# Production of surface agents for limiting the growth of bacteria and fung based on silver nano and microparticles made by electrochemical methods on an industrial scale


**Bartosz STEMPSKI**
*Nanotec Sp. z o.o.Affiliation, Toruń, Poland*

bartosz.stempski@globedata.pl

**Grzegorz GRODZICKI**
*Nanotec Sp. z o.o.Affiliation, Toruń, Poland*

grzegorz.grodzicki@globedata.pl



**Abstract**

*The article presents the basics of a new technology for the production of surface agents for limiting the growth of bacteria and fungi based on silver nano and microparticles made by electrochemical methods on an industrial scale, developed on the basis of the results of industrial research and development works. There is a growing interest in the use of nano and micromaterials in various fields of the economy, including cosmetology, arable and livestock agriculture, and medicine. Silver is a biologically active element that strongly limits the growth of bacteria, fungi and even viruses. In the case of water colloids produced by electrochemical methods on an industrial scale, the processes accompanying the reactions are not controlled with an appropriate level of accuracy. The authors developed methods to automatically control these processes for the first time based on the optical properties of water colloids. As a result of the project, theoretical foundations for control systems as well as functional diagrams along with appropriate program codes were developed. The technology was tested in the production line of water molecular silver colloids used in cosmetics such as creams, mists, cosmetic waters and serums. Thanks to it, the stability of the reaction and the quality of the manufactured intermediates were increased. The technology will also be applicable to silver colloids intended for agriculture and other industries.*



***Keywords:*** *silver, nanomolecules, micromolecules, bacteria, fungi.*

## 1. Introduction

The interest in using silver in the processes of limiting the growth of fungi and bacteria dates back to antiquity. A long sailing tradition has dictated that a silver coin be placed in a potable water tank to keep it fresh for a long time. The aristocracy and nobility used silver utensils and cutlery when the techniques of freezing and preserving food were not yet known. The first observations of nanoparticles also took place in ancient Rome, around 1,600 years ago. Roman craftsmen then created the chalice of King Lycurgus, which changed its color under different lighting conditions. They did this by embedding tiny silver and gold particles in the glass. The unique properties of this object were observed in the 1950s, but only microscopic research in 1990 allowed to discover their essence. Nano and microparticles are produced naturally during many cosmological, geological, meteorological and biological processes. In the 19th century, Michael Faraday [1] provided the first description, in scientific terms, of the optical properties of nanometer-scale metals. In turn, in Turner's work from 1908 we find: "It is well known that when thin leaves of gold or silver are mounted upon glass and heated to a temperature that is well below a red heat (~500°C), a remarkable change of properties takes place, whereby the continuity of the metallic film is destroyed. The result is that white light is now freely transmitted, reflection is correspondingly diminished, while the electrical resistivity is enormously increased." [2] In the 1970s and 80s Granqvist and Buhrman led first fundamental studies with nanoparticles in the United States while in Japan nanopartical materials were studied within an ERATO project. These times researchers used term "ultrafine particles" but till the National Nanotechnology Initiative was launched in the United States in 1990, the term nanoparticles had become more common.

Currently, we know many materials in the form of nanoparticles. They take various shapes such as nanospheres [3], nanorods, nanochains [4], nanostars, nanoflowers, nanoreefs [5], nanowhiskers, nanofibers, and nanoboxes. The shape of nanoparticles very strongly determines the properties of nanomaterials in many areas, such as interaction with light, solvents, behavior in external force fields, participation in chemical reactions, etc. Often, single nanoparticles form larger clusters, creating groups of several dozen to hundreds of thousands of nanoparticles. It also depends on the shapes whether the nanoparticles will form crystal structures (e.g. rods, boxes) or stay amorphous (e.g. spheres). Since the smaller a given structure, the better its surface area to volume ratio is, smaller clusters or individual nanoparticles are usually more reactive than larger clusters. Ex. 1 kg of particles of 1 $mm^3$ has the same surface area as 1 mg of particles of 1 $nm^3$. Nevertheless, it should be remembered that the penetration of single nanoparticles or small clusters in the external material is also significantly greater than in the case of large groups.

