EXTRACTION OF SUPRAMOLECULAR COMPLEXES AND THEIR ACTIVITY AGAINST TUBERCULOSIS BASED ON NATURAL TRITERPENOIDS

[1]Khurramova F. N, [2]Sobirova F.A, [3]Esanov R. S, [4]Matchanov A. D

Postgraduate of Institute of Bioorganic Chemistry named after A.S.Sadikov[1], Teacher of Institute of Bioorganic Chemistry named after A.S.Sadikov[2], Teacher of Institute of Bioorganic Chemistry named after A.S.Sadikov[3] , Teacher of Institute of Bioorganic Chemistry named after A.S.SadikovAddress: 100125 Tashkent city, Mirzo Ulugbek district, Mirzo Ulugbek avenue, 83. [4]

ABSTRACT

Liquorice is a plant that is very popular in traditional Chinese medicine. More than 20 triterpenoids and about 300 flavonoids have been isolated from the licorice plant. Among these isolated components, 73 biologically active components have been identified and 91 of them have been used for various purposes. Studies show that the two main triterpenoids in licorice plant, Glycyrrhizin (GL) and 18b-Glycyrrhetinic acid (GA), have many pharmacological properties. For example, it can treat viruses, inflammation, tumors, bacteria, and even treat tuberculosis.

Keywords: glycyrrhizinic acid, tuberculosis, isoniazid, rifampicin, pyrazinamide, ethambutol hydrochloride and levofloxacin, supramolecular complex.

INTRODUCTION

The plant Glycyrrhiza glabra is one of the most widely used medicinal plants in medicine for centuries. Shennong's book " The Classics of Matter" states that the licorice plant should have life-enhancing properties [1].

The main medicinal parts of the licorice plant are its roots and rhizomes. One of the main substances that give medicinal properties to the composition of licorice root is glycyrrhizic acid [2,3].

Many pharmacological properties of licorice have also been studied in modern medicine. Aqueous extract of liquorice, ethanol extract and highly critical liquid extract have a strong effect in inhibiting the activity of gram-positive and gram-negative bacteria, such as Staphylococcus aureus, Escherichia coli, Pseudomonascan, Pseudomonas [4-6].

These extracts have also been shown to relieve pain, treat dental caries, prevent digestive problems, eliminate phlegm and relieve cough, and treat tuberculosis [7]. The main biological activity of licorice root root is triterpenoids - Glycyrrhizin-GL, and Glycyrrhizinic acid (18b-Glycyrrhetinic acid-GA), as well as four flavonoids in the root - Likvirigigenin (Likviritigin), Likoniritigen (Liquirit) LCA), Licochalcone B (Licochalcone E-LCE) and Glabridin (Glabridin-GLD) may show major antiviral and antimicrobial activity.

It is known that the biological activity of heterocyclic compounds containing a nitrogen atom in the molecule of matter is high. Therefore, this type of compound is widely used in various fields of pharmacy [8]. These compounds can inhibit the growth of microbes and reduce the production of toxins.

Treatment of tuberculosis with synthetic drugs leads to side effects. The use of the main active ingredients of licorice root is one of the promising directions in the development of safe and effective antiviral or antimicrobial agents.

Recent studies suggest that glycyrrhizinic acid and its salts in the licorice plant may serve as the main active ingredient in the treatment of tuberculosis. In this review, we will focus on the causes, consequences, and medications used to treat tuberculosis.

According to the World Health Organization (WHO), one-third of the world' s population is infected with Mycobacterium tuberculosis. This epidemic kills 1.7 million people worldwide every year. There are more

than 60 species of the Mycobacterium family, but several of them can cause diseases in humans, such as Mycobacterium tuberculosis, Mycobacterium leprae, Mycobacterium africanum, and Mycobacterium avium [9]. Tuberculosis is highly contagious when active and is mainly transmitted through the respiratory system. The disease can occur as a result of the entry of at least 10 bacteria into the deep part of the lungs [10]. Although the lung system is the primary target for tuberculosis mycobacteria, it is the skeletal system, the gastrointestinal tractcan spread and enter non-pulmonary systems such as the gastrointestinal tract, central nervous system, and lymphatic system. Early signs of tuberculosis include night sweats, fever, and weight loss, while evening symptoms include chest pain, shortness of breath, and blood in sputum production [11].

