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Д.В.СОКОЛЬСКИЙ АТЫНДАҒЫ «ЖАНАРМАЙ,
КАТАЛИЗ ЖӘНЕ ЭЛЕКТРОХИМИЯ ИНСТИТУТЫ» АҚ

# Х А Б А Р Л А Р Ы

# ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

АО «ИНСТИТУТ ТОПЛИВА, КАТАЛИЗА И
ЭЛЕКТРОХИМИИ ИМ. Д.В. СОКОЛЬСКОГО»

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OF THE REPUBLIC OF KAZAKHSTAN

JSC «D.V. SOKOLSKY INSTITUTE OF FUEL,
CATALYSIS AND ELECTROCHEMISTRY»

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## СЕРИЯ ХИМИИ И ТЕХНОЛОГИИ

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# SYNTHESIS AND STUDY OF PINOSTROBIN OXIME SUPRAMOLECULAR COMPLEXES

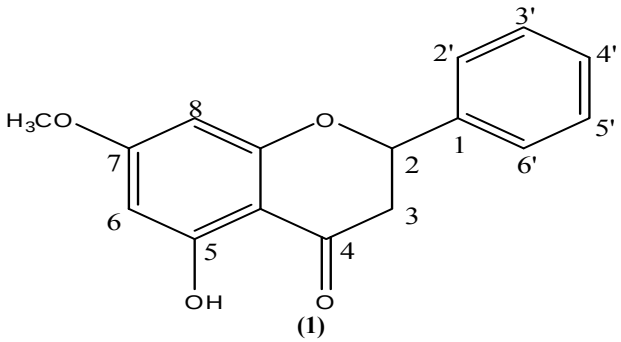
**Abstract.** The results of pinostrobinoxime derivatives studying, andits complexing abilitywith arabinogalactan, disodium salt of glycyrrhizic acid and magnesium carbonate, in particular,were discussed in this article. A supramolecular complex based on pinostrobinoxime with a higher water solubility was synthesized by mechanochemical treatment. Herewith, the complex of pinostrobinoxime with disodium salt of glycyrrhizic acid was shown to be the most effective. As the result, water solubility of this complex increased by 300 times.

**Keywords:** Pinostrobinoxime, arabinogalactan, disodium salt of glycyrrhizic acid, mechanochemical treatment, water solubility, supramolecular complexes.

## 1. Introduction

One of the priorities in the development of native pharmaceutical industry is a phytochemical manufacture development, based on original herbal drugs elaboration. In this case, herbal polyphenolic compounds perform a practical interest, as a new drug substance sources [1].

Flavonoids, including flavones, flavonols, flavanones and isoflavone, make large and important group of plant polyphenolic compounds. Pinostrobin (1), isolated from the *Populusbalsamifera L.* buds, belongs to available flavanones.



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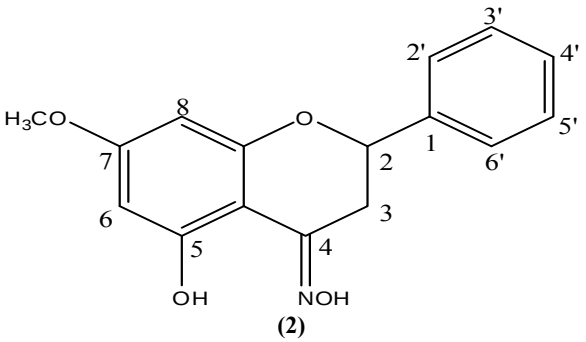


**(2)**

It is known, that flavonoids isolated from *Populusbalsamifera L.* buds have a hepatoprotective activity, a normalizing effect on synthesis of proteins in liver and prevent the cholestasis [2]. Antiproliferative, antimicrobial, neuroprotective and anti-inflammatory properties have been alsofound outin pinostrobin[3]. The development of pinostrobin chemical modification methods opens upnew possibilities for obtaining original agents with specific biological activity.

A new high-potential compound pinostrobinoxime (PO) with hepatoprotective and antioxidant properties has been obtained by the reaction of pinostrobin with hydroxylamine hydrochloride [4].

