



# SYNTHESIS AND ANTIMICROBIAL PROPERTIES OF NEW POLYMERIC MATERIALS WITH IMMOBILIZED PEROXYACID GROUPS

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## ABSTRACT

A method has been developed for the modification of polymer materials of the FIBAN brand, which allows to immobilize up to 5.5% of peroxyacid functional groups on them without significant changes in their physical and mechanical properties. It has been shown that the concentration of active groups and the stability of the synthesized materials depend strongly on the nature of the polymer carrier. Method for chemical analysis of the concentration of immobilized peroxyacid groups have been proposed, and the features of the IR spectra of modified materials have been described. It has been proved that all synthesized materials exhibit pronounced antimicrobial activity. Such materials can be used for manufacturing goods with increased resistance to microbial colonization, including medical masks, respirator filters, wound dressings, etc.

**Keywords:** FIBAN polymers, immobilization, peroxides, peroxyacid groups, antimicrobial activity.

## 1. INTRODUCTION

Different types of polymeric materials with different chemical structures and functional purposes are becoming more prominent in all fields of medicine [1-3]. They are most commonly used for making containers for storage of medical goods and drugs, medical tools, implants, dressing materials, artificial membranes, etc. Synthesis of polymers with antimicrobial properties is a separate, rapidly developing direction [4-6]. Such materials have a number of advantages: provide prolonged effect, don't have residual toxicity, are chemically stable, non-volatile, and cannot absorb through the skin [7]. As such these materials can be used to manufacture goods with increased resistance to microbial colonization (clothing, underwear, furniture in public places) or as effective local antiseptics, which is especially important considering the global tendency of increasing antibiotic resistance [8, 9]. Such polymers could also be used as active components of the individual (medical masks, respirators, special clothing) and collective (air filtration elements) protection means against infectious diseases of different etiology, the necessity of mass adoption of which was demonstrated by recent events due to COVID-19 pandemic [10, 11].

Based on the source of the biocide effect, antimicrobial polymers can be split into two major groups. The first group includes materials impregnated with antimicrobial agents of various origin. They are easy to

produce and depending on antiseptic they can provide immediate and strong effect, however, the effect is short, they are often unstable and cannot be regenerated [12, 13]. The second group includes materials that contain chemically bound biocide fragments. Some of these possess innate antimicrobial activity, which often due to positively charged groups in polymer structure or other bound biocide molecules [14-16]. Such material effectively neutralizes pathogen on direct contact, but cannot release their agent into the environment. However, the most promising are polymers with immobilized functional groups-donors of antimicrobial agents. While they are more technologically complex, they have advantages of the first two types: have a broad antimicrobial effect, disinfect environment, are stable during storage, and can be regenerated repeatedly [6, 7].

## 2. LITERATURE REVIEW

Various functional groups can be used as antimicrobial fragments for immobilization onto a carrier. Polymers with immobilized active chlorine donor groups are well known [17-20]. They exhibit high oxidative and biocide activity, and are rather stable, which lead to their wide use for water purification from chemical and biological contaminants, and as a promising material for protective clothing [21-23]. Another common approach for adding biocide properties to polymers is the immobilization of quaternary ammonium groups [14, 24,



25]. Being positively charged, they are capable of electrostatically attracting many organisms to their surface and disrupt their metabolism. The main disadvantage is the inability to release active elements into the environment. In recent years there have been active developments towards immobilization of enzymes and antibiotics on polymer surface [26-28]. Such polymers are especially effective against specific microorganisms and processes, but lack systematic antimicrobial effect, are difficult to manufacture and expensive.

