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Determination Of Optimal Conditions For Obtaining Conjugate On Based Haptens And Macromolecular Matrics

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Abstract. *In this scientific work we developed the optimal conditions on the basis of the antibiotic cefipime and macromolecular matrix. Here, as the macromolecular matrix is albumin BSA (bovine albumin serum). The scientific workdisplayed properties produce specific antibodies in the animal body in order to determine the relationship of the macromolecular matrix of the antibiotic, and the relationship of the antibiotic to the macromolecular matrix*

Key words. hapten, conjugate, macromolecular matrix, antibody, antigen, cefipime,ELISA

Introduction. Medical reforms carried out under the leadership of our president in the field of public health, serve as an important factor in ensuring a healthy and long life of our people. The new edition of the Law of the Republic of Uzbekistan “On medicines and pharmaceutical activity”[1], which entered into force on January 5, pays special attention to raising the reforms in the sector to a new level. As a result of these reforms more than 100 of pharmaceutical enterprises have been carrying out activity in our country. About 30 enterprises have introduced the international system of quality management. Thanks to the year-over-year increase of the volume of foreign investments directed to the provision of quality drugs, in order to further develop the pharmaceutical industry a wide range of measures are being implemented.

According to statistics, a third of the country's domestic market is supplied by the medicines manufactured by the local pharmaceutical industry. The local companies produce more than 1700 medical drugs related to 90 pharmaceutical groups.

The gradual development of the pharmaceutical industry, circulation of medical drugs, and many innovations emerging in the process of their use in medicine require an improvement of the legislation in this sector. As a result, it is very important to control the quality of produced medical products.

Today, it is no secret about the sale of counterfeit or illegal medicines in the country and in the world scale. Therefore, the provision of the country’s population with high quality medicines is one of the significant problems today. The Article 14 Act on the procedure for the import and export of medicines of the Law of the Republic of Uzbekistan "On medicines and pharmaceutical activity" states that

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importing into Uzbekistan of poor-quality and counterfeit medicines, as well as illegal analogues of medicines registered in the Republic is prohibited.

Therefore, it is very important to work out a new modern high-speed and high accuracy test systems in quality control of medicines. The followings can be shown as examples of modern high speed and high precision analysis methods: High performance liquid chromatography, mass spectroscopy, ion exchange chromatography gel chromatography and a number of other methods of analysis. However, physical and physicochemical test systems complexity, expensive prices, requirement of separate qualified specialist create a number of inconveniences. In this respect, it should be noted that, among the modern biotechnological analytical directions the immuno enzyme analysis methods are widely used in various fields, especially in practice. They considerably speed up the processes of diagnostics and biochemical analysis. In comparison with the above indicated physicochemical analysis methods, immuno enzyme analysis is characterized by its simplicity, cost effectiveness, high accuracy, and capability of analyzing 100 samples at one time. This is considered very important in controlling the quality of medicines.

Today, one of the main directions of biotechnology science, the immuno biotechnology and its research work are focused on creating new fast methods. In the past years, one of the most widely used sensitive and accurate methods is surely immuno enzyme analysis. In terms of sensitivity the immuno enzyme analysis is considered as a medium-high method, and identification of antigens, antibody components is realized on the basis of ferment molecules. This method is widely used in medicine, agriculture, food industry, but now we also aim to use it in wide scales in improving the qualitative and quantitative analysis of medicines in practice. Immuno enzyme analysis method is based on mutual relations and effects of antigens and antibodies. Therefore, the information about formation, structure and functions of these two components is given in details in our chapter called literature survey.

The antigen is a stranger substance which right after penetration into an organism comes into interaction with a specific antigen against itself, and it is considered the immunogen active material forming new proteins. As various antigens bacteria, viruses, polysaccharides, proteins, cells and tissues can play definite role. Generally, antigens are called the substances leading to formation of immunoglobulins antibodies which are part of glycoproteids class in an organism. If, stranger substance gets into an organism, and antibodies are not formed, then such substances on serological aspect are not considered as the active substances. Nucleic acids, oils, some pharmaceuticals, for example antibiotics, vitamins, hormones and others can be examples of low substances of properties of antigenicity. Antigenicity or immunogenicity low property substances are usually considered small molecular compounds, which are called haptenes. Small molecular compounds combining with other macromolecules, they can synthesize

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substances with big molecular weight – conjugates. And this leads to forming of an antibody in organism.

Results. On the basis of an antibiotic ceftazimide we developed optimum conditions for synthesis of a macromolecular matrix with a conjugate. At the same time as a macromolecular matrix the albumin of the blood serum of a bull (BSB) was used. In the work we showed the ratio of a macromolecular matrix to an antibiotic, and also the ratio of atntibiotic to a matrix through their features of formation of a specific antibody in an animal organism.