On the basis of quantum chemical calculations [5], it can be said that the pinostrobin is a nucleophilic reagent with basic reaction centers at C3, C6, and C8 atoms, and the nucleophilic centers of pinostrobinoximeare O and N atoms, with the oxygen atom of the hydroxyl group at C5 possessing a relatively high electron density in comparison with the nitrogen atom of oxime.

The major disadvantage of PO, as well as other flavonoids, is its poor water solubility, which affects the bioavailability and inhibits pharmacological and preclinical studies, which makes it necessary to modify the molecules of active substances by transferring them to water-soluble salts. In this case, the emergence of drug side effects is possible, such as increased toxicity, which leads to the inexpediency of using original phytopreparations.The chemical behavior of flavonoids and their derivatives is unpredictable and unexpected in many cases, due to their polyfunctional nature.Various methods [6-13] of biologically active substances (BAS) water solubility enhancement are known,including a mechanochemical treatment.Mechanochemical treatment (MT) is a solidphase reaction, it's a one-stage process with high efficiency and relative simplicity and it allows to avoid using solvents. The authors of [13-16] obtained supramolecular BAS complexes by mechanochemical treatment to increase bioavailability and noted the effectiveness of this method.

As a result of BAS mechanochemical treatment with an auxiliary component, which has an oligomeric, polymeric, or other macromolecular structure, a spontaneous association of an undetermined number of components occurs aspontaneous association of an indefinite number of components with the formation of supramolecular ensembles. Supramolecular ensembles have quite definite structural, conformational, thermodynamic, kinetic and dynamic properties; different types of interactions can be distinguished in them, differing in their strength, direction, dependence on distances and angles: coordination interactions with metal ions, electrostatic forces, hydrogen bonds, van -der-Waals interactions, donor-acceptor interactions, etc. The strength of the interaction can vary over a wide range, depending on the type of bonds. In general, intermolecular bonds are weaker than covalent bonds, therefore they are less stable thermodynamically, but more flexible dynamically [17].

High-intensiveMT can lead to rupture of strong covalent bonds, whereas low-intensiveMT allows BAS molecules to "penetrate" into space inside the macromolecule or self-associative auxiliary substances, forming a supramolecular complex due to hydrogen bonds and van der Waals forces. Considering the absence of a covalent interaction between the molecules of BAS and the auxiliary substance, we can say that the structure of BAS remains unchanged.

Based on this, the actual task is the synthesis of a water-soluble complex with a biocompatible agent by MT, which allows to enhance water solubility of the substance and to preserve the molecule original structure.

## 2. Experimental part

### 2.1 PO complexes obtaining

The obtaining of solid dispersions was carried out in a BM-1 ball mill with a cylindrical vessel that has a fluoroplastic lining.Treatment mode: grinding media acceleration - 1g, total loading of processed mixture components – 18-22 g, vessel volume – 300 mL, grinding media – steel balls (steel gradeШХ-15, diameter 22 mm, loading 675 g). Treatment duration was from 1 to 16 hours. Regardless of the barrel volume, the filling value of grinding media should be approximately 40%, and the filling value with processing material is 10-40%.

As complexing agents, we used:

- Arabinogalactan (3) (a good water soluble polysaccharidearabinogalactan (AG) isolated from*Larixdahurica*) produced by CJSC Ametis(Blagoveshchensk, Russia), Technical Specifications 9325-008-70692152-08. General formula $[(C_5H_8O_4)(C_6H_{10}O_5)_6]_n$. It is a solid amorphous substance, light brown in color, odorless, has a sweetish taste. Melting point is 240-250 ° C. Easily soluble in water.