Drugs, medications and cosmetics with silver nano or microparticles have a strong biocidal or biosuppressive effect against a wide range of harmful microorganisms, including pathogenic bacteria, fungi and even viruses (e.g. HIV). The multidirectional interaction of silver nano and microparticles impairs the defense mechanisms of microorganisms and inhibits the growth of bacterial resistance. Silver particles interfere with the energy metabolism of cells, damage their structures and nucleic acids. The activity and effectiveness of silver nano and microparticles depends on their shape, size of clusters, solvents and the method of their production. Silver, in the form of nano and microparticles, is currently used in wound dressings and preparations because research has shown that it reduces the burden of microorganisms on wounds, heals or prevents local infections [6]. Silver has also been used in the food industry, air mist decontamination, inhalation systems, and food preservation and storage. Studies have also shown that silver nano and microparticles show relatively low toxicity to humans and a low degree of deposition.

During the research presented in this article, a number of research tasks were carried out within the established research agenda. The main issues concerned the following areas:

- Registration and processing of large-volume (nephelometric and turbidimetric) data along with the processes of their transmission.
- Statistical analysis of large-volume measurement data with extraction processes for key data sets.
- Machine learning mechanisms in the classification processes of nano fractions and microparticles.
- Process feedback loop modifying the parameters of the electrochemical reaction.
- Creation of a new surface technology for limiting the growth of bacteria and fungi with the use of silver nano and microparticles produced by electrochemical methods.

As a result of the research agenda, new knowledge was created in the form of a new technology for the production of surface agents for limiting the growth of bacteria and fungi based on silver nano and microparticles made by electrochemical methods on an industrial scale. Our own research equipment was also used for research. A utility model application was also filed and a new technology was implemented in test production line.

## 2. Measuring system

The principle of operation of the apparatus is based on nephelometric and turbidimetric methods, where not only the total luminous flux, i.e. turbidity, is measured, but also the distribution of luminous fluxes resulting from the absorption and scattering of the incident light wave. For this purpose, classic galvanometers were replaced with photosensitive matrices with a specific resolution (e.g. 3648x2736 px, i.e. 10Mpx). One of the matrices registers the light transmitted through the colloidal sample, while the other registers the light scattered by the colloid particles. An alternative may be the use of one matrix with a variable position (such a system was used in the apparatus described). The light sources are semiconductor (light-emitting) diodes and laser diodes (ie semiconductor lasers). Light emitting diodes give a relatively wide spectrum distribution with a maximum of a given wavelength. An example may be the Epistar power LEDs with spectra shown in the figures: Fig. 1 (maximum at 660nm), Fig. 2 (maximum at 515nm) and

Fig. 3 (maximum at 445nm). Appropriate collimators were used to narrow the beam to a cone with an angle of 5°. The spectra of semiconductor diodes should be as well separated as possible. The small size and very low price (including collimators) allow for the construction of an efficient lighting matrix. In turn, laser diodes give light similar to monochrome, with relatively high efficiency. An example can be LambdaWave point modules. They have a good quality of workmanship, which is important due to the independence from the influence of ambient temperature and good power control. Laser diodes allow the use of practically monochrome waves with complete spectral separation. Hence, practically all available options can be used, such as: 405nm, 450nm, 520nm, 638nm. Laser diodes are characterized by a much higher price (modules that meet certain quality parameters and with permanent characteristics are not cheap), but they ensure high stability of the light parameters. One of the advantages of using laser modules is that no additional collimators are needed.

