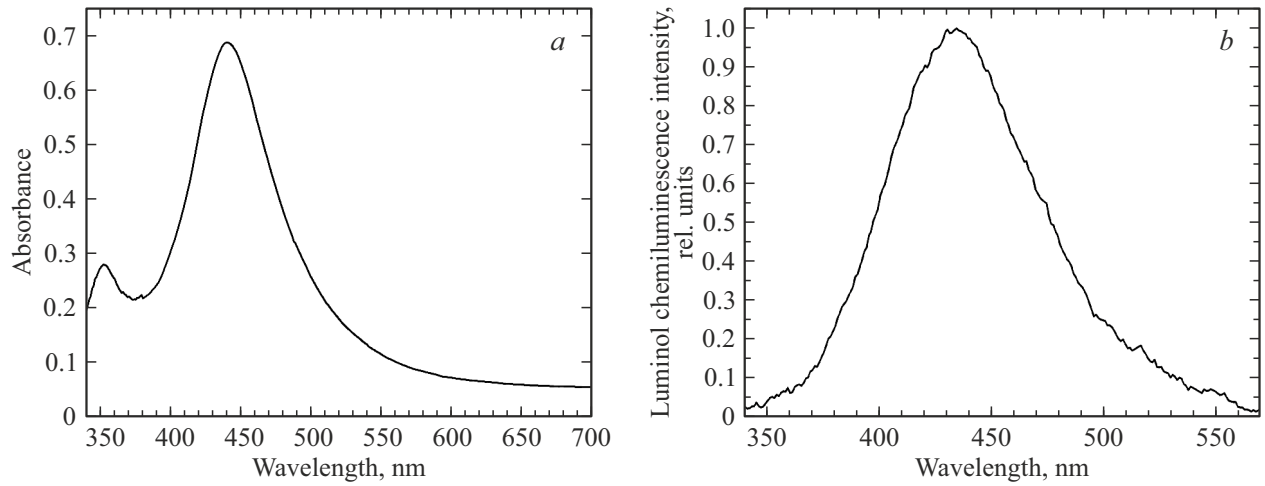

Figure 1. (*a*) Extinction spectrum of silver island film formed on bottom of microfluid channel, (*b*) chemiluminescence spectrum of luminol in process of oxidation with sodium hypochlorite.

Formation of assembly of silver nanoparticles

Silver nanoparticles were formed on a glass substrate by thermal sputtering in vacuum chamber PVD-75 (Kurt Lesker, USA). Equivalent thickness of granulated film varied within the range from 8 to 10 nm according to the readings of quartz microscale. After sputtering the film was annealed in vacuum for 30 min at 200 °C. Sputtering and annealing processes are described in more detail in [14].

Fig. 1, *a* presents the extinction spectrum of island silver film measured on spectrophotometer SF-56 (LOMO, Russia), and fig. 1, *b* — the chemiluminescence spectrum of luminol recorded in the process of oxidation with sodium hypochlorite with multichannel photon analyzer PMA-12 (Hamamatsu, Japan). Evident spectral proximity of luminol chemiluminescence spectrum and absorption band of granulated silver film makes it possible to rely on effective interaction between the excited products of luminol oxidation and collective electron excitations in nanoparticles forming a silver film.

For use of the granulated silver film within the microfluid chip it was necessary to provide for the possibility of tight connection of the glass substrate carrying silver nanoparticles with the wafer from PDMS, on which channels and functional elements were created. Since the tested technology suggested PDMS connection to glass, a metal mask was created, which made it possible in the conditions of vacuum silver sputtering leave the parts on the substrate that are not coated with silver. Besides, it was checked that treatment of the glass substrate with solvents and corona discharge necessary to provide for reliable connection of microfluid chip, insignificantly reduces extinction of the granulates silver film [15] and has no effect on the spectral position of the plasmon resonance.

Design and manufacturing of microfluid chip

The designed microfluid chip consists of two tightly connected wafers: the necessary functional elements were created on one wafer formed from PDMS, and on the second one — the glass one which usually only performs the protective function [16], — a granulated silver film was created, which was described above.