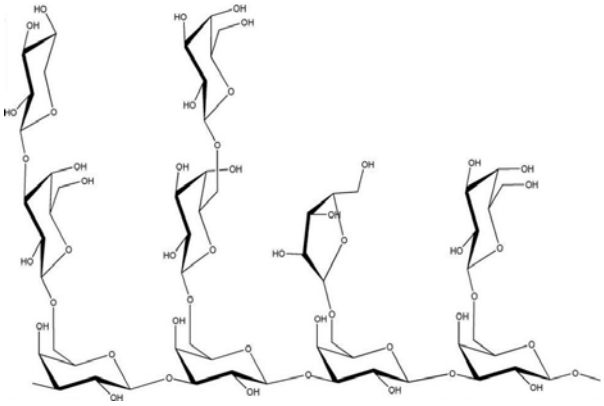
- Disodium salt of glycyrrhizic acid (4) (Na2GA) – a natural saponin derivative, (CFS, 98%) produced by Shaanxi Sciphar Biotechnology Co., Ltd (Xi'an, China). The gross formula is $C_{42}H_{60}O_{16}Na_2$. It is a gray powder with a mustard tint. Does not melt.Tempered at a temperature of ~ 400 ° C. Easily soluble in water.

- Polyvynilpyrrolidone (5) (PVP) is a synthetic polymer of HuangshanBonsunPharmaceuricals Co., Ltd. production (Huangshan, China). Common formula is $C_6H_9NO)_n$. It is a white, yellowish white powder

with no smell. It has sweetish taste. Fusion temperature is 150 °C. It is soluble in water, ethanol, and methanol.

- Magnesium carbonate substance ($MgCO_3$) of pharmacopeic purity (FSP42-3989-08)

Optimal mass ratio of components: PO/AG – 1:10, PO/Na2GA – 1:10, PO/MgCO3 – 5:1



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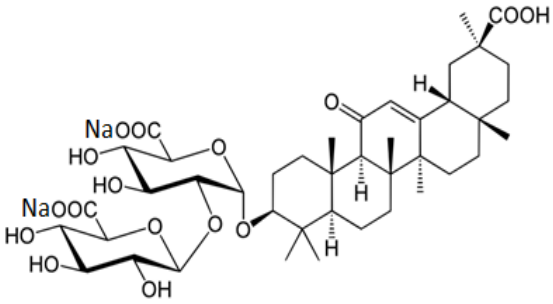



(4)

**2.2 High performance liquid chromatography (HPLC)**

Agilent 1200 HPLC system with a reverse phase column (Zorbax Eclipse XDB-C18, 4.6×50 mm) was used to determine the concentration of PO. Column temperature is +30 °C. HPLC system equipped with diode-array detector and two pumps. The mobile phase is consisted of acetonitrile:acetate buffer (acetic acid/sodium acetate) pH=3,4, ratio is 60:40, flow rate is 1 mL/min and detector operated at 279 nm.PO concentration was determined in relation toa specially prepared alcohol solutions.

The dissolution kinetics of the initial PO was measured as a function of time to determine PO solubility, according to these data the optimal solution time was chosen. Then the sample weights of physical mixtures and mechanically treated PO mixtures\ auxiliary substance with pledged excess of PO were taken for obtaining of saturated solution. Sample weights were mixed in 10 ml of distilled water in incubator-shaker (37°C, 200 rmp) during the chosen period of time of solution (90 min). The the solution was centrifuged for 10 min (12000 rmp), supernatant liquid was filtered through the paper filter.

PO concentration in solution was determined by HPLC. PO concentration was determined in relation with prepared on purpose alcohol solutions.

**2.3 Gel permeation chromatography (GPC)**

The molecular weight distribution of the AG in complexes was analyzed by gel permeation chromatography on Agilent 1260. The solvent was a 0,1 M aqueous solution of LiNO3, flow rate was 1 ml/min, sample concentration was 1 mg/mL. The calibration was based on standard dexstrans with molecular weights of 25,12,5 kDa and D-galactose.

**2.4 Scanning electron microscopy**

Electronic images were acquired using a Hitachi TM-1000 microscope (Tokyo, Japan). Coating of samples with gold was performed by JEOL JFC-1600 auto fine coater. The coating parameters were as follows: amperage - 30mA, sputtering time - 30 s and film thickness was 15 nm.

**3. Results and discussion**

**3.1 Obtained PO complexes**

During the MT, the crystal particles of PO and the spherical particles of AG and $Na_2GA$ are destroyed and a polydisperse powder is formed, consisting of 5-20 μm particles and their aggregates.