It is known that low-molecular substances, that is hormones, various medicines and including antibiotics are called haptents, and they are not capable to form antibodies in an organism. Because they do not carry out an inductor task for synthesis of an antibody in an organism. However, for today in the analysis of quality of the pharmaceuticals imported to our Republic an important role is played by the modern test systems. Because, these test systems are characterized by low cost, speed and high-sensitivity. Such modern test systems can include the immunoenzymatic analysis. In this case it should be noted that for the qualitative and quantitative analysis of the above-stated low-molecular substances, including antibiotics on the basis of IEA as a component a specific antibody will be required[2].

It is a very difficult task to carry a specific antibody to low-molecular substances [3,4]. Therefore in this work, for macromolecular matrixes of an antibiotic ceftazidime, as a macromolecular matrix we used albumin of blood serum of a bull and connected to it an antibiotic of cefipime in the covalent way. At the same time we took an antibiotic of cefipime in different volumes, and did not change the volume of a macromolecular matrix. Then we administered 4 synthesized miscellaneous type of conjugates into an animal organism and studied the process of formation of an antibody. The received results are shown in the first table.

1-table

Results of the research of conjugate's feature in formation of an antibody by means of immunodiffusion on the basis of an antibiotic cefipime and a macromolecular matrix.

	Haptene, Ceftazimide, mole	**Macromolecular matrix, мg**	**GA, %**	**Volume, %**	**Titre Haptene: antibody**
C1	6-8	50	0,1	3	1:16
C2	10-12	50	0,1	3	1:16
C3	16-18	50	0,1	3	1:64

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C4	20-22	50	0,1	3	1:64

From the table it is visible that when the volume of antibiotic cefipime as a part of a conjugate (C1) synthesized in the first option made 6-8 moles and the volume of a molecular matrix (BSA) made 50 mg, it was revealed that the titre of an antibody of the animal formed in the organism was equal to 1:8. And in the second option of synthesized conjugate (C2) we can see that when the volume of a cefipime made 10-12 moles, the titre of the received antibody was equal to 1:16. In C3, C4 options of the conjugate it was revealed that due to increase in volume of cefipime the antibody titre formed against it, also raises. But it was noted that when the volume of cefipime reached 20-22 moles, the titre of the antibody did not change. From this it is possible to make the conclusion that when receiving a specific antibody for an antibiotic cefipime, its volume makes 16-18 moles and when the volume of the matrix makes 50 mg, the antibody formed in an animal organism on the basis of this conjugate will have high serological activity. It was revealed that increase in volume of cefipime (20-22 mol) does not influence positively serological activity of the antibody of the animal which is formed in an organism.

At the next stage of the experiment we set a goal – without changing the volume of cefipime (18-20 mol), to change the volume of the macromolecular matrix and to carry out synthesis of the conjugate. And in this situation, the conjugate was synthesized in four different options, the synthesized conjugates were injected into an organism of the animal and thus, the feature of conjugates to form a specific antibody was studied. The received results are shown in 2- table.

2-table

Relation of conjugate synthesized on the basis of antibiotic ceftazidime to the volume of macromolecular matrix.

	Haptene, Ceftazimide, мg	**Macromolecular matrix, мg**	**GA, %**	**Volume, %**	**Titre Haptene: antibody**
C1	16-18	20	0,1	3	1:16
C2	16-18	30	0,1	3	1:16
C3	16-18	40	0,1	3	1:32
C4	16-18	50	0,1	3	1:64

From table 2 it is visible that on the basis of various volumes of a macromolecular matrix the synthesized conjugates (C1, C2, C3, C4) form an antibody of various serological activity in an animal organism. It appears, increase