Treatment of tuberculosis is carried out by taking a combination of antibiotics for a period of 6-8 months. The first category of drugs are used in tuberculosis are isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutolhydroxlaride (EMB). If the bacterial strain becomes resistant to these drugs, second-line drugs are used. These include streptomycin, kanamycin, fluoroquinolones, ethionamide, and paraaminosalicylic acid [12,13] Typically, secondary drugs are less effective and more toxic than primary drugs.

Most anti-tuberculosis drugs kill bacteria during division. Isoniazid is used in the treatment of tuberculosis as a major part of treatment regimens in combination with drugs such as rifampicin, pyrazinamide, and ethambutol hydrochloride. Its minimum concentration against tuberculosis mycobacteria is 0.02–0.20 μg / ml [14]. Although isoniazid has a bactericidal effect on fast-growing Cox rods, it has a limited effect on slow-growing (intracellular) and intermittently growing (extracellular) rods. Activation of isoniazid results in the formation of oxygen-derived free radicals (superoxide, hydrogen peroxide, and peroxynitrite) and organic free radicals that inhibit the formation of mycolic acids in the bacterial cell wall, leading to DNA damage and consequent death of the bacillus. Isoniazid is used in doses of 5– 15 mg per kilogram of body weight when used alone to prevent tuberculosis [15]. Rarely, it has a side effect in people without liver disease or kidney failure [16].

Rifampicin is the most important drug in the treatment of tuberculosis. Its minimum concentration against tuberculosis mycobacteria is 0.05-0.50 μg / ml. When rifampicin is used in combination with pyrazinamide, the duration of treatment for tuberculosis can be reduced to six months. Rifampicin blocks DNA-binding RNA polymerase, leading to cell death [14,17]. Patients receiving rifampicin may experience nausea and abdominal pain. Pyrazinamide with an isoniazid-like molecular structure is a derivative of nicotinic acid. Pyrazinamide was synthesized in 1936 and has been used as an anti-tuberculosis drug since 1952. The minimum concentration of this drug for tuberculosis mycobacteria is 6.25-50.0 μg / ml [14,18]. Once administered orally, pyrazinamide is well absorbed and distributed throughout the body. The maximum concentration of the drug occurs 2 hours after taking the drug in the blood plasma. Pyrazinamide is the most effective drug to destroy the population. Pyrazinamide enters Cox's rods slowly, is converted to pyrazinic acid by pyrazinamidase, and reaches high concentrations in the bacterial cytoplasm [18,19].

Ethambutol is another important drug against tuberculosis Ethambutol affects intracellular and extracellular rods, mainly fast-growing rods. The minimum concentration of this drug for tuberculosis mycobacteria is 1-5 mcg / ml. Ethambutol disrupts the biosynthesis of the main polysaccharide arabinogalactan in the cell wall of tuberculosis mycobacteria [14,19,20). The L configuration of ethambutol was found to be a more toxic isomer [21]. Once ethambutol enters the body, it can form chelates with various cations (zinc and copper). The body, on the other hand, requires copper and zinc, several enzymes that are essential for healthy functioning. Thus, the reduction of these cations during treatment with ethambutol affects the normal functioning of various enzymes [22].

Recently, extensive research has been conducted to develop an alternative treatment regimen that effectively eliminates tuberculosis infection, requires less time to treat, and minimizes side effects or no side effects at all. Based on the above data, the development of new drugs based on the licorice plant will increase the safety and effectiveness of confectionery-related products. Based on the literature, drugs used in the treatment of patients with tuberculosis may have some adverse effects on the body and therefore it is possible to develop highly effective drugs by modifying these types of drugs on the basis of natural biologically active substances isolated from plants.