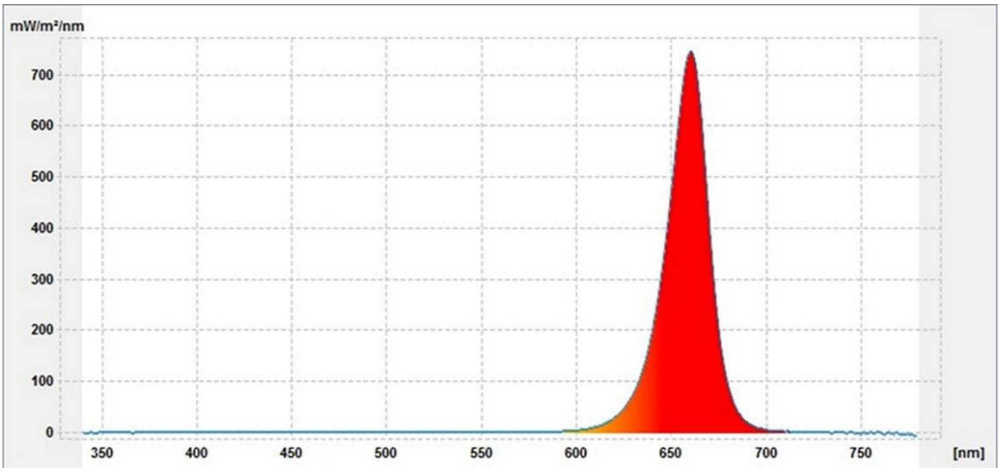


**Figure 1.** The Epistar 660nm diode spectrum
***Source:*** *The Epistar Power LED 1W 45 mil DEEP RED 660nm datasheet*

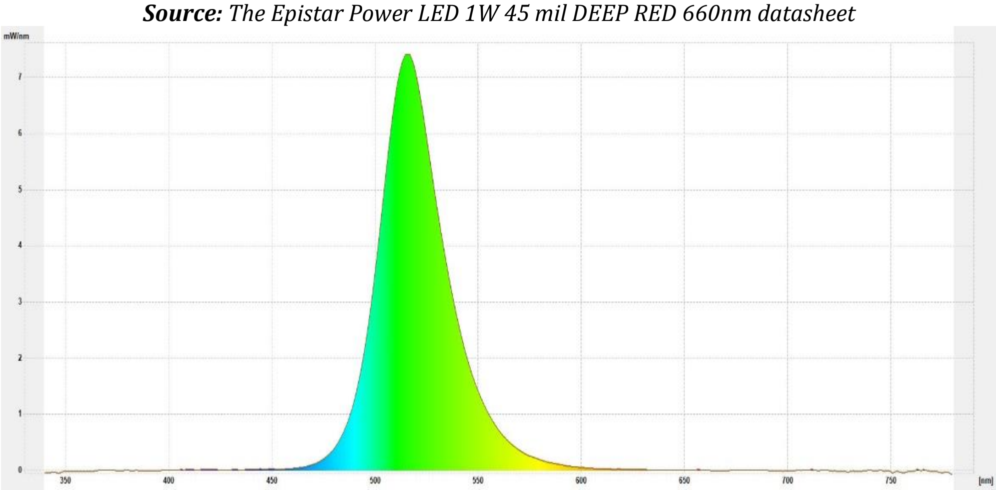

**Figure 2.** The Epistar 515nm diode spectrum
***Source:*** *The Epistar Power LED 1W 45 mil GREEN 515nm datasheet*

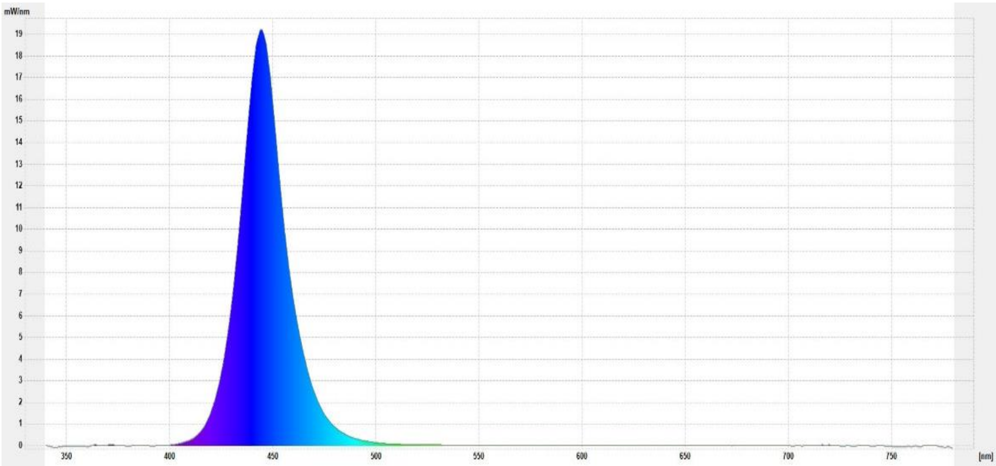


**Figure 3.** The Epistar 445nm diode spectrum
***Source:*** *The Epistar Power LED 1W 45 mil ROYAL BLUE 445nm datasheet*