It is known, that peroxide compounds are powerful antiseptics and disinfectants [29]. The most known - hydrogen peroxide - is a biogenic compound that is synthesized in the human body during phagocytosis, and despite low concentration it is a defensive response of the body against pathogens at inflammation sites [30, 31]. Even small quantities of hydrogen peroxide are sufficient to destroy the majority of disease-inducing microorganisms [32, 33]. However, peroxide solutions are quite unstable, so its functional analogs are used instead - organic peroxyacids, such as peracetic and perlactic acids [34, 35]. Industrially produced reagents based on them, consist of a mixture of free acid, hydrogen peroxide, and peroxyacid itself, in the concentration of 10-25%. However, such mixtures cannot be used as antiseptics as cause irritation of skin and mucus membranes [36]. This, combined with rapid decomposition of peroxides on porous surfaces, make simple impregnation of polymer materials unsuitable for obtaining antimicrobial materials. So, the deposition of peroxides onto the solid substrate surface to achieve a prolonged microbiocide effect is only possible through immobilization of corresponding functional groups. Not many of such materials are known [37, 38]. Immobilized peroxyacid groups exhibit their antimicrobial properties through homolytic fission oxygen-oxygen bond and progressive emission of hydroxyl radical. A significant advantage of polymers with such fragments is that upon reaction with a contaminated environment, the only product released by the carrier is oxygen. This makes them ecologically safe and attractive in terms of consumer properties. As such, immobilization of peroxyacid groups onto surface of available polymer substrates with suitable physico-mechanical and chemical properties is a promising direction for the creation of medical goods, especially in light of frequent epidemics of infectious diseases.

### 3. MATERIALS AND METHODS

#### 3.1 Characteristics of Initial Polymeric Substrates

For polymeric substrates, we have chosen series of mass-produced nonwoven and needle-puncturable materials 2-6 mm thick with a density of 280-600 g/m<sup>2</sup>, which were developed by the Institute of Physical and Organic Chemistry of the NAS of Belarus: FIBAN K-4, FIBAN AK-22, FIBAN AK-22V. FIBAN K-4 is a fibrous cationic exchanger prepared by grafting acrylic acid onto polypropylene fibers (tex 0.33, 65-75 mm long) by irradiating it with  $\gamma$ -rays (<sup>60</sup>Co, using UGU-400 gun) in air atmosphere, and soaking them in the aqueous solution of

acrylic acid [39]. Anion-exchanger FIBAN AK-22 is prepared by as a result of polymer analogues transformation of polyacrylonitrile fiber "Nitron". The fiber is aminated with an aqueous solution of diethylenetriamine or triethylenetetramine, which results in producing amides with weakly basic primary and secondary amine groups [40]. Hydrolysis of nitrile groups occurs in parallel, resulting in a small number of carboxylic groups. Polyampholite FIBAN AK-22V is prepared similarly, but with the addition of sodium carbonate to increase the content of carboxylic groups in materials [41]. All used polymers are chemically stable and possess suitable physico-mechanical characteristics, do not contain monomer admixtures and open a wide range of possibilities for further functionalization. They are currently successfully used for water treatment for removing heavy metal ions, iron, purification of vent gases from ammonia, hydrazine, alkylamines, alkali aerosols, etc. [42].

#### 3.2 Polymer Modification Method for Obtaining Immobilized Peroxiacid Groups

Modification of the aforementioned polymer substrates for immobilization of peroxygroups onto them was implemented according to the general method, which consists of soaking polymer with hydrogen peroxide in the presence of mineral acids until equilibrium is established. A sample of about 20 g of nonwoven material FIBAN K-4, FIBAN AK-22, or FIBAN AK-22V was submerged in 500 ml of distilled water, 20 ml of concentrated hydrochloric acid was added, and the sample was left for a day. Then a sample was thoroughly washed with distilled water and pressed, transferred into a dark glass vessel containing 300 ml of 50% of hydrogen peroxide and 30 ml of phosphoric acid. The material was placed into the vessel in a way so it is completely submerged. The vessel was non-hermetically covered with a lid and was left at 15-25 degrees. Aging time, the time after which no extra formation of peroxyacid groups was observed, for FIBAN K-4, was 6 days, and for FIBAN AK-22 and FIBAN AK-22V - 2 days. After aging in hydrogen peroxide, materials were pressed and washed in distilled water to remove absorbed residue of initial reagents and catalysts, dried underflow of warm air to constant mass. The sample mass increased insignificantly (to 21-21.5 g).

The schematic representation of polymer modification is shown in Figure-1 and Figure-2:

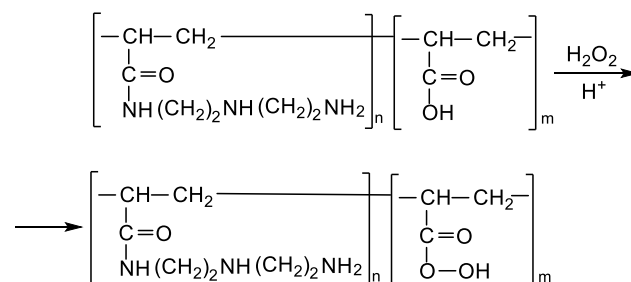


Figure-1. Modification of FIBAN AK-22 and FIBAN AK-22V.