Arabinogalactan is a highly branched polysaccharide. This feature of the structure promotes the formation of strong supramolecular complexes with an active substance, the molecules of which can be bound by intermolecular bonds in the space formed by side chains.

There are hydrophilic and hydrophobic fragments in the molecule $Na_2GA$, so the possible mechanism of interaction of $Na_2GA$ with PO in solution is the inclusion of molecules of these substances into micelles. Most probably, the $Na_2GA$ molecules in a micelle are oriented by hydrophobic fragments inwards, and hydrophilic parts are oriented to the outer surface of the self-associates. In this case, the PO

molecules can be found in the internal hydrophobic part of the micelle andthey also complex with external hydrophilic fragments.

PO refers to flavonoids, which are polyphenolic compounds with acidic properties. In alkaline pH ranges, their molecules are capable of ionization, and the ionized form, as a rule, has a higher water solubility. Thus, by shifting the equilibrium towards ionized molecules, we increased the total concentration of PO in the solution. As an alkaline agent, which allowed to "shift" the pH value to the required pH range of 10.2, we used a "pharmacopeia" substance of magnesium carbonate.

**3.2HPLC analyses result**

The results shown in Fig. 1 display that after16 hour of MT the water solubility of PO in complex with $Na_2GA$ increased by 300 times, and in combination with AG - by 30 times, in complex with PVP by 42 times.After a 4-hour treatment, the water solubility of PO in a mixture with $MgCO_3$ increased 70-fold. After 1 and 2 hours ofMT, the PO/$MgCO_3$ mixture proved to be more effective than other complexing agents, and the PO concentration during the long mechanical action changes insignificantly, and after four hours of treatment begins to decrease. The highest concentration of PO in solutions of the PO/AG complex is observed at 16 hours of MT, however, it does not differ much from the same value at 8 hours, which indicates that there is no need to increase the duration of the process. In the case of PO/ $Na_2GA$, a significant increase in the water solubility of PO is observed with an increase in the processing time. Probably, the 24-hour MT of this complex will be even more effective.

Table 1 – HPLC analysis of supramolecular complexes of oximepinostrobin.

| Substance | Mixing timemin | Mech. treatment time, h | The sample volume, µl. | Peak time, h | Peak area | PO concentrationmg/l |
|---|---|---|---|---|---|---|
| PO | 30 | - | 20 | 4,96 | 3,5 | 0,08 |
| PO | 60 | - | | 4,96 | 12,92 | 0,33 |
| PO | 90 | - | | 4,97 | 13,26 | 0,34 |
| PO | 137 | - | | 4,96 | 22,00 | 0,57 |
| PO | 150 | - | | 4,97 | 11,58 | 0,29 |
| PO | 180 | - | | 4,97 | 11,32 | 0,29 |
| PO | 240 | - | | 4,97 | 16,10 | 0,41 |
| PO | 300 | - | | 4,97 | 16,66 | 0,43 |
| PO+AG1/10 | 90 | 1 | 20 | 4,57 | 194,18 | 5,15 |
| PO+AG1/10 | | 2 | | 4,96 | 229,08 | 6,08 |
| PO+AG1/10 | | 4 | | 4,96 | 529,47 | 14,07 |
| PO+AG1/10 | | 8 | | 4,96 | 669,89 | 17,81 |
| PO+AG1/10 | | 16 | | 4,88 | 686,97 | 18,26 |
| PO+AG1/10 | 120 | 16 | | 4,89 | 781,03 | 20,7 |
| PO+AG1/10 | 150 | 16 | | 4,89 | 288,28 | 7,65 |
| PO+Na2GA 1/10 | 90 | 1 | 5 | 4,95 | 140,39 | 14,92 |
| PO+Na2GA 1/10 | | 2 | | 4,92 | 187,08 | 19,89 |
| PO+Na2GA 1/10 | | 4 | | 4,93 | 397,24 | 42,26 |
| PO+Na2GA 1/10 | | 8 | | 4,94 | 662,89 | 70,53 |
| PO+Na2GA 1/10 | | 16 | | 4,87 | 1693,47 | 180,20 |
| PO+Na2GA 1/10 | 120 | 16 | | 4,87 | 1403,08 | 149,30 |
| PO+Na2GA 1/10 | 150 | 16 | | 4,87 | 1295,06 | 137,80 |
| PO+$MgCO_3$ 5/1 | 90 | 1 | 20 | 4,93 | 1029,95 | 27,39 |
| PO+$MgCO_3$ 5/1 | | 2 | 5 | 4,92 | 248,43 | 26,42 |
| PO+$MgCO_3$ 5/1 | | 4 | 5 | 4,92 | 268,46 | 28,55 |
| PO+$MgCO_3$ 5/1 | | 8 | 20 | 4,94 | 1017,84 | 27,06 |
| PO+$MgCO_3$ 5/1 | | 16 | 20 | 4,95 | 550,76 | 14,64 |
| PO+PVP1/10 | 90 | 1 | 20 | 4,77 | 489,75 | 13,01 |
| PO+PVP1/10 | | 2 | | 4,6 | 914,97 | 24,33 |
| PO+PVP1/10 | | 4 | | 4,62 | 605,35 | 16,09 |
| PO+PVP1/10 | | 8 | | 4,86 | 606,83 | 16,13 |
| PO+PVP1/10 | | 16 | | 4,92 | 963,33 | 25,61 |

