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SYNTHESIS OF ANTIMICROBIAL MODIFIERS BASED ON POLYVINYL ALCOHOL ACYLATED WITH CHLOROANHYDRIDE OF MALEOPIMARIC ACID

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Abstract. The water-soluble form of of maleopimaric acid (MPA) as its ester with polyvinyl alcohol (PVA) has been prepared by reaction of (PVA) with chloroanhydride of MPA in the conditions of interphase catalysis. The optimal conditions of the acylation reaction of PVA with chloroanhydride of MPA have been determined, the catalyst allowing to obtain the water-soluble polyester with yield to 52 % has been chosen. The tests of samples of modifiers for antibacterial activity against Gram-positive and Gram-negative strains of bacteria have been carried out.

Keywords: *Synthesis, maleopimaric acid, PVA, water-soluble polyester, interphase catalysis, antimicrobial activity.*

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1. Introduction

It was known that the durability of products made from polymer materials is determined by their resistance to external environment. The various microorganisms (macroscopic fungi, bacteria, etc.), acting on the product, cause damage (biological and microbiological) of its surface, changing the structural and functional characteristics of the product up to its destruction (Zhang, 2017). For prevention of these undesirable processes, the biocidal compositions containing low-toxic antimicrobial additives are used. Currently, on ecological reasons, the use of arsenic, zinc and other heavy metal compounds is being restricted. Instead of them, more effective synthetic or natural biocidal compounds are used (Pawłowska & Stepczyńska, 2022). There are also requirements for biocidal additives regarding the absence of an unpleasant odor. In some cases, the soluble antimicrobial polymer materials, for the manufacture of which the natural polymers - starch, cellulose, chitosan, etc., modified with antimicrobial compounds are used. The structural peculiarity of these polymers allows to achieve good compatibility and adjustability of migration of the antimicrobial compound (Mymrin, 2013; Lee *at al*., 2005). Therefore, the development of new effective protective agents with use of the natural compounds is also perspective from the ecological point of view.

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The PVA selected by us as the object of investigation is a water-soluble carbochain polymer characterized by nontoxicity, biocompatibility, bio-destructibility, which allows its use in surgery, cosmetic and pharmacological industries (Olewnik-Kruszkowska *et al*., 2019; Takács *et al*., 2022). PVA is one of the accessible and inexpensive polymers, which are often used for modification in order to give them specific properties (Sedlařík *et al*., 2006; Liu *et al*., 2021). For example, the composition materials based on such polymers exhibit improved ability to biological decomposition, which is preferable from an economic and ecological point of view.

The resinous acids and their derivatives have great potential as biologically active substances. Their antimicrobial activity has been connected with the availability of the functional groups such as hydroxyl, ketone and aldehyde in the structure of the molecule, and also the ability to take *cis*- and *trans*- configurations, due to which they have a wide range of activity against various types of microorganisms (Savluchinske-Feio *et al*., 2006; Bell *et al*., 2024). However, in spite of their high antimicrobial activity, the low solubility in an aqueous medium restricts their use. For solution of this problem, new modification technologies using resinous acids are currently being used. (Santovito *et al*., 2018; Zhang *et al*., 2022). Thus, the resinous acids and their derivatives are valuable raw materials of natural origin for the synthesis of various means of fighting of infection agents (Popova *et al*., 2021; Ito *et al*., 2020). The maleopimaric acid used for the modification of PVA has been obtained from rosin possessing wide range of biological activity and widely used in the compositions for the biological protection of materials (Kluev *et al*., 2014). MPA itself and some of its derivatives also show the antimicrobial and antifungal activity (Vafina *et al*., 2019).

In this paper the results of the synthesis of polymer on the basis of PVA, modified with chloroanhydride of maleopimaric acid have been presented and their antimicrobial properties have been investigated.

2. Experimental part

The IR spectra of the synthesized monomers and the obtained modifiers were taken on the “Cary 630 FTIR” device from Agilent Technologies (ZnSe crystal). The PMR spectra were taken on “Fourier” spectrometer (frequency 300 MHz) from “Bruker” in DMSO as solvent, the internal standard is hexamethyldisiloxane. The chemical shifts of the signals are given in the scale δ (ppm.).