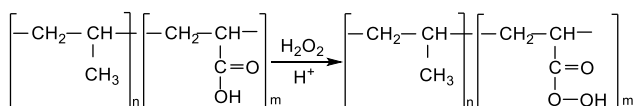


Figure-2. Modification of FIBAN K-4.

### 3.3 Determination of Presence and Content of Immobilized Peroxyacid Groups

The content of peroxygroups immobilized on a polymer substrate was evaluated using iodometric titration. For this, 0.5-1.0 g of sample, weighted with 4 digit precision, was placed in 250 ml flask with 100 ml of distilled water, then 10 ml 20% sulfuric acid, and 10 ml 10% aqueous potassium iodide were added and the flask was placed into a dark place for 30 min. Flask was then placed on a water bath and heated to 60-70°C, and released iodine was titrated with 0.1 N sodium thiosulfate. The solution was heated periodically until all released iodine diffuses into solution. At the end of process 2 ml of 0.05% of starch solution was added and titrated again. Content of peroxyacid groups (% mass) PA was calculated by the formula (1):

$$\text{PA} = \frac{0.0044 \cdot V}{m} \cdot 100 \quad (1)$$

where

0.0044 - the mass of peroxyacid, corresponding to 1 cm<sup>3</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O with the concentration of 0.1 mole/L;

V - the volume of 0.1 N sodium thiosulfate solutions used in a titration, ml;

m - the mass of analyzed sample, g.

In addition to chemical analysis, the IR-spectra of all synthesized materials were studied to investigate their chemical composition.

### 3.4 Study of Antimicrobial Activity of Synthesized Materials in Vitro

Studies were conducted using the nutrient agar medium and modified method of agar plates [43]. Tests were conducted using museum microorganism strains: *E. coli B - 906* and *S. aureus B - 904*. Suspensions of daily cell cultures were added to molten nutrient agar cooled to ~40°C, to an obtained cell concentration of 10<sup>6</sup> in 1 ml of the medium. 20 ml of medium contaminated with microorganisms were poured into Petri dishes. Before agar solidified, 10x10 mm pieces of modified polymer samples were placed into dishes so they would be fully submerged. After medium is solidified and dried up dishes were placed into a thermostat for 24 hours at 37°C. After that, the results were evaluated by measuring zones of inhibition of microorganisms growth around samples.

## 4. RESULTS AND DISCUSSION

### 4.1 Physico-Chemical Properties of Synthesized Materials

It has been found that during the treatment process, the materials did not undergo oxidative destruction and maintained their deformation-durability characteristics. The visual appearance of modified FIBAN K-4 is almost the same as that of initial material, and modified FIBAN AK-22 and FIBAN AK-22V changed color to white from their initial light yellow-green color (Figure-3):

