

Abstract of
dissertation for the degree of Doctor of Philosophy (PhD) in the educational
program 8D07108 - "Basic processes of synthesis and production of new
organic and polymeric materials" Kabdrakhmanova Ainur Kanatovna
"Development of biostimulants based on complexes of succinic acid
derivatives with silver ions"

General characteristics of the work. The dissertation is aimed at the synthesis of complexes based on succinic acid and its derivatives, in particular dimethyl ester of succinic acid with silver, as well as a mixed-ligand complex based on ecobioligands, i.e. succinic acid and glycine in combination with copper ions for a comparative study of its potential with silver, in the study of their physicochemical properties, bioactivity, as well as in the study of the adaptogenic properties of the composition based on the obtained complexes, activated bentonite, kalzhat deposit and modified corn starch.

Relevance of the research topic. One of the main tasks of chemistry today is to solve urgent problems of the agro-industrial complex, aimed at improving the quality and productivity of agricultural crops. It is known that one of the main conditions for its formation is the treatment of substances with compounds that have bioactive properties that are harmless to plants, a complex synthesized in the presence of metal ions, using the concept of "green" chemistry. This in turn allows to get a clean harvest without harming the soil biota and the ecosystem-crop-soil-atmosphere chain.

Ecobioligand complexes of d-elements, in particular succinic acid and its derivatives, are widely used in medicine, production of catalysts, pesticides, synthesis of ion-exchange and porous materials, as well as in the field of bioengineering. In addition, succinic acid and its derivatives are non-toxic and do not have a mutagenic effect on a living organism, being an intermediate product of the Krebs cycle metabolism. Succinic acid is a ligand with a chemical structure with two carboxyl groups, which can be used as coordination points for obtaining complex compounds. In literary sources it has been established that intracellular absorption of complexes based on succinic acid and its derivatives occurs better compared to the participation of other ligands, in combination with silver ions. It has also been revealed that microelements in combination with organic ligands, i.e. as part of chelate complexes are easily absorbed by plants. In addition, oxygen atoms in succinic acid easily bind inorganic substances, allowing them to form a block through carboxyl groups.

Another pressing problem of today's reality is the alarming growth of bacterial resistance to antibiotics due to their widespread use, which in turn contributes to the formation of antibiotic-resistant bacteria. This problem can be solved by using the biological activity of d-element complexes with eco-bio-ligands of antibacterial and biofungicidal action. This will allow them to be used as environmentally and biologically effective products that reduce the use of fungicides and pesticides that have a negative impact on living organisms and environmental objects. It should be noted that in addition to the adverse effects on living organisms and the environment,

fungicides and pesticides contribute to the rise in the cost of the final product. Therefore, the development of effective methods for increasing crop yields and reducing crop costs is a priority today.

It has been established that succinic acid (SA) and its derivatives act as inhibitors of bacteria, pathogenic fungi that cause phytopathogenic rot in agricultural crops. There are studies that have revealed activity against such pathogens as alternaria, curvularia, pseudocercosporan fungi, rod-shaped and anaerobic thermophilic bacteria. Despite studies on the use of succinic acid and its derivatives as biostimulants and protective agents, fungicidal and antibacterial properties of substances based on complexes of succinic acid and its derivatives with silver ions, the method of their application, identification of optimal concentration, as well as the effect on the germination energy of crops, have not been sufficiently studied. Therefore, obtaining new drugs with significant resistance to bacteria (resistant strains), as well as activity against pathogenic microbes, becomes an urgent problem. In this regard, it is necessary to note the importance of succinic acid complexes and its derivatives with silver ions, which have a cytotoxic effect against gram-positive and gram-negative bacteria and fungi. The activity of succinic acid complexes with silver ions is directly related to their good solubility and stability in water, lipophilicity, reducing capacity and the rate of release of silver ions. The main factors influencing this property of silver include the choice of effective ligands and small modulations of their electronic, steric action. This contributes to their long-term bioavailability and prevention of recovery or resistance. Therefore, a large variety of new classes of silver complexes attracts the attention of scientists to the development of new electrolytes and biologically active silver compounds from the point of view of electro- and biocoordination chemistry. In this regard, the use of complexes of succinic acid and its derivatives with d-elements as plant adaptogens with their biostimulating and antibacterial properties is currently a promising direction.