Studies have shown that supramolecular complexes of the biologically active substance glycyrrhizic acid (GA) and glycyrrhizinic acid monoammonium salt (GAMAS) with anti-tuberculosis drug substances may have synergistic effects against this disease. The development of effective and inexpensive drugs based on liquorice can significantly improve the pharmaceutical market of our country. The development of new drugs for liquorice will increase the safety and effectiveness of liquorice -related products.

PRACTICAL PART

Obtaining supramolecular complexes of glycyrrhizinic acid (GA) and monoammonium salt of glycyrrhizic acid (GAMAS) with anti-tuberculosis drug substances (isoniazid, rifampicin, pyrazinamide, ethambutol hydrochloride and levofloxacin) (Fig. 1).

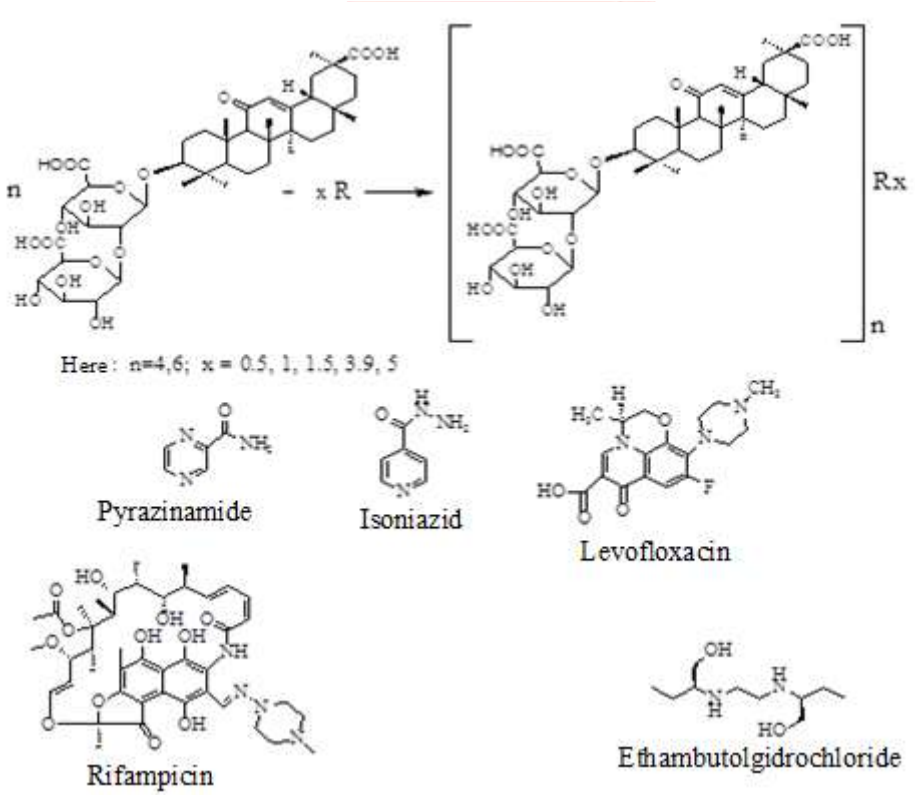

Figure 1. Scheme of obtaining supramolecular complexes based on glycyrrhizic acid.

Here Some physicochemical quantities of the obtained complex, liquefaction temperatures, solubility were determined. Systems for thin-layer chromatography were selected to characterize the purity of the complex, and the Rf values of the substance were calculated. Hydrodynamic properties were studied and UV and IR spectra of the complexes were obtained and the results were analyzed. The results obtained are presented in Table 1. The liquefaction temperatures of the complexes were determined on the device PTPTU 25-11-1144. They are tested on thin layer chromatography UV-254 on brand Silifol paper. Hydrodynamic properties were studied in a

viscometer. Hydrodynamic properties were studied in a viscometer. The UV spectrum was obtained on a SHIMADZU UV-1280 spectrophotometer. The IR spectrum was obtained on a Perkin Elmer IR 10.6.1 spectrophotometer.