The obtained results show that an increase in the water solubility of a substance based on pinostrobinoxime (PO) was achieved by the formation of supramolecular PO complexes by the method of mechanochemical treatment. Water-soluble polysaccharide arabinogalactan (AG) from Dahurian larch and

a vegetable saponin derivative disodium salt of glycyrrhizic acid (Na2GA) were used as complexing agents. Structural features of complexing agents make it possible to form supramolecular complexes with the processed substance, molecules of what form hydrogen bonds in the intermolecular space formed by macromolecules of complexing substances. MgCO3 was also used as an agent for shifting the pH of solid PO dispersions to a slightly alkaline range, what increases water solubility as well. The concentration of PO in the aqueous solutions of the obtained samples was determined by HPLC.

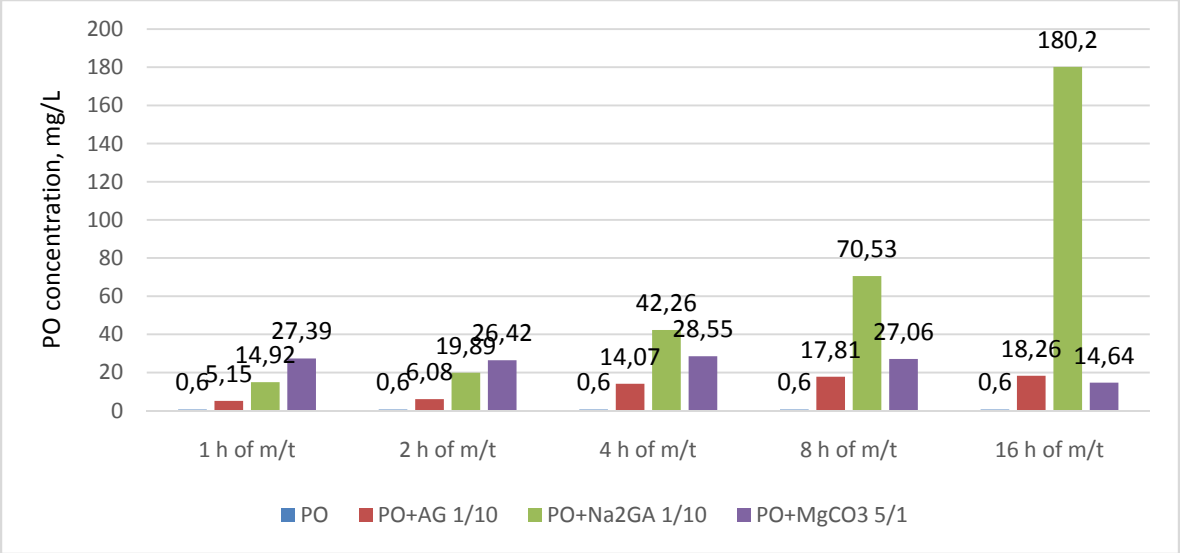



Figure 1 - Results of HPLC analysis of intermolecular complexes of oximepinostrobin. Dependence of the pinostrobinoxime concentration in solutions of intermolecular complexes on the time of MT.