The key stage for the reaction is mixing of chemiluminophore and analyte that launches the oxidation reaction and further results in appearance of excited reaction products and light emission. For effective mixing of reagents in microfluid devices, it is necessary to use special mixers [17–19], since without those there is laminar flow in the channels, which prevents mixing. The topology of the single channel of microfluid chip is presented in fig. 2. In the first part of the channel there are inputs for chemiluminophore and analyte and a mixer, and in the second one — the surface that could have been coated with silver nanoparticles, and output for the spent mixture. In the area of the microfluid mixer the depth of channels is 70 μm, and in the area of location of silver nanoparticles and registration of chemiluminescence — 10 μm. The scheme of one channel to observe chemiluminescence is shown in fig. 2.

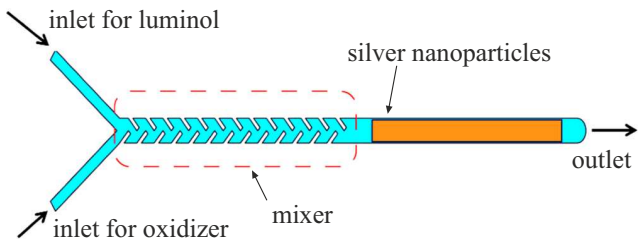

Figure 2. Principle diagram of one channel of microfluid chip for chemiluminescent diagnostics of oxidative stress.

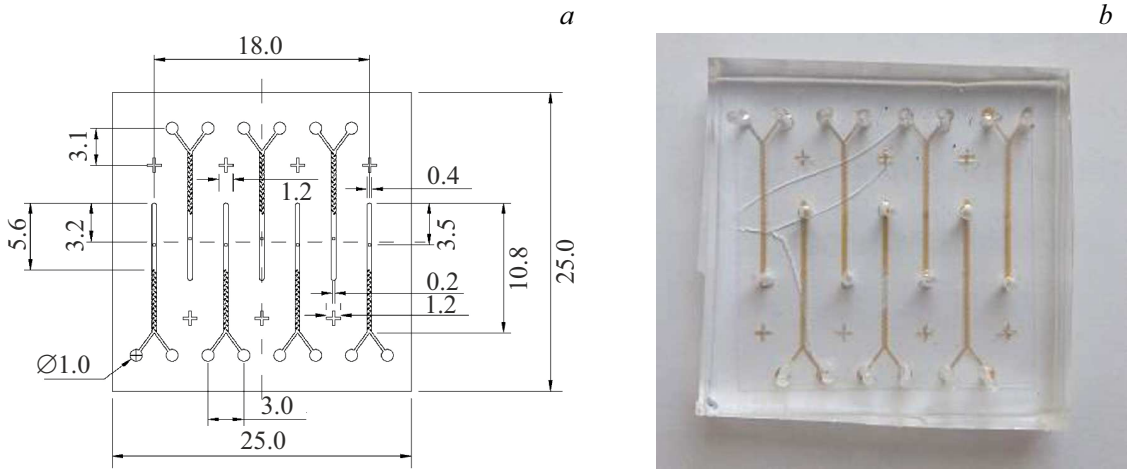

Figure 3. Design and photograph of microfluid chip with seven independent channels used for study of chemiluminescence. Each channel has two inputs to supply chemiluminophore and oxidant and one output for spent mix. The photograph shows a mixer in the form of a snake, where intense mixing of chemiluminiphore and oxidant takes place.

For convenience of experiments on one substrate with size of 25×25 mm 7 independent microfluid channels were made, the scheme of location of which is shown in fig. 3, *a*. Fig. 3, *b* presents the photograph of the manufactured microfluid chip.

Measurement procedure

To measure chemiluminescence, the chip was placed into a special jacket, which removed spurious lighting. The microfluid chip was fixed above active area of photomultiplier tube H11890 (Hamamatsu), operating in the photon count mode, to collect the maximum of the emitted light. After that reagents were fed through a syringe pump with speed of $10\,\mu$L/min along two silicon tubes: luminol and mix of sodium hypochlorite with sodium hydroxide, concentrations of which varied. Reagents were mixed in the channel of the microfluid chip and then removed from the chip along the third silicon tube. The signal of the photomultiplier tube was displayed on the monitor screen. Since the time of luminol and oxidant feed to the mixer is dozens of seconds, during which the intensity of chemiluminescence increases, was the stabilized value of the chemiluminescence intensity was accepted as the measurement result.

