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Antimicrobial Activity of Native and Nanoencapsulated Cephalosporin Antibiotics

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***Abstract*—The analysis of the inhibiting effect of antibiotic from the cephalosporin series, nanoencapsulated gellan and xanthan gum in comparison with native antibiotics was performed. Differences in the effect of drugs depending on concentration were studied as well as positive effect of prolongation of activity nanoencapsulated form in low concentration. The expediency of using gellan and xanthan gum nanoencapsulation of cephalosporin antibiotic was shown.**

Keywords—cefalosporin antibiotics, microencapsulation, Escherihia coli, gellan and xantan gum

I. INTRODUCTION

Cefalosporin antibiotics have low toxicity, high selectivity in antimicrobial effect, slow development of resistance of pathogens of drug and are used parenterally, for intramuscular and intravenous administration. For other methods of administration, a new method of manufacturing antibiotics in microcapsules has been proposed. Microcapsules ranging in size from 100 to 500 microns are the most widely used in medicine. Nanocapsules are a special type of microcapsules, they are particles of medicinal substances with a size of 62.5-262.5 nm [1]. Micro-and nanocapsules are successfully used for targeted delivery of drugs, and to regulate their prolonged action in the body, as well as contribute to the protection of the drug from oxidation and other types of destructive effects of the environment [2]. There is evidence of using of sodium alginate, carboxymethylcellulose and other natural polymers to form micro-and nanoparticles containing antibiotics [1, 3].

The aim is to study the effect of nanostructured cephalosporin antibiotics of the 3rd generation on bacterial test cultures of *Escherichia coli* and *Staphylococcus sp*. compared to their native forms.

II. EXPERIMENTAL

The materials for the study were antibiotics of the 3rd generation cefazolin, cephalosporin, cefotaxime and ceftriaxone in the native form and in nanoshell of gellan and xanthan gum (core-shell ratio 1: 3) [4, 5].

Initial concentration was 0.25 mg/ml. It was decreased stepwise by dilution with water in the ratio 1:1 till the absence of inhibitory effect, defined by disc-diffusion method. The inhibitory effect was compared between the native and nanocapsulated forms of each drug; between freshly prepared and daily solutions for each sample in different concentrations of drugs.

To determine the minimum inhibitory concentration (MIC) liquid nutrient medium GMF-broth was used. Titers of the studied drugs in the amount of 9.9 ml were prepared in test tubes. The concentrations for the experiment were selected based on the literature data [6-9]. 0.1 ml of overnight culture of the test object was added to test tubes with prepared solutions, cultured for one day in an incubator at a temperature of 37 °C. Culture growth was assessed by direct observation and using microscopy.

III. RESULTS AND DISCUSSION

The disk-diffusion method ensures the formation of the concentration gradient of the test substance in the agarized medium. If this substance is able to have a significant impact on the physiological state of microbial cells, as diffusion occurs, the formation of a zone of complete suppression of the proliferation of the microorganism, whose cells are seeded with a Petri dish takes place. The use of nanocapsulated antibiotic particles or even larger structures does not contradict the essence of the method, since their diffusion effectively occurs in a layer of 1.5 % agarose gel. For substances with similar diffusion rates, the method allows quantitative comparative evaluation of the effectiveness of their antimicrobial action by measuring the diameters of the zones of inhibition of microbial growth.

In the experiment to determine the bactericidal activity of freshly prepared solutions of the studied antibiotics at the

initial concentration, a decrease in the activity of the nanocapsulated forms of cefotaxime and ceftriaxone was observed compared to the native form with respect to *E. coli*. No significant differences were found between native (n) and nanocapsulated gellan (g) and xanthan (k) gums forms of the same preparations in relation to *Staphylococcus sp*. (Fig. 1,2).

Presumably, this effect is caused by the formation of encapsulated conglomerates with a high concentration that causes gelation of the solution, which was found in earlier studies [10].

In the experiment with solutions of preparations in the initial concentrations, a higher antibiotic activity of nanocapsulated preparations was observed compared to native ones, which may be due to the gradual dissolution of the shells and the release of the active substance into the solution. At the beginning, the effect is shown by capsules of the smallest size, which reduces the effectiveness at the beginning of the exposure, but prolongs the effect of the drug. With a sequential decrease in the concentration of solutions, a tendency was revealed to manifest a more significant inhibitory effect of nanocapsulated forms in comparison with native forms (Fig. 3).

When diluting the initial solutions to 0.25 mg/ml, cefotaxime and ceftriaxone in their native form did not act on bacterial test cultures but preparations in gellan and xanthan gum still showed antibacterial activity. Cefazolin in all concentrations in the native form showed significantly higher activity than in the nanocapsulated form (Fig. 4).