The rosin (~80% consisting of abietic acid) was used with the softening temperature of 70°C acid number - 162.8 mg KOH/g; d=1.06 g/cm^3. The maleic anhydride (MA) was purified by recrystallization from benzene. PVA (GOST 10779-78) for decrease of content of the sodium acetate in it to 0.5% was washed 3 times with five-fold quantity of ethanol for 60 min. at 40°C.

The maleopimaric acid (MPA) was obtained by twofold recrystallization from an adduct solution in acetic acid, obtained by the interaction of rosin and MA at 180°C for 6 h (Pirguliyeva, 2022). For removal of the acetic acid, the solvate of maleopimaric acid and acetic acid was kept for 30 min. at 140°C. Maleopimaric acid has m.p. = 220-230ºC. The yield of maleopimaric acid by this method has a disadvantage - low yield (64 %).

The chloroanhydride of MPA was synthesized from MPA and thionyl chloride in methylene chloride according to the method (Wulfson, 1964).

The acylation of polyvinyl alcohol (PVA) with chloroanhydride of MPA was carried out on the model reaction of PVA esterification on the Schotten-Bauman method

(Bey *et al*., 2019; Kogai *et al*., 2014). An aqueous solution of PVA was placed in a three-necked flask with volume of 50 ml, equipped with a mechanical stirrer, a thermometer and a dropping funnel, at 0°C in an inert gas atmosphere, then an aqueous solution of NaOH and catalyst of an interphase catalysis (TEBA-Cl) were added. After that, the chloroanhydride solution of MPA in a mixture consisting of acetone (or methyl ethyl ketone) with benzene was added dropwise to the solution in intensive stirring. The mixing was continued for 2 hours. On completion of the reaction, the organic phase was separated, the aqueous part was extracted twice with sulfuric ether, neutralized with diluted acid (1:10) to a neutral medium, and planted twice in ethanol. The precipitate was filtered, washed with ethanol and dried in air.

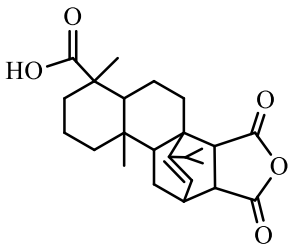
The hydrolysis of PVA modified with chloroanhydride of MPC was carried out as follows: 10% aqueous solution of sodium hydroxide was added to the modifier at 70°C, the reaction was carried out for 0.5 h, after which the reaction mixture was cooled to room temperature and neutralized with a diluted solution of the hydrochloric acid. The polymer was purified by its precipitation twice in ethanol and dried in air.

The antimicrobial properties of the modified PVA films were evaluated using the diffusion method. For this purpose, the samples as discs with a diameter of 1 cm were cut from the films of modifiers. For the tests, the bacteria strains of *Staphylococcus aureus* and *Escherichia coli* were used as the suspensions with bacterial concentrations in the range from 10^6 to 10^7 CFU/ml. The film samples were placed in Petri dishes with Muller-Hinton agar (pH 7.4 at 25°C) inoculated with bacterial suspensions. The samples were incubated at 37°C for 24 h. After incubation, the width of the inhibition zone for each sample was measured with hand caliper. The selected bacterial strains are standard test cultures for the reveal of antimicrobial properties of compounds). The film samples were placed in Petri dishes with Muller-Hinton agar (pH 7.4 at 25°C) inoculated with suspensions of the bacteria. The samples were incubated at 37°C and after 24 and 72 hours, the width of the inhibition zone for each sample was measured with a caliper. For each type of bacteria, the tests were carried out on three samples, the average value of the width of the inhibition zone was found.