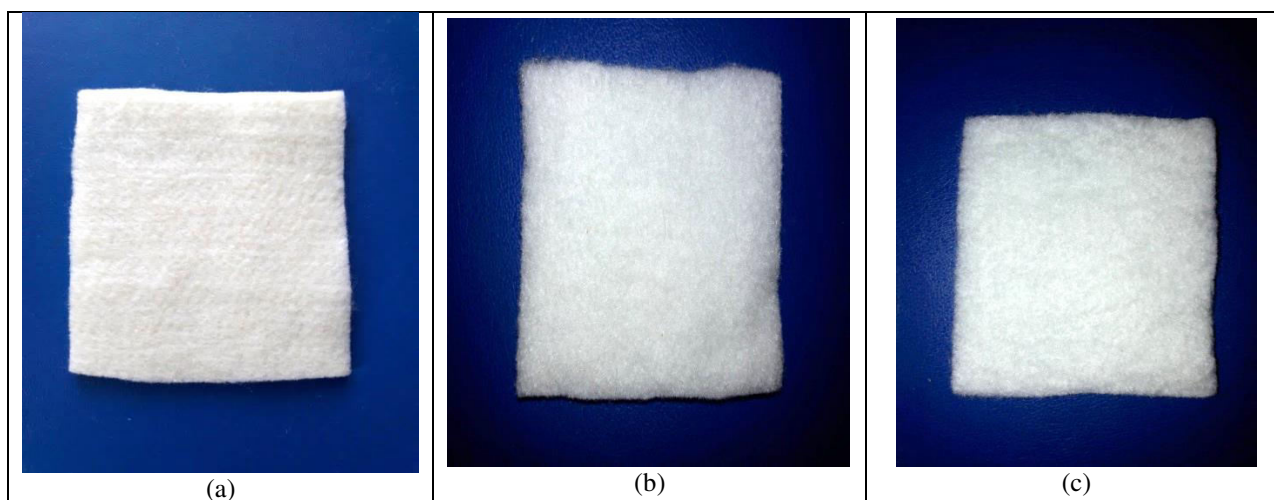


Figure-3. The visual appearance of synthesized materials: a - modified FIBAN K-4, b - modified FIBAN AK-22, c - modified FIBAN AK-22V.

Modified polymers practically have no smell, unlike our previous modified polymers with immobilized N-chlorosulfonamide groups, that have characteristics smell of "active chlorine" [19, 20]. This makes these materials more promising from the consumer point of

view, for production, for example, the components of medical masks.

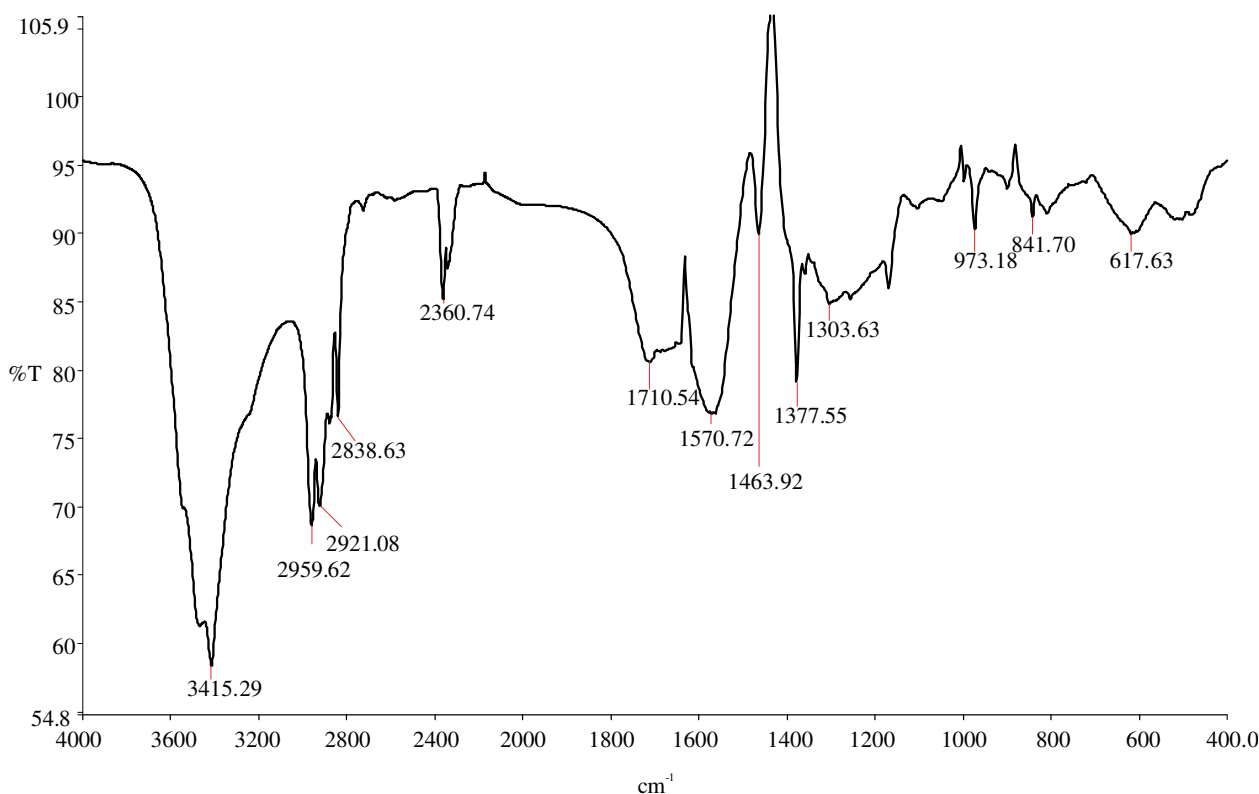
The main physico-chemical properties of synthesized polymeric materials with immobilized peroxyacid groups are listed in Table-1.