During sequential decrease of concentrations, a tendency was revealed to more significant inhibitory effect of nanocapsulated forms in comparison with native forms (Fig. 5, 6). In the experiment to determine the MIC also obtained results indicating the gradual release of the active substance in the case of nanocapsulated forms. Microscopy of the preparations revealed a slight growth of test cultures after the first six hours of cultivation (+), which stopped as the microcapsules dissolved and the active concentration of the active substance in the solution increased. When a sample with *E. coli* was introduced into the indicator medium of the Code after a day, a positive reaction was observed. From table 1 it can be seen that the inhibitory effect on the growth of test cultures of drugs in the native form was manifested directly after the introduction of microorganisms into the medium with a solution having a minimum effective concentration (++). No cells were detected on the micropreparations, and no growth was observed when the samples were introduced into the indicator medium.

During the preparation of solutions, a faster dissolution to a homogeneous state of the drug nanocapsulated with gellan gum was observed. Preparations in xanthan gum formed a thick gel at a concentration of 0.25 mg/ml, in large concentrations they were not completely dissolved.

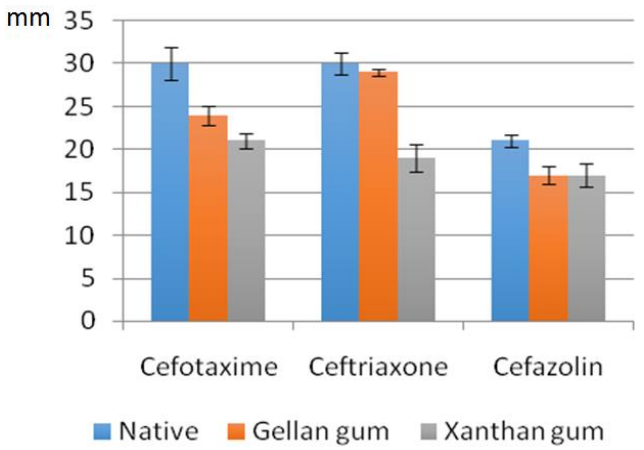

Fig. 1. The effect (suppression zone width) of freshly prepared solutions of antibiotics at concentration of 2.5 mg/ml on *E. coli*

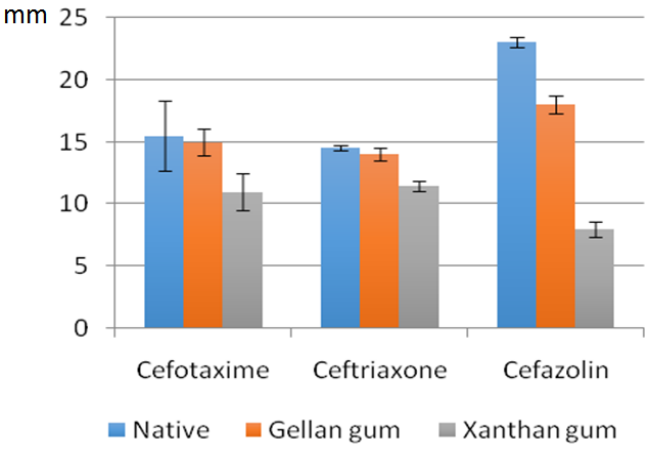

Fig. 2. The effect (suppression zone width) of freshly prepared antibiotic solutions at concentration of 2.5 mg/ml on *Staphylococcus sp.*

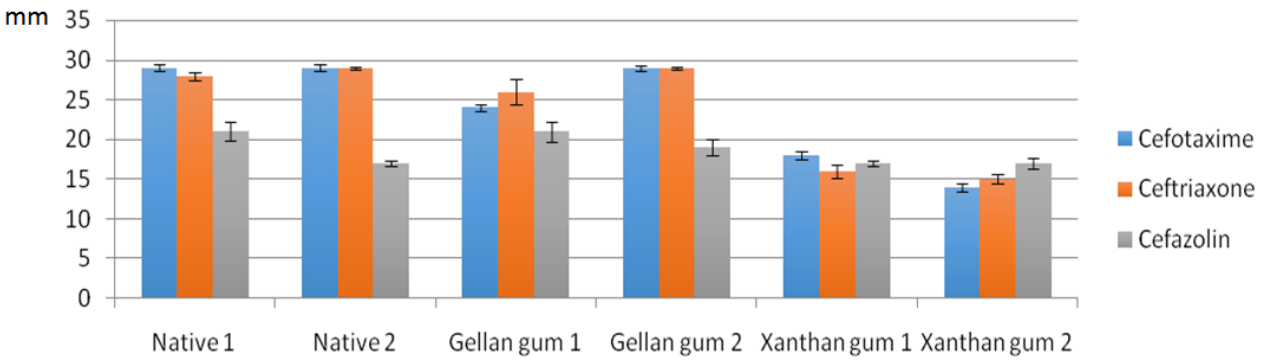

Fig. 3. Comparative analysis of the inhibitory effect (suppression zone width) of freshly prepared and daily solutions of antibiotics in concentration of 2.5 mg/ml on *E. coli*.