3. Results and discussion

It was known that the main part (up to 95%) of rosin consists of various resin acids and their isomers. The main of them - abietinic acid - at higher temperatures (~180-200°C) is isomerized with conversion to levopimaric acid, which easily reacts with Diels-Alder cycloaddition (Bey *et al*., 2019). The levopimaric acid, reacting with maleic anhydride, is converted into maleopimaric acid (MPA). MPA contains two reactive groups - carboxylic and anhydride one in its molecule, which makes it a convenient compound for preparation of the products used in a number of branches of technology (in the manufacture of printing inks, in the production of alkyde resins and binders, paper industry, etc.). Some derivatives of MPA possess expressed biological activity and can also be used in medical practice (Kanerva *et al*., 2019; Jindal *et al*., 2017; Majeed *et al*., 2020).

During carrying out of condensation of levopimaric acid with maleic anhydride, the forming anhydride-containing adduct has the structure shown below, established by spectral data:



In the IR spectrum of the compound (Figure 1), the absorption bands characteristic for valence vibrations of the carbonyl group of the acid fragment at 1710 cm^{-1}, two characteristic bands of the carbonyl group of anhydride at 1757 and 1828 cm^{-1}, two bands: wide - at 1225 cm^{-1} and narrow - at 1086 cm^{-1}, characteristic for ether bonds and also a low-intensity band of unsaturation at 1633 cm^{-1} are clearly observed.

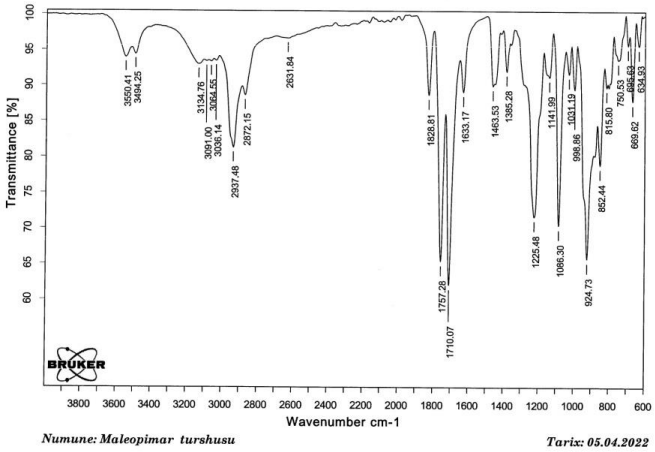


Figure 1. IR spectrum of maleopimaric acid

In the PMR spectrum of the compound (Figure 2), the chemical shifts of methyl protons in cycles are appeared by a group of the signals in the field of 2.0-2.6 ppm., the methyl protons in tertiary carbon atoms by a singlet signal at 0.5-0.7 ppm. Methylene protons of cycles are appeared by a signal in the field of 1.3-1.6 ppm. The chemical shifts of the proton of the carboxyl group and proton of the internal double bond of MPA are appeared by singlet signals with maxima at 12.1 and 5.5 ppm, respectively. The chemical shifts of the methine protons in the anhydride and aromatic cycles are appeared by a group of the signals in the field of 2.2-2.7 and 3.2-3.6 ppm, respectively.

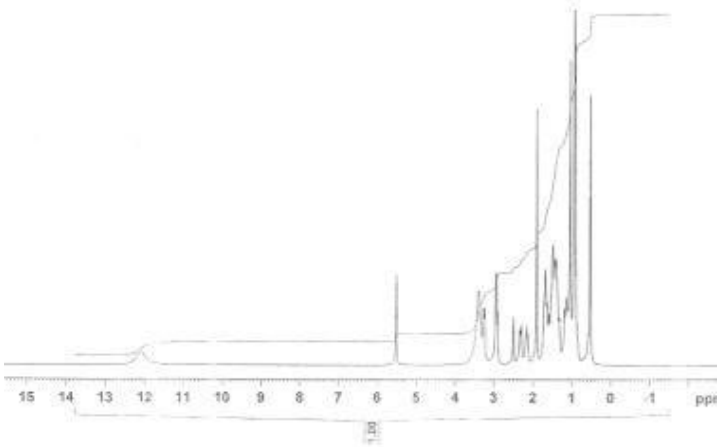
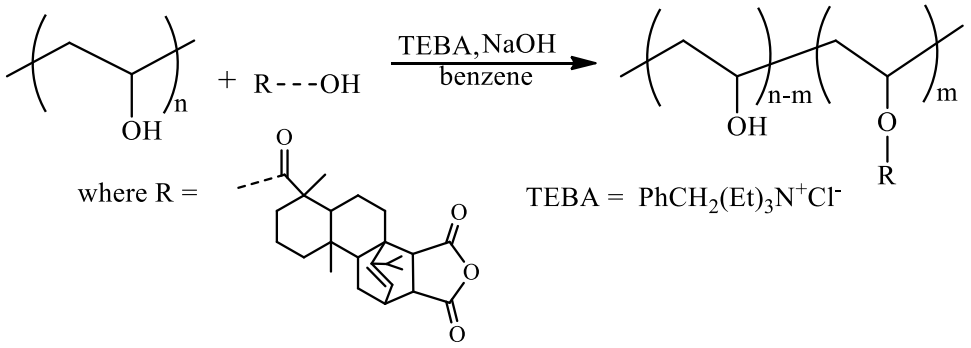