Only water colloids were used in the research due to biological safety and a wide range of applications. Ultimately, the tested colloids are used for the production of cosmetics with dermatological properties, such as creams, mists, cosmetic waters and serums.

A 23.5 x 15.6 mm sensor with a pixel number of 24.72 million (effective pixels number is 24.2 million) was used as the CMOS recording matrix with a variable position. The NEF (RAW) 14 bit uncompressed format was used to record the nephelometric and turbidimetric spectra with 35mm DX-type optics. A 23.5 x 15.6 mm sensor with a pixel number of 24.72 million (effective pixels number is 24.2 million) was used as the CMOS recording matrix with a variable position. The NEF (RAW) 14 bit uncompressed format was used to record the nephelometric and turbidimetric spectra with 35mm DX-type optics. Figure 4 shows a functional diagram of the basic version of the measurement system. The principle of operation is presented in the section below.

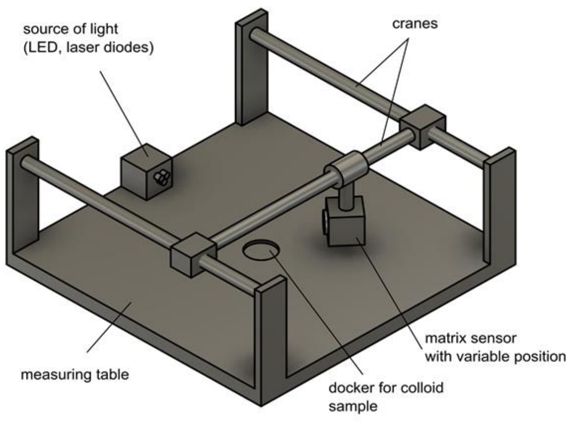


**Figure 4.** The functional scheme of the measuring apparatus used during research
***Source:*** *own materials*

*2.1. The principle of operation of the measuring system*

The concept of measurement has two main aspects. The first is based on a rough measurement of the luminous flux. The efficiency of the light source is known, besides, the device can be calibrated each time by directing the beam to the first matrix (without a colloid in the light path) and then to the second (the result should be similar, because the matrices will be identical, and the calibration beam would be directed e.g. at using a mirror inserted in the place of the later colloid container). By controlling the supply current, the intensity of the light beam can be changed, besides, the calibration can take place in several wavelengths (both polychromatic and monochromatic), which will increase the accuracy of the measurement. Then, after placing the colloid in the device, it is possible to estimate the reduction of the source beam intensity (the sum of the measurements from both matrices together with the analysis of the light distribution reaching the matrices). The measuring container should be symmetrical, preferably in the form of a cylinder or orb-like. One matrix is mounted perpendicular to the axis of the container, opposite the light source, the other one parallel to the horizontal axis of the container, on its side (similar to galvanometers in a classic device). By summing up the estimated intensities of the secondary beams recorded by both matrices, it is possible to determine how much light was scattered on the particles of the suspension. From the nephelometric and turbidimetric measurements, the mean size of the scattering particles can be estimated.

*2.2. Control of particle size changes regardless of the concentration of the solution.*

Based on the Wells formula, the amount of turbidance can be determined:

$$T = k \cdot \frac{d^3}{d^4 + a\lambda^4} \cdot l \cdot c \qquad (1)$$

***Where:***

*k - proportionality factor depending on the type of suspension and the measurement method*