Table 1

Some physicochemical quantities of raw materials and obtained supramolecular complexes

№	Complexes	mole ratio	R_f value	T.c.,^{0}C (decomp.)	UV, λmax, nm(lgε)	Solubility
1	GAMAS Pyrazinamide Isoniazid	4:2:1	0,51	187-189	260 (5,02)	Acetonitrile, water, water + alcohol
2	GAMAS Isoniazid Levofloxacin Pyrazinamide Rifampicin	6:0,5:1,5:2:1	0,54	175-180	259 (5,15)	Acetonitrile, water, water + alcohol
3	GAMAS Isoniazid Rifampicin Pyrazinamide Ethambutolgid-rochloride	6:1:2:5:3,9	0,46	160-165	226 (4,06)	Acetonitrile, water, water + alcohol
4	GAMAS Isoniazid Ethambutolgid-rochloride	4:1:2	0,52	180-185	262 (4,90)	Acetonitrile, water, water + alcohol
5	GA Isoniazid Rifampicin Pyrazinamide Ethambutolgid-rochloride	6:1:2:5:3,9	0,48	155-160	265 (5,23)	Acetonitrile, water, ethanol

System: Acetonitrile: Ethanol: Water (5: 2: 1)

UV and IR spectra of complex compounds were obtained and analyzed. To study the nature of the bonds, UV spectra of substances were used in a 50% ethanol solution, and their absorption peaks were observed at 255– 260 nm, i.e., in the near-ultraviolet field.

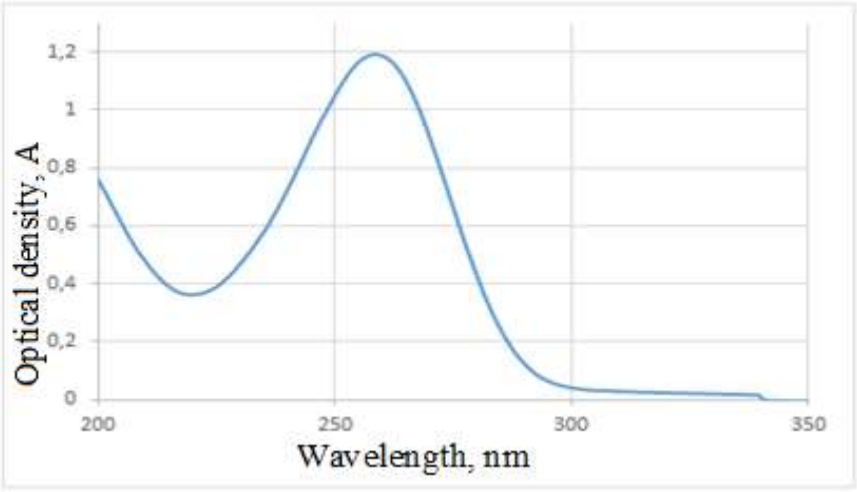

Figure 2. UV spectrum of GAMAT: pyrazinamide: isoniazid (4: 2: 1) complex

Absorption peaks of similar wavelengths (250-265 nm) were observed in other obtained complexes. The results obtained are given in Table 1. IR spectra of the complexes were also obtained and analyzed in order to determine what types of interactions were present during the complex formation process. Based on the change in the fundamental oscillation frequencies of the functional groups in the IR spectra of the starting materials, it is possible to consider what types of interactions between molecules occur in the formation of molecular complexes. In particular, the valence oscillation frequencies of the OH groups in the GAMAS molecule were observed at 3209 cm-1, and in the complex at 2926 cm-1. The difference in the valence oscillation frequencies of the ON groups indicates that hydrogen bonds are formed in the complex. (Figure 3).

