

ABSTRACT

**of the dissertation by Tuleyeva Rysgul Nurlanovna
submitted for the degree of Doctor of Philosophy (PhD)
in the educational program «8D07105 – Chemical Technology of Organic
Substances» entitled «Preparation of gold nanoparticles in the presence of
water-soluble polymers for inhibition of Ehrlich cancer cell growth using
plasmonic photothermal therapy»**

General description of the research. The dissertation is focused on the synthesis and stabilization of gold nanoparticles intended for use in plasmonic photothermal therapy through the application of synthetic polymers and polyampholytic polymers obtained by chemical modification of natural polymers, as well as on the investigation of their inhibitory effect on tumor cell growth.

Relevance of the research topic. In recent years, as a result of collaborative efforts by researchers in the fields of chemistry and physics, a wide variety of nanoscale structures containing metal nanoparticles and functional polymers have been developed and extensively studied. The creation of next-generation materials and nanotechnologies based on such structures represents one of the most pressing scientific and practical challenges for the country. Gold nanoparticles, in particular, have attracted significant attention due to their unique physicochemical properties and their considerable potential for application in biomedicine, especially in the diagnosis and treatment of cancer. However, the instability and strong tendency of gold nanoparticles (AuNPs) toward aggregation significantly limit their practical use. To address this issue, the stabilization of nanoparticles using biocompatible, non-toxic, and water-soluble polymers is considered one of the most promising approaches.

Photothermal therapy, particularly plasmonic photothermal therapy, is widely investigated as a modern and effective method that enables the selective destruction of cancer cells. The ability of gold nanoparticles to absorb light in the visible and near-infrared regions of the spectrum allows them to convert light energy into heat, thereby inducing thermal damage to malignant cells. A key advantage of this approach is its high spatial precision, which enables targeted treatment while minimizing damage to surrounding healthy tissues.

In this context, the development of efficient systems for photothermal therapy based on polymer-stabilized gold nanoparticles represents one of the current challenges in nanomedicine. Moreover, research in this area paves the way for the development of innovative and highly effective strategies for cancer treatment. The outcomes of such studies can make a significant contribution to the fight against oncological diseases and to the advancement of biomedical applications of nanotechnology.

Aim of the research. The aim of the research is to synthesize gold nanoparticles in the presence of water-soluble polymers and to apply them in plasmonic photothermal therapy for inhibiting the growth of Ehrlich tumor cells.

Objectives:

1) Synthesize polyampholytes through chemical modification of natural polymers and investigate their structural and physicochemical properties.

- 2) Synthesize gold nanoparticles stabilized with synthetic, natural, and polyampholytic polymers.
- 3) Evaluate the cytotoxicity of polymer-stabilized gold nanoparticles.
- 4) Investigate the photothermal effect of polymer-stabilized gold nanoparticles under visible light irradiation using *ex vivo*, *in vitro*, and *in vivo* models.
- 5) Develop a technology for the production of polymer-stabilized gold nanoparticles, to calculate the material balance of the process, and to perform an economic feasibility assessment.

Objects of the research. Gellan gum, chitosan, and their derivatives (quaternized gellan gum and carboxymethyl chitosan), as well as polymer-stabilized spherical and rod-shaped gold nanoparticles.

Subject of the research. The synthesis and physicochemical properties of modified polymers, polymer-stabilized gold nanoparticles, and the process of inhibiting the growth of Ehrlich tumor cells using plasmonic photothermal therapy.

Scientific novelty and main results of the research.

1. For the first time, spherical and rod-shaped gold nanoparticles stabilized with modified polyampholyte polymers based on gellan and chitosan were synthesized. The structural and physicochemical properties of the obtained polyampholytic polymers were investigated using Fourier-transform infrared (FTIR) spectroscopy, ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, thermogravimetric analysis, and elemental analysis. The degree of quaternization of gellan with trimethylammonium groups was determined to be approximately 38% according to ^1H NMR spectroscopy, 34.55% based on potentiometric titration, and 38.87% according to elemental analysis. Similarly, the degree of carboxymethylation of chitosan was estimated to be approximately 37% from ^1H NMR spectral data and 35.20% based on back potentiometric titration.

2. For the first time, gellan was purified using fractional dissolution and ultrasonic treatment. By applying two different fractionation methods – fractional dissolution and ultrasonic treatment – nine gellan fractions with molecular weights ranging from 38,400 to 90,300 Da were isolated.