### 3.3GPC analyses of PO/AG

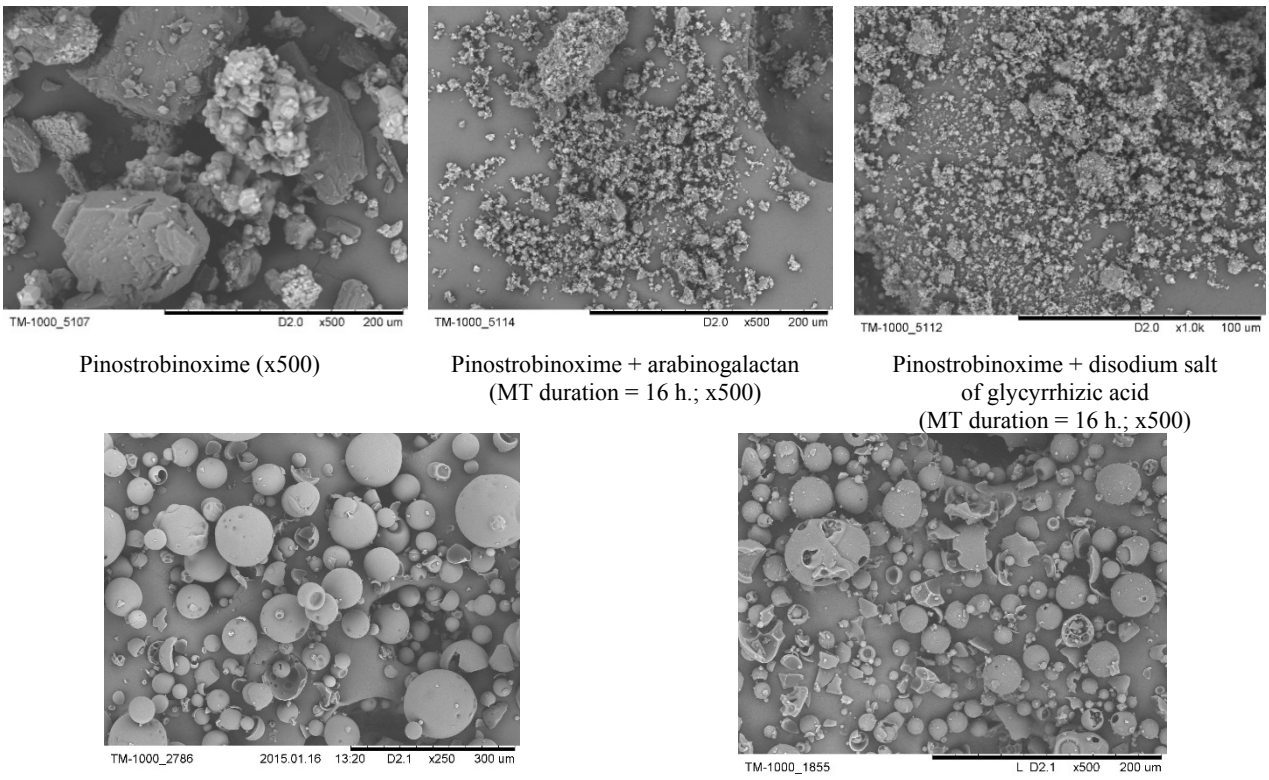
According to the data presented in Table 1, after MT ofPO with AG, the average molecular weight of AG and the degree of polydispersity (Mw / Mn) change slightly. Also, MT of PO with AG leads to a slight decrease in the proportion of arabinose fragments in the molecule of the polysaccharide arabinogalactan, which does not dependon the time of mechanical action.