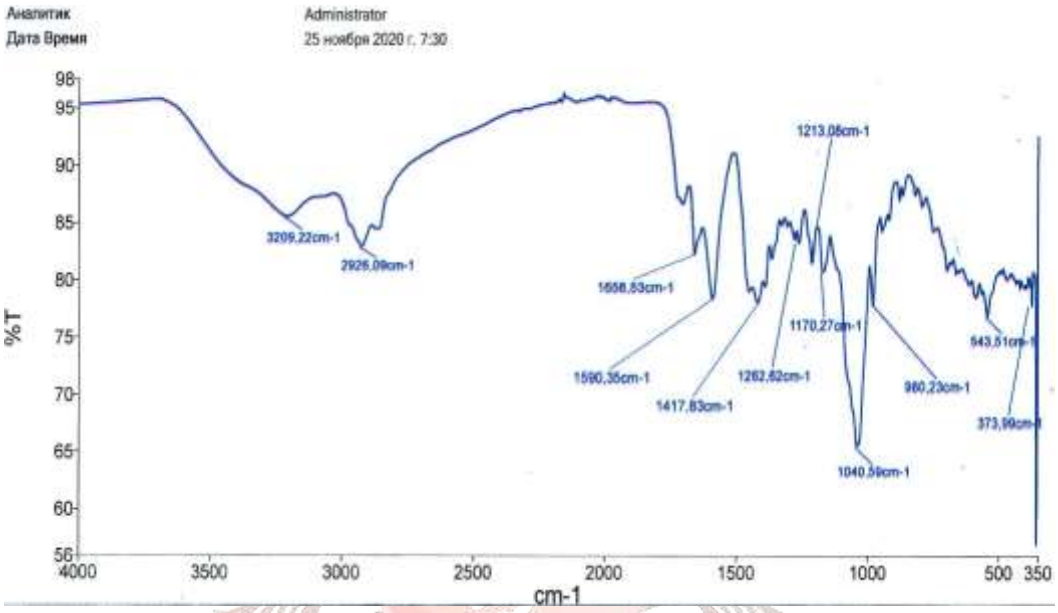

Figure 3. IR spectrum of GAMAS: pyrazinamide: isoniazid (4: 2: 1) complex

The valence oscillation frequency of the carbonyl group in the S-11 part of the aglycone part of the GAMAS molecule was observed at 1658 cm-1. An increase in the complex spectrum of vibration frequencies at 1590 cm-1 belonging to the carboxyl group in the GAMAS molecule means that electrostatic (-SOO- • • • + NH3-) interactions are also involved in the formation of the complex.

IR spectra of the remaining complexes were also obtained and analyzed: 2 complexes; (OH, NH_4^+)=3203, ν(CH, CH_2, CH_3)=2927, ν(C=O)=1694, ν(C_{11}=O,C=C)=1658, ν(COO^-)=1593, δ(CH_2, CH_3)=1453, δ(NH_4^+)=1385, δ(CH)=1213, 1167, δ(C-O-C, C-OH)=1042, δ(=CH)=979, δ(CH)=807 (Ar). 3 complex; (OH, NH_4^+)=3253, ν(CH, CH_2, CH_3)=2935, ν(C=O)=1686.6, ν(C_{11}=O, C=C)=1657, ν(COO^-)=1585, δ(CH_2, CH_3)=1450, 1419, δ(NH_4^+)=1328, δ(CH)=1214,1167, δ(C-O-C, C-OH)=1042, δ(=CH)=978, δ(CH)=868(Ar). 4 complex; (OH, NH_4^+)=3209.6, ν(CH,CH_2, CH_3)=2927, ν(C=O)=1699, ν(C_{11}=O, C=C)=1658, ν(COO^-)=1590, δ(CH_2, CH_3)=1456, 1414, δ(NH_4^+)=1387.7, δ(CH)=1212, 1172, δ(C-O-C, C-OH)=1033, δ(=CH)=979. 5 complex; (OH)=3306, ν(CH, CH_2, CH_3)=2926, ν(C=O)=1687, ν(C_{11}=O, C=C)=1658, ν(COO^-)=1585, δ(CH_2, CH_3)=1450, 1419, δ(NH_4^+)=1371, δ(CH)=1214, 1167, δ(C-O-C, C-OH)=1024, δ(=CH)=978, δ(CH)=868(Ar).