*a - constant characteristic for a given measurement method*

*c - weight concentration of the analyzed substance, expressed in units of ppm*

*d - the average diameter of the colloid particles*

*λ - length of the source light wave*

*l - sample layer thickness*

If we assume that:

$$\alpha = k \cdot \frac{d^3}{d^4 + a\lambda^4} \qquad (2)$$

then for the monochromatic wave the formula above takes the form of Lambert-Beer low:

$$T = \alpha \cdot l \cdot c \qquad (3)$$

***Where:***

*α is a constant*

From the definition of turbidance we get:

$$T = \log\frac{I_0}{I_t} \tag{4}$$

***Where:***

*$I_0$ - intensity of the source of light falling on the sample*

*$I_t$ - the intensity of light passing through the sample (since we have a turbidimetric measurement also)*

We can introduce following markings:

$$I_i = I_t^{(i)} for\, \lambda_i \tag{5}$$

is the measured light intensity of wavelength $\lambda_i$ passing through the sample.

$$T_i = \log\frac{I_0}{I_i} \tag{6}$$

i.e.

$$T_i = k \cdot \frac{d^3}{d^4 + a\lambda_i^4} \cdot l \cdot c \tag{7}$$

For a single measurement of a sample for which the process of precipitation of new nanoparticles is not currently taking place, virtually all quantities can be treated as constant. By using two different monochromatic waves in the measurement, i.e. $\lambda_1 \neq \lambda_2$, we can eliminate the concentration *c* from the equations:

$$c = T_i \cdot \frac{d^4 + a\lambda_i^4}{k \cdot l \cdot d^3} \tag{8}$$

$$T_1 \cdot \frac{d^4 + a\lambda_1^4}{k \cdot l \cdot d^3} = T_2 \cdot \frac{d^4 + a\lambda_2^4}{k \cdot l \cdot d^3} \tag{9}$$

Ordering by *d* we get:

$$d^4 = a \cdot \frac{T_2\lambda_2^4 - T_1\lambda_1^4}{T_1 - T_2} \tag{10}$$

If we mark subsequent measurements with index *j*, we can write:

$$d_j^4 = a \cdot \frac{T_2^{(j)}\lambda_2^4 - T_1^{(j)}\lambda_1^4}{T_1^{(j)} - T_2^{(j)}} \tag{11}$$

Finally we get:

$$D_j = \frac{T_2^{(j)}\lambda_2^4 - T_1^{(j)}\lambda_1^4}{T_1^{(j)} - T_2^{(j)}} \tag{12}$$

where

$$D_j = \frac{d_j^4}{a} \tag{13}$$

The stability of the process in terms of generating nanoparticles means that $D_j = const$ over time. An increase in the average particle diameter *d* means a proportional increase in the size of *D*. Therefore, it is enough to measure the turbidances at successive moments of the nanoparticle generation process using two different monochromatic waves and calculate the ratio:

$$\frac{T_2^{(j)}\lambda_2^4 - T_1^{(j)}\lambda_1^4}{T_1^{(j)} - T_2^{(j)}} \tag{14}$$

It can be seen that the greater the difference between wavelengths $\lambda_1$ and $\lambda_2$, the role of nominator increases and the whole fraction should take values from a wider range, especially if $T_1$ and $T_2$ are not close to each other.

## 3. Application of new technology in test production line of silver water colloids

The new technology developed during the project was tested on the production line of water colloids containing silver nano and microparticles. The production system consists of:

- a reverse osmosis demineralizer connected to the local water network,
- jacket reactor with a stirrer, maintaining the reaction temperature,
- equalizing mixer,
- buffer tank with a mixer,
- mechanical filtration system,
- the peristaltic pump anti-air system,
- peristaltic pump with a system for pouring the solution into bottles,
- electronic system for pouring the solution into bottles,
- computer system for controlling electrochemical reaction processes, reactor parameters and the flow of liquid reaction environments,
- reactor reaction feed system,
- power supply system of the jacket heating system of the reactor,
- a system for regulating reaction parameters based on nephelometric and turbidimetric measurements,
- device communication system using the TCP/IP secure network protocol.

The functional scheme of the system for regulating reaction parameters based on nephelometric and turbidimetric measurements implemented to test production line has been presented on Fig. 5.