Table 1 - Molecular weight distribution of machined mixture of oxyne pinostrobin with arabinogalactan

| Sample | The conditions of MT, the ratio of OPB / AG | MM | $M_w/M_n$ | Area of peaks with different MM |
|---|---|---|---|---|
| AG init.. reprecipitated | - | 18060<br>445 | 1,44 | 99,9<br>0,1 |
| PO/AG | 1:5, τ=4 h | 18245<br>585 | 1,21<br>1,35 | 94,4<br>5,6 |
| PO/AG | 1:10, τ=4 h | 18105<br>550 | 1,37<br>1,13 | 98,4<br>1,6 |
| PO/AG | 1:15, τ=4 h | 18105<br>775 | 1,40<br>1,09 | 99,1<br>0,9 |
| PO/AG | 1:5, τ=8 h | 18290<br>570 | 1,38<br>1,2 | 94,9<br>5,1 |
| PO/AG | 1:10, τ=8 h | 18220<br>660 | 1,42<br>1,23 | 93,7<br>6,3 |
| PO/AG | 1:15, τ=8 h | 18200<br>570 | 1,42<br>1,1 | 99,0<br>1,0 |
| PO/AG | 1:5, τ=16 h | 17995<br>600 | 1,45<br>1,21 | 93,9<br>6,1 |
| PO/AG | 1:10, τ=16 h | 18155<br>570 | 1,44<br>1,12 | 98,6<br>1,4 |
| PO/AG | 1:15, τ=16 h | 18015<br>570 | 1,46<br>1,11 | 98,8<br>1,2 |

**3.4Electronic micrographs of samples**

These micrographsshown in Fig.2 describe the morphology of the sample surface. The pure PO consists of crystalline particles and their agglomerates. AG is in the form of spherical particles with a porous surface. $Na_2GA$ consists of spherical hollow particles with a smooth surface. After MT, the original shape of the particles of the original components has changed and it is impossible to separate the individual components, except the formed agglomerates. As it can be seen in Fig. 2, the obtained substances are polydisperse powders with particles with size of 5-20 μm and their aggregates.




Pinostrobinoxime (x500)

Pinostrobinoxime + arabinogalactan (MT duration = 16 h.; x500)

Pinostrobinoxime + disodium salt of glycyrrhizic acid (MT duration = 16 h.; x500)

Arabinogalactan (x250)

Disodium salt of glycyrrhizic acid (x500)

Figure 2- Electron micrographs of pinostrobinoxime and obtained complexes

**4. Conclusion**

Synthesis of supramolecular complex of pinostrobin oxime with arabinogalactan, disodium salt of glycyrrhizic acid and basic magnesium carbonate, selection of complexing agents, optimal compositions of water-soluble pinostrobin oxime complex have been carried out. The regime of obtaining a supramolecular complex is determined by the method of mechanochemistry. The obtained complexes have increased water solubility in comparison with pinostrobin oxime. The high water solubility of the pinostrobin oxime showed a complex with the disodium salt of glycyrrhizic acid. After mechanochemical treatment of the pinostrobin oxime with arabinogalactan, the average molecular weight of arabinogalactan changes insignificantly.

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## СИНТЕЗ И ИЗУЧЕНИЕ СУПРАМОЛЕКУЛЯРНОГО КОМПЛЕКСА ОКСИМА ПИНОСТРОБИНА

**Аннотация.** В статье обсуждаются результаты изучения оксимпроизводногопиностробина, в частности, способность комплексообразования их с арабиногалактаном, динатриевой солью глицирризиновой кислоты и основным карбонатом магния. Методом механохимической обработки на основе оксимапиностробина получен супрамолекулярный комплекс, обладающий повышенной водорастворимостью. При этом наибольшую эффективность показал комплекс оксимапиностробина с динатриевой солью глицирризиновой кислоты, в котором растворимость полученного комплекса повысилась в 300 раз.

**Ключевые слова:** Оксимпиностробина, арабиногалактан, динатриевая соль глицирризиновой кислоты, механохимическая обработка, водорастворимость, супрамолекулярный комплекс.

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