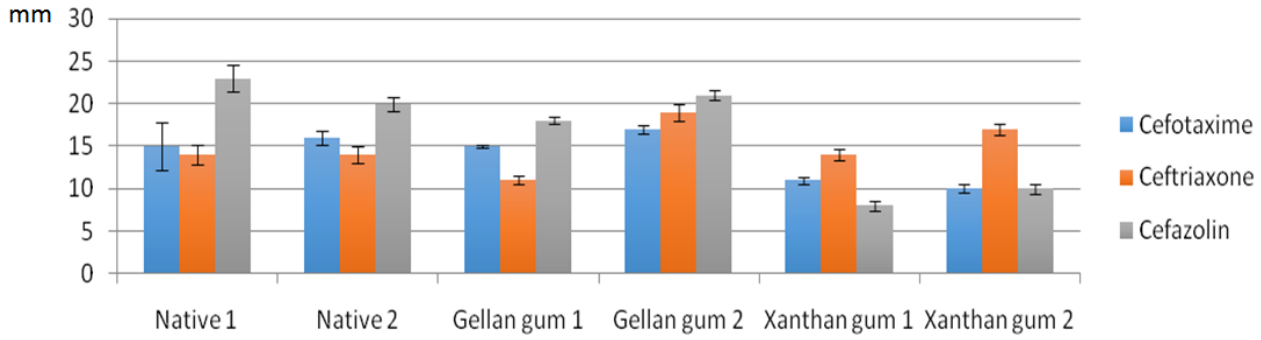

Fig. 4. Comparation of the inhibitory effect (suppression zone width) of freshly prepared and diurnal solutions of antibiotics in concentration of 2.5 mg/ml on *Staphylococcus sp.*

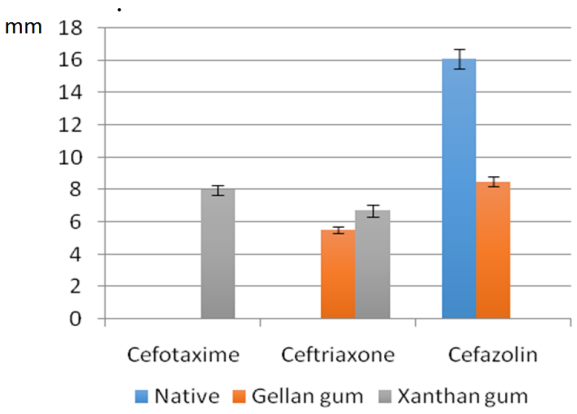

Fig. 5. The effect (suppression zone width) of freshly prepared solutions of antibiotics in concentration of 0.25 mg/ml on *Staphylococcus sp.*

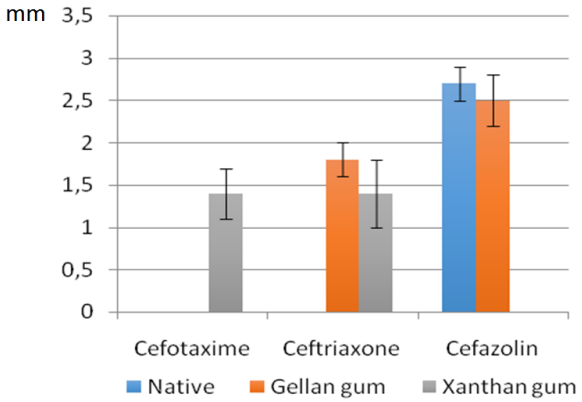

Fig. 6. The effect (suppression zone width) of freshly prepared solutions of antibiotics in concentration of 0.25 mg/ml on *E.coli.*

IV. Conclusion

The preservation of the activity of nanoencapsulated preparations in comparison with the native ones when using diurnal solutions may indicate the influence of properties that affect the interaction with the core and the different rate of release of the active substance from the capsules. In the literature, there are also data on the presence of compounds between drugs and composite polymer shells [11]. Various manifestations of the antibacterial activity of various drugs in the same shell are also superimposed on the core-shell. The obtained experimental data show the expediency of using nanoencapsulation in concentrations approaching to the threshold concentration.

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TABLE I. Results of Determination of The Minimum Inhibitory Concentration of Native and Nanocapsulated Antibiotics

Antibiotic	Test culture	*E. coli*					*Staphylococcus sp.*				
	Concentration, µg/ ml	1	2	4	8	16	4	8	16	32	64
Cefotaxime	Native	-	-	-	++	++	-	-	-	++	++
	Gellan gum	+	+	+	++	++	-	-	-	+	+
	Xanthan gum	-	-	-	+	++	-	+	+	+	++
Ceftriaxone	Native	-	-	-	++	++	-	-	+	++	++
	Gellan gum	-	-	+	++	++	-	-	+	++	++
	Xanthan gum	-	-	+	+	+	-	+	+	++	++
Cefazolin	Native	++	++	++	++	++	++	++	++	++	++
	Gellan gum	-	-	-	+	+	-	-	+	++	++
	Xanthan gum	-	-	+	+	+	-	-	-	-	+

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