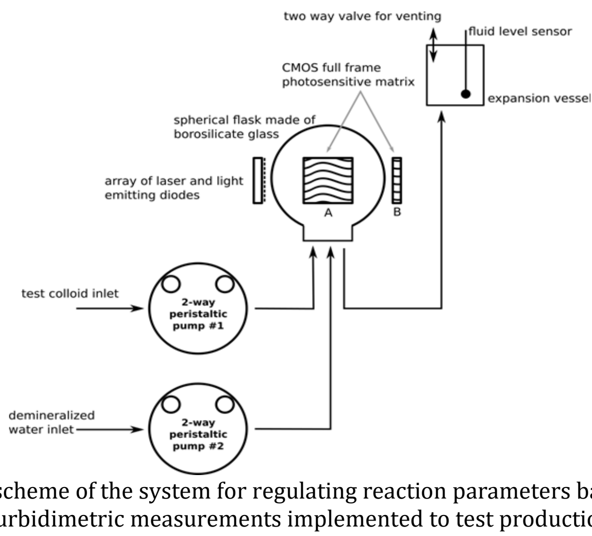


**Figure 5.** Functional scheme of the system for regulating reaction parameters based on nephelometric and turbidimetric measurements implemented to test production line
***Source:*** *own materials*

## 4. Summary

Thanks to the application of a system controlling and regulating the parameters of the electrochemical reaction, in which the new technology was used, the quality and repeatability of the produced water colloids of silver nano and microparticles were stabilized. Colloids are the basis for the production of cosmetic products with dermatological properties, such as creams, cosmetic waters, mists or serums. They help fight and limit the growth of harmful bacteria and fungi that infect skin cells. Initial attempts were also made to limit the growth of harmful fungi on the surface of useful flowers. The obtained results encourage further tests and research in other agricultural sectors, such as the cultivation of cereals, vegetables and fruit. Livestock farming, including pig farming, may also be promising.

**Acknowledgements**

The project was co-financed by the European Regional Development Fund under the Regional Operational Program of the Kujawsko-Pomorskie Voivodeship for 2014-2020. Project title: "Implementation of industrial research and development works aimed at developing a new technology for the production of surface agents for limiting the growth of bacteria and fungi based on silver nano and microparticles made by electrochemical methods on an industrial scale".

(PL: Projekt współfinansowany ze środków Europejskiego Funduszu Rozwoju Regionalnego w ramach Regionalnego Programu Operacyjnego Województwa Kujawsko-Pomorskiego na lata 2014-2020. Tytuł projektu: "Realizacja badań przemysłowych i prac rozwojowych mających na celu opracowanie nowej technologii produkcji środków powierzchniowego ograniczania rozwoju bakterii i grzybów na bazie nano i mikrocząstek srebra wytwarzanych metodami elektrochemicznymi w skali przemysłowej".)

### References

[1] Faraday, Michael (1857). *Experimental relations of gold (and other metals) to light*. Phil. Trans. R. Soc. Lond. 147: 145 181.National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov, date: 02.03.2016.

[2] Turner, T. (1908). *Transparent Silver and Other Metallic Films*. Proceedings of the Royal Society A. 81 (548): 301–310.

[3] Agam, M. A.; Guo, Q (2007). *Electron Beam Modification of Polymer Nanospheres*. Journal of Nanoscience and Nanotechnology. 7 (10): 3615–9.

[4] Kralj, Slavko; Makovec, Darko (27 October 2015). *Magnetic Assembly of Superparamagnetic Iron Oxide Nanoparticle Clusters into Nanochains and Nanobundles*. ACS Nano. 9 (10): 9700–7.

[5] Choy J.H.; Jang E.S.; Won J.H.; Chung J.H.; Jang D.J. & Kim Y.W. (2004). *Hydrothermal route to ZnO nanocoral reefs and nanofibers*. Appl. Phys. Lett. 84 (2): 287.

[6] Chmielowiec-Korzeniowska A.; Krzosek Ł.; Tymczyna L.; Pyrz M.; Drabik A. (2013). *Bactericidal, fungicidal and virucidal properties of nanosilver. Mode of action and potential application. A review*. Ann. Universitatis M. Curie-Skłodowska Lublin (Polonia). vol. XXXI (2): 1-11.