Figure 2. PMR spectrum of maleopimaric acid

By the interaction of MPA with thionyl chloride in the presence of methylene chloride, the chloroanhydride of MPA was obtained. The purity and structure of the obtained product has been confirmed by data of elemental analysis (on chlorine content) and IR spectroscopy.

The PVA acylation reaction with chloroanhydride of MPA was carried out using a method known as the Schotten-Baumann method under conditions of interphase catalysis according to the scheme presented below:



PVA acylation by the Schotten-Baumann method provides for the reaction to be carried out under interphase conditions, which allows to achieve the highest yield of the purposeful product. The solvents immiscible with water have been selected as the organic phase. Since PVA is not dissolved in organic solvents and chloroanhydrides of the organic acids are insoluble in water, the reaction was carried out in a mixture of the solvents - in an aqueous acetone-benzene solution at 0°C. As a result, PVA with various degrees of modification, which is easily dissoluble in alcohol, was obtained.

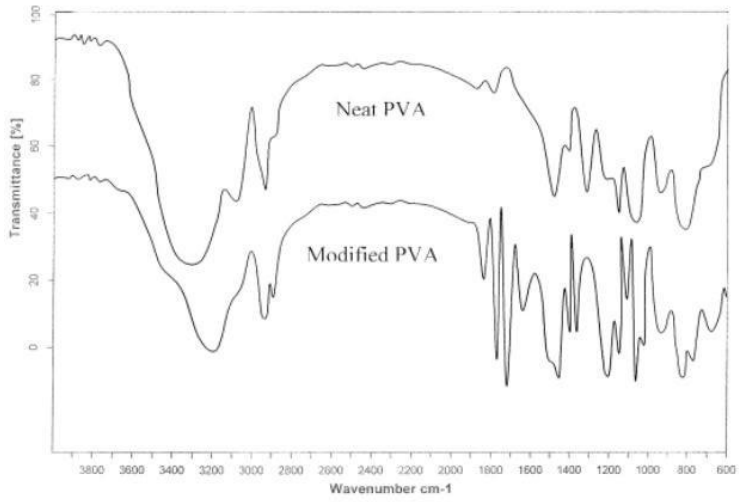


Figure 3. IR spectrum of PVA modified with chloroanhydride of maleopimaric acid

In the IR spectra of the obtained modifiers, there are absorption bands characteristic for the carbonyl group at 1720 cm^{-1} (ester fragment), the intensive absorption band at 1170 cm^{-1}, referring to vibrations of C-O-ether bond, which is characteristic for mixed ethers. In the text of the article, IR spectra of maleopimaric acid and PVA modified with maleopimaric acid are presented. The text is also supplemented with a description of the spectrum of the end product:

In the IR spectra of the obtained modifiers, the strong absorption band characteristic for the carbonyl group at 1720 cm^{-1} (ester fragment) and two bands characteristic for the carbonyl group of the anhydride fragment at 1835 and 1766 cm^{-1} are appeared. The intense absorption band at 1086 cm^{-1}, referred to vibrations of -C–O–C- (ether bond), is characteristic for mixed ethers. In addition, the band at 1644 cm^{-1}, attributed to the double bond, located in the cyclic fragment of maleopimaric acid and the bands at 1145, 1385 cm^{-1}, referring to the methyl groups of maleopimaric acid are appeared. In the spectrum one can also observe the availability of the wide bands characteristic for intramolecular hydrogen bonds in the ranges of 3100-3550 cm^{-1} and 1210-1270 cm^{-1}.