The purpose of the dissertation: Synthesis of complexes of succinic (Succ) and dimethyl ester of succinic acid (DmSucc) with silver ions and a mixed-ligand complex based on ecobioligands - glycine and succinic acid with copper ions, study of their physicochemical properties and biological activity, as well as development of an effective composition for pre-sowing treatment and encapsulation of seeds of agricultural crops based on the synthesized complex $[Ag_2(Succ)]$, activated bentonite clay kalzhat and modified corn starch.

To achieve this goal, the following tasks were set:

- study of the formation of a complex of succinic acid with silver ions and the study of the physicochemical properties of the synthesized complex;
- study of the formation of a complex of dimethyl ether of succinic acid with silver ions, the study of the physicochemical properties of the resulting complex;
- synthesis of a mixed-ligand complex based on ecobioligands - succinic acid and glycine with Cu^{2+} ions for the purpose of a comparative study with Ag^+ ions;
- study of the biological activity of complexes of succinic acid and its derivative - dimethyl ether of succinic acid with silver ions and a mixed-ligand

complex based on succinic acid and glycine with copper ions against gram-positive and gram-negative bacteria;

- study of the biological activity of complexes of succinic acid and its derivative - dimethyl ester of succinic acid with silver ions and a mixed-ligand complex based on succinic acid and glycine with copper ions on the growth and development of agricultural crops, including soybean seeds;

- Development of an effective composition and technology for pre-sowing treatment and encapsulation of agricultural crops based on a complex of succinic acid with silver ions, as well as activated bentonite clay and modified corn starch.

Research objects: complexes based on succinic acid and dimethyl ether of succinic acid with silver ions, as well as ecobioligands with copper ions, i.e. complexes of succinic acid and glycine and its bioactivity.

Research subject: Methodology for obtaining a complex of succinic acid and dimethyl ether of succinic acid with silver ions, as well as obtaining a copper ion with succinic acid and glycine to obtain a substance with bioactive properties and studying their physicochemical, bioactive properties.

Research methods: in the course of the research work, classical and modern physical and chemical research methods were used. Complexes of succinic acid and dimethyl ether of succinic acid with silver and copper ions were synthesized, their physical and chemical properties were studied on the basis of the engineering profile laboratory of the Kazakh National Research Technical University named after K. Satbayev, on the basis of the Department of Chemical and Biochemical Engineering and during a scientific internship at the Center for International and Interuniversity Nanoscience and Nanotechnology of Mahatma Gandhi University (India) and on the basis of the national scientific laboratory for collective use of the East Kazakhstan University named after S. Amanzholov.

In the course of the dissertation work, modern methods of synthesis and physicochemical methods of analysis were used, in particular, the following methods of sample preparation and research were used:

- the formation of complexes, the chemical structure of succinic acid, dimethyl ester of succinic acid and their complexes with silver and copper ions were studied using IR Fourier spectroscopy (SDR) (Nicolet iS12 400 FTIR Thermo Scientific) in the range of 450-4700 cm^{-1} ;

- using the FT-Raman spectrometer (Bio-Rad), the chemical structure was established, the formation of complexes with an excitation power of 100 mW, obtained at a rate of 1064 kV. Each spectrum is measured by adding 1200-fold scanning of one sample of 8 cm^{-1} size (data collection time is 20 minutes);

- the optical characteristics of the synthesized complexes were determined by UV spectroscopic method (PE-5400UV, Russia, quartz cuvette thickness 10 mm) in the wavelength range of 190 - 1000 nm;

- the chemical structure of the complexes, the structural formula of ^1H (500.15 MHz) and ^{13}C (125.77 MHz) were determined using nuclear magnetic resonance (NMR) spectroscopy JNM-ECA-500 (Jeol, Japan);