**Table-1.** Physico-chemical properties of synthesized polymeric materials.

No	Label	Modified peroxyacid forms of materials		
		Fiban K-4	Fiban AK-22	Fiban AK-22V
1	Static exchange capacity (SEC), mg-eq/g:			
	- ammine groups	-	4.5	2.1
	- COOH groups	3.0	0.3	0.8
2	Surface density, g/m <sup>2</sup>	310	430	270
3	Humidity, %	0.5	0.5	0.5
4	Water absorption, g <sub>H2O</sub> /g	0.70	0.95	0.90
5	Thickness, mm	3.0	3.0	3.0

Results of the chemical analysis revealed that the content of peroxy groups varies significantly. For modified FIBAN K-4, their content is only 1.0-1.2%. Data on the low content of peroxyacid groups in this material is

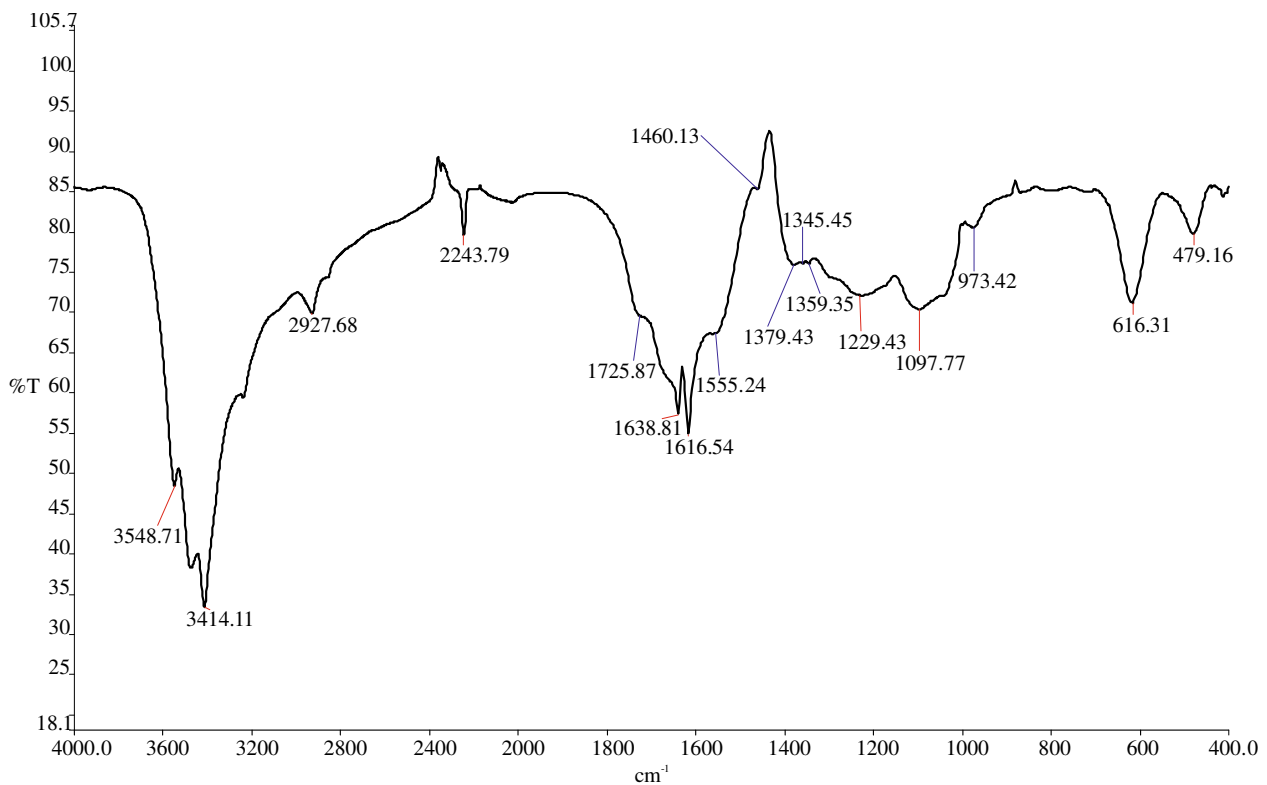
also supported by the analysis of its IR-spectrum, which showed no significant difference from the original form (Figure-4):