In order to study the hydrodynamic changes of the obtained complexes, the viscosities of the complexes obtained on the basis of GA and GAMAS were studied. The dependence of the given viscosity of the complex obtained on the basis of the initial GAMAS depends on the concentration. The result was that the observed viscosity increased moderately when the concentration reached 0.2%, and the observed viscosity increased

relatively rapidly from 0.2% to 0.4%. Hence, as the concentration of the aqueous solution of the obtained complexes increases, the viscosity also increases. (Figure 4).

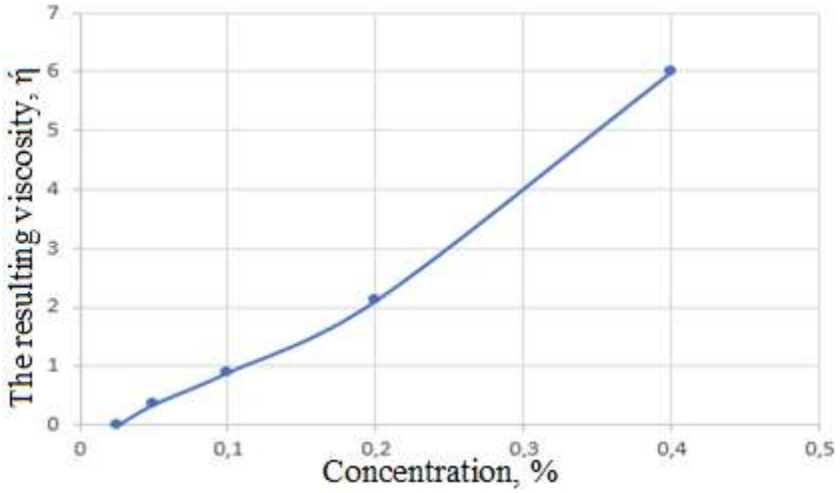

Figure 4. GAMAS: Pyrazinamide: Isoniazid 4: 2: 1 concentration dependence of the viscosity of the aqueous solution (25ºC)

The effects of environment and temperature on the viscosity of complex compounds were also studied. The viscosities of the obtained supramolecular complexes in different environment: urea (breaking down intermolecular hydrogen bonds), xylose (an agent prone to hydrophobic interaction in the system) and KCl (electrolyte) solutions were studied. (Figure 5).

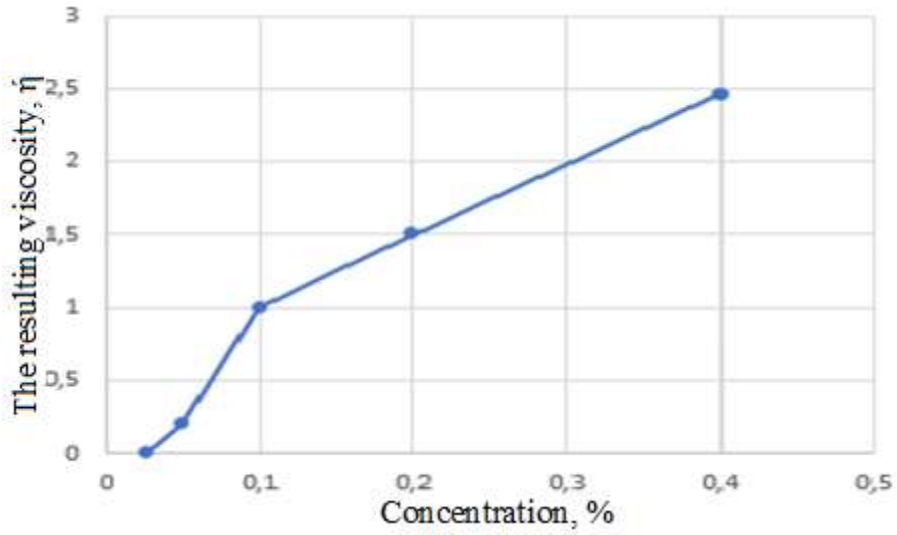

Figure 5. GAMAS in 0.01M urea medium: Pyrazinamide: Isoniazid 4: 2: 1 ratio obtained viscosity concentration-dependent curve (25ºC)

The temperature dependence of the viscosity of a 0.1% aqueous solution of the obtained complexes was studied. At the same time, the applied viscosity of the complex decreased in the temperature range from 25^0C to 35^0S, the viscosity of the complex decreased sharply in the range of 35^0C to 40^0C, and gradually decreased in the range of 40^0C to 45^0C. (Figure 6).