3. Spherical and rod-shaped gold nanoparticles stabilized with poly(N-vinylpyrrolidone) (PVP), gellan gum (GG), quaternized gellan (QGG), chitosan (Ch), and carboxymethyl chitosan (CMCh) were synthesized, and their sizes were confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The average diameters of spherical AuNPs stabilized with QGG and CMCh were approximately 45 nm and 85 nm, respectively, while the rod-shaped gold nanoparticles exhibited lengths of 50-55 nm and widths of 12-14 nm.

4. The photothermal effect of gold nanoparticles, namely their ability to convert light into heat, was investigated *ex vivo* using porcine stomach tissues. When gold nanoparticles were irradiated in the absence of tissue, the maximum temperature reached 43.5 ± 0.2 °C. Upon incorporation of AuNPs–PVP, GG, QGG, Ch, and CMCh into the tissue samples, the maximum temperatures were 42.6 ± 0.1 , 42.8 ± 0.1 , 42.7 ± 0.1 , 42.5 ± 0.1 , and 42.5 ± 0.1 °C, respectively, whereas the temperature of tissues without gold nanoparticles increased only to 39.0 ± 0.1 °C.

5. In *in vitro* experiments, Ehrlich carcinoma cells were exposed to polymer-stabilized spherical (AuNPs) and rod-shaped (AuNRs) gold nanoparticles under various laser irradiation conditions. Upon irradiation with visible light at a wavelength of 530 nm, a significant decrease in tumor cell viability was observed in the presence of AuNPs ($p < 0.0001$), while a reduction in cell number was also detected in non-irradiated samples.

6. Based on the previously obtained results, *in vivo* studies were conducted to further evaluate the therapeutic efficacy of the proposed method. In samples treated with AuNPs-PVP without subsequent irradiation, tumor growth decreased by $19 \pm 10\%$ after 3 days, with the inhibitory effect increased by $42 \pm 5\%$ after 7 days. In contrast, in samples treated with AuNPs-PVP followed by irradiation, tumor growth inhibition reached $90 \pm 6\%$ after 7 days. Over a 9-day observation period, the tumor growth inhibition coefficient was $43 \pm 23\%$ in the non-irradiated AuNPs-PVP group, whereas in the irradiated group this value increased to $85 \pm 3\%$.

7. The effect of gold nanoparticles on the viability of SW620 cancer cells was evaluated using the Cell Counting Kit-8 (CCK-8) metabolic assay. The results demonstrated that the SW620 cancer cell line exhibited pronounced sensitivity when interacting with AuNPs-GG, leading to a decrease in cell viability. A statistically significant difference was observed between the control group and the AuNPs-GG treated group ($p < 0.01$). However, no significant changes in cell viability were detected in samples treated with other types of nanoparticles.

8. A technological scheme for the production of polymer-stabilized gold nanoparticles was developed. A material balance was calculated for an annual production capacity of 1,000 kg, and the main economic indicators were determined. It was established that the production cost of the final product is 2,037.77 KZT, the payback period is 3.47 years, and the profitability reaches 28.80%.

Validity and reliability of the obtained data. The validity and reliability of the research results are substantiated through discussion based on up-to-date literature sources relevant to the dissertation topic, as well as through the application of physicochemical analytical techniques, including Fourier-transform infrared (FTIR) spectroscopy, UV/Visible spectroscopy, ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, thermogravimetric analysis, dynamic light scattering (DLS), transmission electron microscope (TEM) and elemental analysis. All research methodologies employed are certified; laboratory glassware and solutions were prepared and used in accordance with state standards (GOST); laboratory equipment underwent metrological verification; and the research materials comply with applicable regulatory documentation.

Relation of the research topic to research plans and governmental programs. The dissertation research was carried out within the framework of grant-funded projects supported by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan: AR08052594 (2020–2022), “Production of gold nanoparticles for the treatment of cancer cells by plasmon photothermal therapy”, and AR13067773 (2022–2024), “Development of scientific basis and preparation technology of prolonged-release eye drops based on gellan and ofloxacin”.

Key statements submitted for defense:

1. Spherical gold nanoparticles synthesized by a one-pot method and rod-shaped gold nanoparticles obtained by a seed-mediated growth method, stabilized with gellan, quaternized gellan, chitosan, and carboxymethyl chitosan, have dimensions of 45–99 nm for spherical particles and 50–55 nm in length with widths of 12–14 nm for rod-shaped particles.