Experimental results

First of all, the optimal concentration of the luminol aqueous solution was determined. For this purpose chemiluminescence was measured in the chip without nanoparticles in the alkaline medium with $\mathrm{pH} = 12$, at which chemiluminescence is rather high enough for the confident registration at low concentration of oxidant equal to $100\,\mu$M. As the luminol concentration increases up to 0.3 mM, the chemiluminescence intensity grows. However, saturation, and then reduction of chemiluminescence intensity is observed at high luminol concentrations. Further experiments were carried out at luminol concentration of 0.3 mM.

Amplification of luminol chemiluminescence under the effect of silver nanoparticles is of greatest interest in neutral conditions at $\mathrm{pH} = 7$, when the radiation intensity without nanoparticles at low concentration of oxidant drops down to noise level. Fig. 4 presents the results of luminol chemiluminescence intensity measurements in microfluid channels without nanoparticles and with silver nanoparticles produced by the above method. You can see that despite the significant spread in the measurement results, the chemiluminescence intensity in the chips with silver nanoparticles is higher than in the chips without silver nanoparticles.

Results and discussion

The enhancement of luminol chemiluminescence in the chip with silver nanoparticles was on average 50 %. This value seems to be low at first, since if you follow the theoretical calculations [20] and numerial modeling [21], at the small distances from metal nanoparticles with plasmon resonance in the emission band of excited luminol reaction products, one could expect the acceleration of the radiation transitions and corresponding increase in the intensity of chemiluminescence dozens of times. According to [22], the averaging of Purcell factor by orientation of the emitter is equivalent to averaging by polarizations of incident emission, so that the results [21] may be used to assess the average Purcell factor. However, the geometry of the microfluid chip is such that only a small share of excited molecules is located at such distances from the metal nanoparticles, which are comparable to their dimensions, and when the emitter is removed from the nanoparticle, the Purcell factor drops quickly. Indeed, the depth of channels

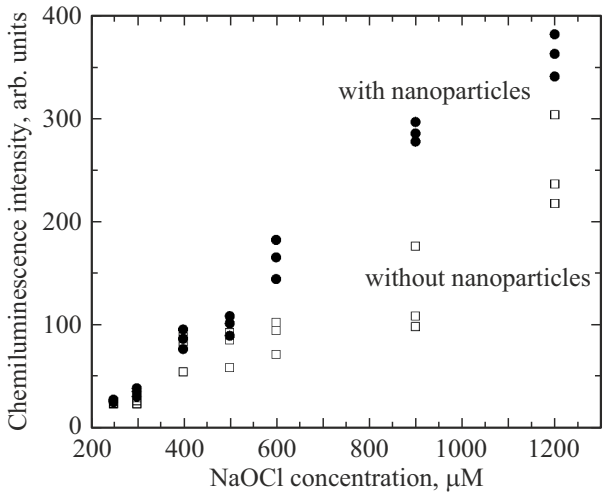

Figure 4. Increase of luminol chemiluminescence intensity in microfluid chips with the silver film applied on the channel bottom. Chemiluminescence intensity in channels without silver film is shown with small open squares, and intensity of chemiluminescence in the channels with silver film applied on the bottom of the channel — with filled circles. Luminon concentration is 0.3 mM, pH = 7.

of the manufactured microchips in the area of emission recording was 10 μm. Since the dimensions of the silver nanoparticles and distances between them after annealing are around 100 nm [14], approximately the hundredth share of the emitting molecules is located at the optimal distance from the surface coated with nanoparticles. Further reduction of the channel depth is limited by rising hydrodynamic resistance, requring increase of fluid pressure, which, in its turn, is limited to the yield strength of the microfluid system parts connection. Reduction of the channel depth is also limited by the precision of making a master die for soft lithography, which was used to make a chip of PDMS. Under the specified conditions the found value of luminol chemiluminescence enhancement by an average of 50% appears to be consistent with the observation conditions.

Conclusion

Use of the specially designed microfluid system made it possible to observe the luminol chemiluminescence in the controlled and reproducible conditions. The coating of the bottom of microfluid channels with silver nanoparticles having plasmon resonance in the luminol chemiluminescence band caused increase in the luminescence intensity when oxidized in the neutral medium with pH = 7, which is most relevant for biomedical applications, on average by 50 %.