- the structural and phase characteristics of the complexes were studied by X-ray diffractometry using an XpertPRO diffractometer (Malvern Panalytical Empyrean, Netherlands);

- the scanning electron microscope (SEM) (JSM-6390 JEOL, Japan) was used to determine the surface morphology of the complexes and particle shapes, the study was carried out in a standard configuration. The measurements were determined in high vacuum mode using a second electron detector at an acceleration voltage of 5 kV;

- thermal stability characteristics of samples were carried out on a differential thermogravimetric analyzer SKZ1060A (Synchronous thermal analyzer);

- the types of plant pathogenic microorganisms were examined under a microscope XSZ 146 (Hinotek, China). Incubation was carried out at a temperature of +23°C for 14 days;

- The antibacterial activity of the complexes was studied in vitro in a sterile environment using the diffusion method for bacteria and pathogenic fungi:

- GOST (further SS) 12038-84 "Seeds of agricultural crops. Methods for determining germination";

- SS 12039-82 "Seeds of agricultural crops. Methods for determining viability";

- SS 12044-93 "Seeds of agricultural crops. Methods for determining infection with diseases".

Scientific novelty and main results of the study:

- biostimulating substances consisting of complexes based on succinic acid and dimethyl ester of succinic acid (DmSA) with silver ions were synthesized and their physicochemical properties and bioactivity were studied;

- the [Cu(succ)(gly)]_n complex was synthesized for the first time, based on ecobioligands - succinic acid and glycine, as well as Cu²⁺ ions for a comparative study with Ag⁺ ions, its physicochemical and bioactive properties were studied;

- an effective composition for pre-sowing treatment and encapsulation of agricultural crops based on SA, DmSA and silver ions, as well as complexes based on SA, glycine and copper ions, in combination with activated bentonite clay Kalzhat and modified corn starch was developed;

- a technology for obtaining biostimulating substances based on a complex of DmSA and silver ions, as well as a mixed-ligand complex of succinic acid, glycine and copper ions [Cu (succ) (gly)]_n has been developed;

- a technology for pre-sowing treatment and encapsulation of soybean crops based on complexes of succinic acid, DmSA and glycine with silver and copper ions, activated Kalzhat bentonite clay and modified corn starch has been developed, and the effect of the complexes on the growth and development of soybeans in laboratory and field conditions has been studied.

The main provisions submitted for defense:

- The effective volume ratio required to obtain complexes based on succinic acid and dimethyl ester of succinic acid with silver ions is 1:1 ml/ml, respectively, the formation of the [Ag₂(Succ)] complex occurs due to the coordination of silver ions with the carboxyl group and the formation of the [Ag(DmSucc)] complex is achieved due to the coordination of silver ions with the carbonyl group in the

dimethyl succinate molecule. The obtained complexes $[\text{Ag}_2(\text{Succ})]$ and $[\text{Ag}(\text{DmSucc})]$ are biologically active for agricultural crops and the effective concentration of the $[\text{Ag}_2(\text{Succ})]$ and $[\text{Ag}(\text{DmSucc})]$ complexes required for pre-sowing treatment of soybeans is $5 \cdot 10^{-3}$ mol/l.

– When synthesizing the mixed-ligand complex $[\text{Cu}(\text{Succ})(\text{Gly})]_n$ based on ecobioligands – succinic acid and glycine, as well as copper, the coordination of the nitrogen atom and oxygen of the carboxyl group in the glycine molecule, as well as two oxygen atoms of the carboxyl groups in succinic acid, is carried out.

– As a result of pre-sowing treatment of soybean crops with a solution of the mixed-ligand complex $[\text{Cu}(\text{Succ})(\text{Gly})]_n$ based on ecobioligands and Cu^{2+} ions, concentration of $5 \cdot 10^{-3}$ mol/l, germination is 100%, the number of healthy shoots increases by 14.7%, pathogenic organisms of bacteriosis and fusarium decreases by 8.35% and 10%, compared with the control plants and with the samples treated only with a solution of succinic acid, respectively.