2. *Ex vivo* irradiation of porcine stomach tissue in the presence of gold nanoparticles stabilized with modified and unmodified polymers results in an increase in tissue temperature from 37 °C to 41–43 °C.

3. The application of plasmonic photothermal therapy leads to significant inhibition of tumor growth ($85 \pm 3\%$ – $90 \pm 6\%$) compared with non-irradiated groups ($19 \pm 10\%$ – $43 \pm 23\%$).

Theoretical significance of the research results.

The research was aimed at a comprehensive study of the physicochemical properties of gold nanoparticles stabilized with water-soluble polymers and modified polyampholytic polysaccharides, as well as their inhibitory effect on the growth of tumor cells under plasmonic photothermal therapy. The obtained results expand theoretical understanding of the interaction mechanisms in polymer–gold nanoparticle systems and provide a scientific rationale for their potential applications in biomedicine.

Practical significance of the research results.

The practical significance of this study lies in the potential use of gold nanoparticles prepared on the basis of natural, synthetic, and modified polyampholytic polymers for plasmonic photothermal therapy of tumor cells. Thus, the results can make a significant contribution to the development of strategies for combating oncological diseases, as well as to the biomedical applications of nanotechnology.

Dissemination of the research results.

The findings of the dissertation have been presented at the following international conferences and forums: the International Scientific and Practical Conference “Modern Aspects of Chemical Science and Chemical Education: Theory and Practice” (Almaty, 2021); the International Scientific and Practical Conference “Synthesis and Study of Properties, Modification, and Processing of High-Molecular-Weight Compounds” (Kazan, Russian Federation, 2021).

Publications. Based on the results of the dissertation, six scientific works have been published, including three articles in international peer-reviewed journals: two articles in *Polymer for Advanced Technologies* (WoS Q2, 69th percentile), one article in *Macromolecular Chemistry and Physics* (WoS Q3, 63rd percentile), one article in *Pharmacy of Kazakhstan*, included in the list recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, and materials and abstracts of two reports presented at international scientific and practical conferences.

Author’s contribution.

All experimental work presented in the dissertation, including conducting these experiments, data acquisition, processing and analyzing the results obtained during the

research, as well as preparing and publishing the research outputs, were carried out solely by the author.

1. ***Tuleyeva R.N.**, Tatykhanova G.S., Gizatullina N.N., et al. Preparation and characterization of amphoteric polysaccharides derived from chitosan and gellan gum // Polym. Adv. Technol. – 2024. – Vol. 35, № 12. – P. e70033. In this work, the PhD candidate is the first and corresponding author who conducted the experimental studies, performed the processing and analysis of the obtained data, prepared the initial draft of the manuscript, and revised it taking into account the reviewers' comments.

2. Tatykhanova G.S., **Tuleyeva R.N.**, Nurakhmetova Z.A., Gizatullina N.N., Krasnoshtanov V.K., Kaldybekov D.B., Aseyev V.O., Khutoryanskiy V.V., Kudaibergenov S.E. Polymer-protected gold nanoparticles for photothermal treatment of Ehrlich adenocarcinoma: in vitro and in vivo studies // Macromol. Chem. Phys. – 2025. – Vol. 226, № 4. – P. 2400128. In this work, the PhD candidate conducted experimental studies, performed physicochemical analyses of the synthesized substances, carried out the processing and interpretation of the obtained data, and also conducted the collection and analysis of scientific literature.

3. Tatykhanova G.S., **Tuleyeva R.N.**, Gizatullina N.N., et al. Characterization of biocompatible gellan gum fractions for prolonged retention in ocular drug delivery systems // Polym. Adv. Technol. – 2024. – Vol. 35, № 11. – P. e6635. In this work, the PhD candidate conducted experimental studies, performed physicochemical analyses of the obtained compounds, processed the results, and carried out a review of the relevant scientific literature.

4. Tokpanova A.A., Turgumbayeva A.A., Abuova Z., Rakhimov K.D., **Tuleyeva R.N.**, Tatykhanova G.S., Jusupkaliyeva A.E. Preparation and standardization of antimicrobial eye drops with gold nanoparticles based on the safflower seed extract // Фармация Казахстана. – 2022. – Vol. 3. – P 231-236. In this work, the PhD candidate performed the synthesis of gold nanoparticles and conducted physicochemical analysis of the obtained samples.

5. In conference proceedings: acquisition and description of experimental data, obtaining and discussion of evaluation results, preparation of materials, and the PhD candidate's participation in international conferences.