**Figure-4.** IR-spectrum of modified FIBAN K-4.

It has absorption bands of OH-groups of carboxylic fragment at 3515 cm<sup>-1</sup>, 3464 cm<sup>-1</sup>. There are also absorption bands CH<sub>2</sub>- and CH<sub>3</sub>- at 2960 cm<sup>-1</sup>, 2920 cm<sup>-1</sup> and 2839 cm<sup>-1</sup>. The band at 1710 cm<sup>-1</sup> is due to the oscillation of C=O group of the carboxylic fragment and absorption band at 1570 cm<sup>-1</sup> is due to valence oscillation of ionized carboxylic group. Spectrum contains absorption bands characteristic for polypropylene: 2839 cm<sup>-1</sup>, 1468 cm<sup>-1</sup>, 1378 cm<sup>-1</sup>, 1303 cm<sup>-1</sup>, 973 cm<sup>-1</sup>, 842 cm<sup>-1</sup>. At the

same time, there is no band at 890-830 cm<sup>-1</sup>, which is characteristics of the -O-O- fragment.

According to chemical analysis, the content of peroxyacid groups in FIBAN AK-22 is significantly higher and is about 3.5-3.7%. However, analysis of its IR-spectrum (Figure-5) also does not allow to identify presence of absorption bands of peroxy groups, which is like in the first case likely due to relatively low content:



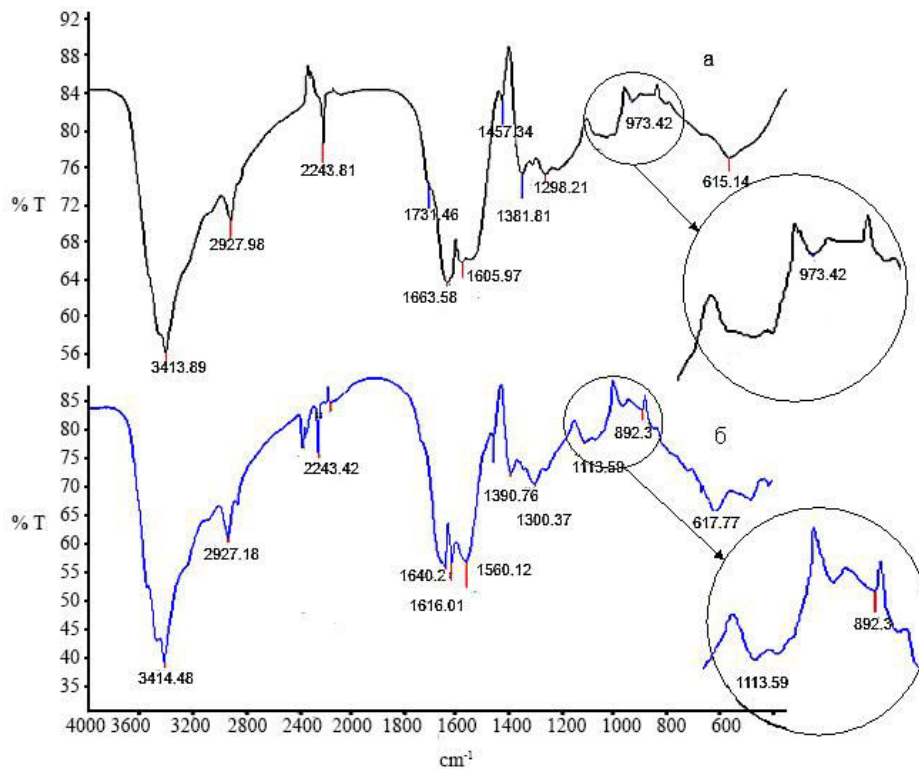
**Figure-5.** IR-spectrum of modified FIBAN AK-22.