– A technology for pre-sowing treatment and encapsulation of agricultural crop seeds based on a complex composition, in particular a silver complex with succinic acid, activated bentonite kalzhat and modified corn starch, is proposed. The yield of early-ripening soybeans subjected to pre-sowing treatment using this technology increased by 3.1 c/ha, the weight of 1000 g of soybeans is 152.3 g, the protein and fat content increases by 2.1% and 1.45%, respectively, biometric indicators improve, in particular, the height of soybeans increases by 8 cm, lodging of lower pods increases by 2 cm, and the vegetation period of soybean development is reduced by 7 days.

Theoretical and practical significance of the work:

The results obtained during the dissertation work on the study of complexes of succinic acid and dimethyl ester of succinic acid with silver ions can be used as materials in teaching chemistry, ecology and biology in secondary specialized and higher educational institutions, as well as in the branches of the agro-industrial complex as biostimulants and adaptogens for plants.

Due to the high bactericidal and bacteriostatic functional activity of the complex containing silver ions, the complex with a non-toxic concentration of silver ions can also find wide application in the medical field. The fact that silver ions have a much higher level of activity against antibiotic-resistant strains of bacteria and fungi compared to strong antibiotics, as well as the fact that silver ions are less dangerous for human cells, indicates that the obtained complexes of succinic acid and dimethyl ester of succinic acid with silver ions can be used in medicine as important metabolic stimulants of the Krebs cycle. In the pharmaceutical industry, given the bioactive properties of the complexes, including the adaptogenic activity of succinic acid, predisposes to their use in the preparation of drugs and biologically active additives for pathogenic conditions of the body.

The work carried out to determine the effective composition of a biostimulating substance based on complexes of succinic acid derivatives with silver ions will expand theoretical knowledge on the effective use of silver ions with bactericidal and bacteriostatic properties in the field of general industrial synthesis. The composition of the complex obtained by the method of "green" chemistry is

environmentally friendly, does not have a toxic effect, has a low concentration of silver ions, is characterized by the shortest synthesis time, contains only two different substances, and is therefore cost-effective for production. That is, optimization of the technological mode for obtaining bioactive complexes based on succinic acid and dimethyl ether of succinic acid with silver ions will contribute to the field of natural science in general.

Compliance with scientific development directions or state programs.

The dissertation was completed at the Kazakh National Research Technical University named after K.I. Satpayev within the framework of grant funding from the Ministry of Science and Higher Education of the Republic of Kazakhstan, in particular No. AP09260644 "Development of an effective encapsulating composition for multifunctional purposes to increase the yield of legumes" for 2021-2023 and within the framework of the program targeted funding project BR24993105 "Creation of a biotechnological R&D center for the development and commercialization of agricultural products and technologies" for 2024-2026.

Publications. The main results of the dissertation work were published in 14 co-authored works, including 4 articles in international scientific journals included in the Scopus and Web of Science databases; 2 articles in a scientific publication recommended by the Committee for Quality Assurance in Science and Higher Education (CQASHE) of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 1 article and 4 abstract of the report in the materials of international and national scientific conferences; 1 patent of the Republic of Kazakhstan for invention, 1 patent for utility model and 1 act of implementation of the results of scientific research, scientific and technical work or the results of scientific and scientific and technical activities.

Personal contribution of the doctoral student to the preparation of each publication:

During the research, the PhD candidate independently conducted a literature review on the topic of the dissertation. Conducted experimental studies in accordance with the set goals and objectives. Synthesized and studied the obtained complexes based on succinic acid and dimethyl ester of succinic acid with silver ions, as well as a mixed-ligand complex of succinic acid with glycine and Cu^{2+} $[\text{Cu}(\text{succ})(\text{gly})]_n$ using physicochemical and biological methods. Conducted a theoretical and practical analysis of the obtained results of experimental work, prepared manuscripts of scientific articles and formed the dissertation work.