The optimal conditions of carrying out of the reaction have been revealed: the molar ratio of chloroanhydride of MPA and PVA was changed from 1:5 to 1:10 (on the basis of one element link of PVA). The concentration of PVA in water was varied from 4% to 6%. With an increase in the concentration of PVA in water above the optimal one (4-6%), the initial polymer precipitates upon contact with other reacting components (chloroanhydride, alkali, catalyst). The molar ratio of NaOH: PVA was constant in all cases and corresponded to 1.5. The molar ratio of TEBA-Cl: chloroanhydride of MPA in all experiments was 0.70.

For unambiguous proof of the availability of an ester group in the PVA macrochain, the modifier had been subjected to a hydrolysis reaction. It has been established that the IR spectrum of the hydrolyzed modifier the absorption bands characteristic for the carbonyl group at 1720 cm^{-1} and the ether bond at 1170 cm^{-1} are absent.

The antimicrobial activity of the films obtained from modified PVA was determined against both Gram-negative (*Escherichia Coli*) and Gram-positive (*Staphylococcus Aureus*) bacteria. The samples obtained at ratio MPA: PVA = 1:5 and 1:10 after incubation at 37°C, the inhibition zone was demonstrated after 24 and 72 hours.

Table 1. The width of the inhibition zone of test-microorganisms growth by samples of modified PVA after 24 and 72 h

Sample	Width of the inhibition zone, mm			
	Escherichia coli		*Staphylococcus aureus*	
	24 h	72 h	24 h	72 h
PVA	+*	+	+	+
PVA:MPA=5:1	2±0.5	3±0.5	8±0.7	11±1.2
PVA:MPA=10:1	1±0	2±0	3±0.7	5±0.8

* - The growth of microorganisms has been noted

As can be seen from the data in Table 1, the modifications showed the greatest effectiveness against Gram-positive bacteria - they caused sufficiently clear zone of *S. Aureus* growth inhibition. In relation to Gram-negative bacteria, the effect of *E. coli* growth inhibition of was insignificant. After 72 hours, the antibacterial effectiveness of the modifier films did not decrease, however, the bacteria growth inhibition in relation to *S. Aureus* was clearly observed. Apparently, this has been connected with the difference in the structure of the bacteria cell wall: Gram-negative bacteria have a more complex cell wall structure than Gram-positive ones.

4. Conclusion

The acylation reaction of PVA with chloroanhydride of MPA on the Schotten-Baumann method has been carried out. PVA acylation was carried out under previously found optimal conditions: the reaction proceeds smoothly at 0°C in an aqueous acetone-benzene solution in the presence of an interphase catalyst TEBA-Cl, the concentration of an aqueous solution of PVA is 4÷6%, the ratio of initial PVA and chloroanhydride of MPA was taken = 5:1.

It has been established that the water-soluble modifiers obtained as a result of the esterification reaction of PVA with chloroanhydride of MPA have a higher antimicrobial potential in comparison with the films of pure, unmodified PVA. The modifiers showed the highest activity in relation to *Staphylococcus aureus* and somewhat less efficiency in relation to *Escherichia coli*, which is apparently caused by the various structure of the cell wall of these bacteria. On the other hand, the water-soluble properties of the obtained modifiers facilitate the physical contact of bacteria with the polymer surface, which inhibits their reproduction on the product surface. PVA used by us is a relatively inexpensive, easily accessible and ecolocally safe polymer and maleopimaric acid is the rosin derivative having unlimited reserves in nature. Therefore, the synthesis of modifiers based on these compounds is also profitable from an economic point of view. The obtained modifiers can be used as components in the compositions for preparation of the antibacterial packaging.

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