As seen, spectrum contains bands at  $3549\text{ cm}^{-1}$ , which are characteristics of unassociated OH-groups of the carboxylic fragment. The presence of  $\text{NH}_2$ -groups is proved by absorption bands at  $3456\text{ cm}^{-1}$  and  $3414\text{ cm}^{-1}$ , and NH- groups - at  $3200\text{ cm}^{-1}$ . Presence of  $-\text{CH}_2-$  groups is proved by the absorption band at  $2928\text{ cm}^{-1}$ . The evidence of the fact that the nitrile fiber is not aminized and hydrolyzed completely is the band at  $2244\text{ cm}^{-1}$ , which is characteristics of  $\text{C} \equiv \text{N}$ -group. Presence of  $\text{C}=\text{O}$  group of the carboxylic fragment is evidenced by the band at  $1726\text{ cm}^{-1}$ , bands at  $1639\text{ cm}^{-1}$  and  $1617\text{ cm}^{-1}$  are due to valence oscillations of  $\text{C}=\text{O}$ -group of secondary amides

(amide band I), and  $1555\text{ cm}^{-1}$  – is due to deformation oscillation of NH- and C-N-bonds of secondary amides (amide band II).

The highest content of immobilized peroxyacid groups of 5.2-5.5%, was achieved for polyampholite FIBAN AK-22V. Such allows identifying their presence on the IR-spectrum with certainty. The spectrum of modified FIBAN AK-22V (Fig. 6b), in addition to bands described for FIBANAK-22, also contains an absorption band at  $892\text{ cm}^{-1}$ , which characterizes oscillation of  $-\text{O}-\text{O}-$  groups, which is absent in the spectrum of original material (Figure-6a):





**Figure-6.** IR-spectra of FIBAN AK-22V:  
*a* – initial, *b* – modified

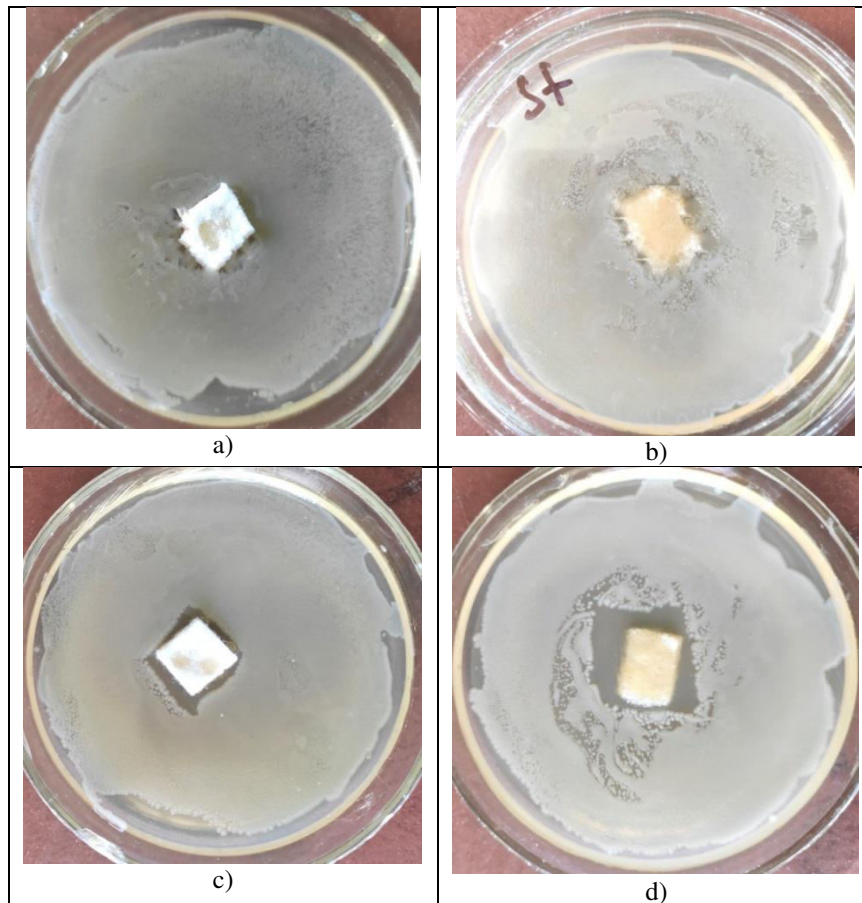
As such it should be mentioned that identification of peroxy- and hydroperoxy-groups in polymeric materials using IR-spectroscopy is complicated by their low concentration, and because of oscillation of –O–O–groups are rather symmetrical and have low intensity, while oscillation of OH-groups from hydroperoxy fragment can overlap with oscillation of OH-groups from the carboxylic fragment. Thus, the chemical identification method is more reliable for identification and quantitative analysis of immobilized peroxyacid groups.