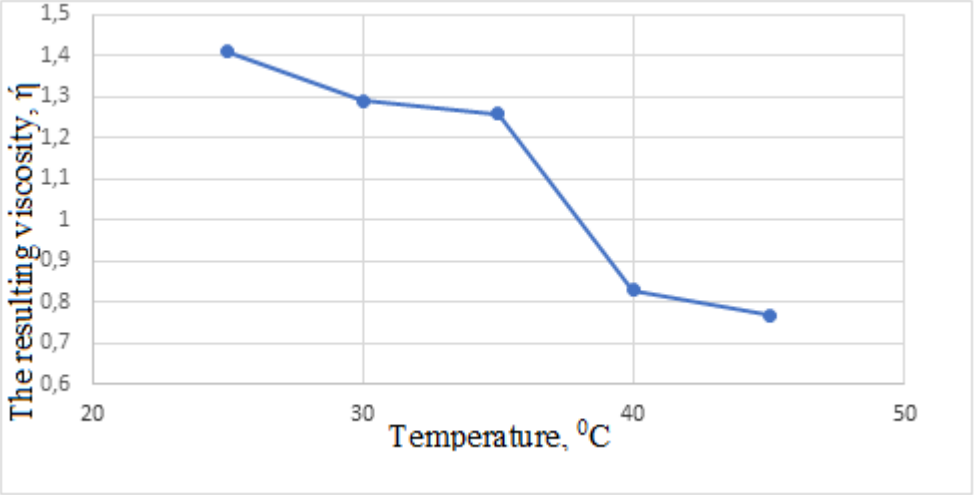

Fig. 6. GAMAS: Pyrazinamide: Isoniazid 4: 2: 1 temperature-dependent curve of viscosity of an aqueous solution of the obtained complex.

Typically, the viscosity of substances is specific to polymeric substances, which is due to their swelling. However, suramolecular complexes are submolecular substances. From this it can be concluded that in the formation of a complex there are forces other than the usual interactions, which are formed when given a certain temperature, and can change as the temperature increases. This is similar to the "orientation effect" between molecules. Under the influence of heat, the charges on the polar parts of the molecule increase in part, resulting in an increase in the size of the micelles due to the repulsion of opposite charges, a decrease in fluidity, and an increase in viscosity.

Experiment part.

GAMAT: pyrazinamide: obtaining a molecular complex of isoniazid (4: 2: 1);

First, 3.36 g (4 mmol) of GAMAT was completely dissolved in 50 ml of ethanol in 200 ml of a flat flask and 0.246 g (2 mmol) of pyrazinamide and 0.137 g (1 mmol) of isoniazid were added, then 50 ml were stirred vigorously in a magnetic stirrer. water was added and stirred for 5– 6 hours at room temperature. The alcohol portion was then sublimated in a rotor evaporator at a temperature of 50^0 C and the aqueous portion was frozen in liquid nitrogen medium and dried using a lyophilic device. The substance obtained is a light yellow powder.

Based on the above method, supramolecular complexes of GA and GAMAS with anti-tuberculosis drug substances were used: rifampicin, pirinamide, isoniide, ethambutol hydrochloride and levofloxacin.

CONCLUSION

For the first time, water-soluble supramolecular complex compounds of different molar ratios of drug substances used against tuberculosis were obtained with GA and GAMAS. Physicochemical properties and chemical structure of the obtained complex compounds, as well as the nature of the bonds involved in their formation were analyzed on the basis of optical spectroscopy methods. The biological activity of the complexes is being studied.

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