Funding

This study was supported financially by grant № 23-72-00045 from the Russian Science Foundation, https://rscf.ru/project/23-72-00045/.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Y. Ando, K. Niwa, N. Yamada, T. Irie, T. Enomoto, H. Kubota, Y. Ohmiya, H. Akiyama. Photochem. Photobiol., **83** (5), 1205 (2007). DOI: 10.1111/j.1751-1097.2007.00140.x
[2] A.F. Koenderink. Opt. Lett., **35** (24), 4208 (2010). DOI: 10.1364/OL.35.004208
[3] V. Chandrakala, V. Aruna, G. Angajala. Emergent Materials, **5** (6), 1593–1615 (2022). DOI: 10.1007/S42247-021-00335-X
[4] M. Kim, J.H. Lee, J.M. Nam. Advanced Sci., **6** (17), 1900471 (2019). DOI: 10.1002/ADVS.201900471
[5] T. Liu, Y. Song, Z. Huang, X. Pu, Y. Wang, G. Yin, L. Gou, J. Weng, X. Meng. Colloids Surf. B: Biointerfaces, **207**, 112023 (2021). DOI: 10.1016/J.COLSURFB.2021.112023
[6] Y. Gao, Y. Zhou, R. Chandrawati. ACS Appl. Nano Mater., **3** (1), 1–21 (2020). DOI: 10.1021/ACSANM.9B02003
[7] H. Yang, W. Xu, Y. Zhou. Microchimica Acta, **186** (12), 1–22 (2019). DOI: 10.1007/S00604-019-3904-9
[8] H. Malekzad, P. Sahandi Zangabad, H. Mirshekari, M. Karimi, M.R. Hamblin. Nanotechnol. Rev., **6** (3), 301–329 (2017). DOI: 10.1515/NTREV-2016-0014
[9] D.R. Dadadzhanov, I.A. Gladskikh, M.A. Baranov, T.A. Vartanyan, A. Karabchevsky. Sens Actuators B Chem., **333**, 129453 (2021). DOI: 10.1016/J.SNB.2021.129453
[10] G.A. Sotiriou. Wiley Interdiscip. Rev. NanomedNanobiotechnol., **5** (1), 19–30 (2013). DOI: 10.1002/WNAN.1190
[11] F. Jiang, P. Li, Ch. Zong, H. Yang. Analytica Chimica Acta, **1114**, 58–65 (2020).
[12] K.L. Kelly, E. Coronado, L.L. Zhao, G.C. Schatz. J. Phys. Chem. B., **107** (3), 668–677 (2003). DOI: 10.1021/JP026731Y
[13] M.H. Chowdhury, K. Aslan, S.N. Malyn, J.R. Lakowicz, Ch.D. Geddes. Appl. Phys. Lett., **88** (17), 173104 (2006). DOI: 10.1063/1.2195776
[14] N.B. Leonov, I.A. Gladskikh, V.A. Polishchuk, T.A. Vartanyan. Opt. Spectrosc., **119** (3), 450–455 (2015). DOI: 10.1134/S0030400X15090179.
[15] T.A. Vartanyan, N.B. Leonov, S.G. Przhibel'skii. Opt. Quant. Electr., **49**, 127 (2017). DOI: 10.1007/s11082-017-0969-8
[16] A.A. Evstrapov. Zhurn. Ros. khim. ob-va im. D.I. Mendeleeva, **55** (2), 99–110 (2011) (in Russian).
[17] K. Ward, Z.H. Fan. J. Micromech. Microeng., **25** (9), 094001 (2015).
[18] Ch.-Y. Lee, Ch.-L. Chang, Y.-N. Wang, L.-M. Fu. Int. J. Mol. Sci., **12** (5), 3263–3287 (2011).
[19] A.S. Bukatin, I.S. Mukhin, E.I. Malyshev, I.V. Kukhtevich, A.A. Evstrapov, M.V. Dubina. ZhTF, **86** (10), 125–130 (2016) (in Russian).
[20] V. Klimov. Nanoplasmonics (Taylor & Francis Group, LLC, 2013). DOI: 10.1201/b15442.
[21] D.R. Dadadzhanov, A.V. Palekhova, T.A. Vartanyan. Opt. Spectrosc., **131** (12), 1646–1650 (2023). DOI: 10.21883/0000000000.
[22] S.I. Maslovski, C.R. Simovski. Nanophotonics, **8** (3), 429–434 (2019). DOI: 10.1515/nanoph-2018-0190

Translated by M.Verenikina