Storage in darkness revealed that the stability of materials differs significantly, too. The highest degradation rate of peroxyacid groups has been observed for modified FIBAN K-4: after 2 months, the remaining concentration was 0.5%, and after two more months - only 0.2%, and after six months of storage, the concentration of active groups was almost zero. The stability of peroxyacid-containing materials based on FIBAN AK-22 and FIBAN AK-22V is significantly higher. After two months, the concentration of active groups decreased by 0.3%, and after six months of storage, concentration for FIBAN AK-22 was 2.5%, and for FIBAN AK-22V - 3.5%.

As can be observed, the concentration of immobilized peroxyacid groups and their stability strongly depends on the nature of the substrate. Despite initial FIBAN K-4 has the highest exchange capacity by carboxylic groups, the concentration of active groups turned out to be lowest. It is possible that the higher degradation rate of peroxide groups is due to the manufacturing process of FIBAN K-4 fibers, which uses Mohr's salt (iron(II)-ammonium sulfate), which resulted in residual amounts of iron (adsorbed or in the form of immobilized salt) catalyzing the decomposition of peroxy compounds during synthesis and analysis. Somewhat lower than in modified FIBAN AK-22V, the content of active peroxide groups in modified FIBAN AK-22, can be explained by a larger number of carboxylic groups in initial FIBAN AK-22V.

#### 4.2 Antimicrobial Activity of Synthesized Materials in Vitro

Studies revealed that modified materials exhibit pronounced antimicrobial activity towards both microorganisms, which wasn't the case of initial materials. Zones of inhibition around initial and modified FIBAN AK-22V samples are shown in Figure-7:



**Figure-7.** Zones of inhibition around the samples of FIBAN AK-22B:  
 a - initial material with *E. coli* B - 906, b - initial material with *S. aureus* B - 904,  
 c - modified material with *E. coli* B - 906, d - modified material with *S. aureus* B - 904

Zones of inhibition around the samples of other materials are listed in Table-2:

**Table-2.** Zones of inhibition around the samples of the studied materials.

Material		Zone of inhibition (mm)	
		<i>S. aureus</i> B - 904	<i>E. coli</i> B - 906
FIBAN K-4	Initial	0	0
	Modified	9 ± 1.2	6 ± 1.3
FIBAN AK -22	Initial	0	0
	Modified	14 ± 1.2	10 ± 1.4
FIBAN AK-22V	Initial	0	0
	Modified	20 ± 1.7	15 ± 1.2

No living bacteria were found on either side of the material surface. Tested samples of modified materials were analyzed for remaining peroxyacid groups, and none were found for all materials. It can be observed, that intensity of the antibacterial effect is proportional to the content of immobilized peroxyacid groups. Lower effects towards *E. coli* can be explained by its increased antioxidant activity. The obtained results are in agreement with literature data on the biocide activity of cellulose material with immobilized peroxyacid groups. In

corresponding work [38] it is noted that a relatively small zones of growth inhibition are due to stability of peroxide groups upon contact of materials with growth media itself. As such *in vitro* tests cannot reflect the real antimicrobial activity of peroxide-containing materials. So it reasonable to propose to study properties of synthesized materials *in vivo* on laboratory animals with modeled infected wounds. In theory, iron in the blood can catalyze the decomposition of peroxide groups, which would lead to increases in antimicrobial activity. Additionally, studied materials



should possess a hemostatic effect due to the presence of carboxylic groups.

## 5. CONCLUSIONS

- a) Treatment of polymeric materials FIBAN K-4, FIBAN AK-22, and FIBAN AK-22V with hydrogen peroxide according to the developed method, leads to the formation of peroxyacid groups immobilized on the polymer, without significant impact on physico-mechanical properties of initial polymers.
- b) Content of immobilized peroxyacid groups in modified materials and their stability strongly depends on the nature of the polymer substrate. Optimal results were obtained from the modification of FIBAN AK-22V.
- c) All modified materials, unlike initial substrates, exhibit antimicrobial properties, the intensity of which is proportional to the content of immobilized peroxyacids groups.
- d) A combination of physico-mechanical, chemical, and antimicrobial properties of synthesized materials and also the possibility of their further functionalization, makes these materials promising for manufacturing medical equipment such as protective mask, special clothing element, dressing materials, etc.

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