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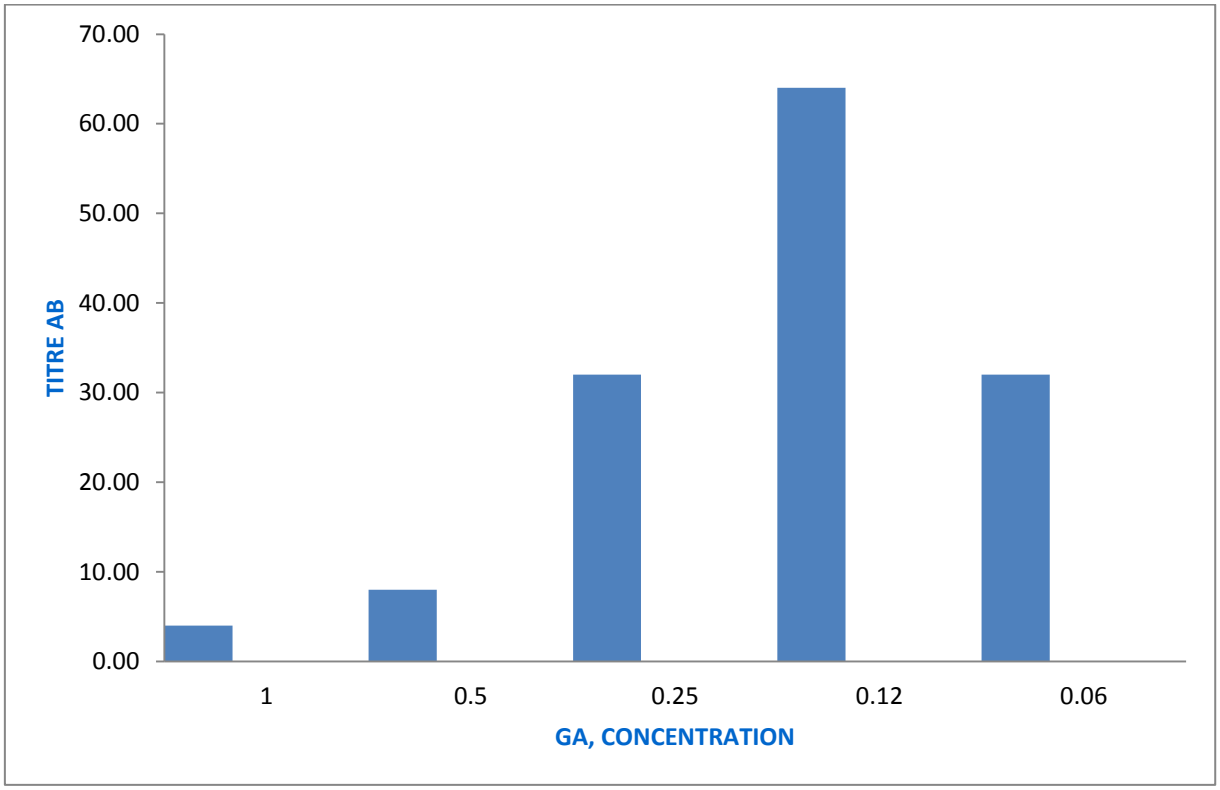
in volume of a macromolecular matrix causes formation of high titre antibodies in an animal organism. According to the received results, the volume of a cefipime that is a conjugate in C4 option made 18-20 mol, and the volume of a macromolecular matrix made 50 mg, and also it was revealed that it forms a high titre antibody in an animal organism. At the same time, as shown in table 2, the titre of an antibody of formed C4 a conjugate makes 1:64. Thus, in this work we investigated optimum conditions for synthesis of a conjugate to receive an antibody for an antibiotic ceftazidime.

At the following stage of our work, we studied influence on synthesis of a conjugate by concentration of glutaraldehyde (a sewing component) which plays an important role in the mutual covalent connection of an antibiotic cefipime and a macromolecular matrix. For this purpose without changing volume of antibiotic cefipime and a macromolecular matrix, choosing a different concentration of glutaraldehyde, we synthesized several different types of conjugates. The received results are shown in picture1.



1-picture. Influence glutaraldehydeon on synthesis of a conjugate on the basis of an antibiotic cefipime and a macromolecular matrix

AB- antibody

GA- glutaraldehydeon

From picture 1 it is visible that without changing the volume of a macromolecular matrix and antibiotic cefipime at synthesis of a conjugate, and having changed concentration of a sewing component (1-0,06%) we synthesized

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conjugates. We administered conjugates into an animal organism by means of immunization and observed process of formation of the antibody. From the picture it is visible that decrease in concentration of a sewing component positively influences process of formation of the antibody in an animal organism. At the same time, conjugates synthesized by 0,12% sewing component form high-titre antibodies. From this it is possible to make the conclusion that at synthesis of a conjugate high concentration of sewing components serves as an evidence that various conjugates in an organism of the animals which do not have inductor properties also can be synthesized.

From the results received on the basis of the above-stated experiments it is visible that the antibodies formed in an organism of a rabbit on the basis of the synthesized conjugate in various volumes do not form an antibody with rather high titre. Therefore, for receiving antibodies with the high content of titres as an object we used a mouse organism. As it is specified in special literature, the glycoproteids synthesized in an organism of a mouse differ on immunologic activity of antibodies synthesized in organisms of other animals. Therefore we used mice organism in our experiment. Results are shown in table 3.

3-table

On the basis of an antibiotic cefipime and a macromolecular matrix inductor features of the synthesized conjugate in a mouse organism

	Haptene Cestazimide, mole	**Macromolecular matrix, мg**	**GA, %**	**Volume, %**	**Titre Haptene: antibody**
C1	6-8	50	0,1	3	1:64
C2	10-12	50	0,1	3	1:128
C3	16-18	50	0,1	3	1:256
C4	20-22	50	0,1	3	1:256

From table 3 it is visible that at synthesis of a conjugate the antibiotic cefipime has a positive impact on formation of an antibody. It is possible to see that when the volume of a haptene reaches 16-18 moles, the formed volume of the antibody titre equals 1:256 (table 3 C3 option). We revealed that increase in volume of cefipime on 20-22 moles, does not change an antibody titre.

Thus, it was revealed that the organism of a mouse is very convenient when receiving an antibody for an antibiotic cefipime and demonstration of high inductor features of the synthesized conjugates in a mouse organism.

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Conclusion:Optimum conditions of synthesis of a conjugate to an antibiotic cefipime related to cephalosporin group were studied.

Concusion. When in work the volume of antibiotic cefipime equals to 16-18 mol and at the same time the volume of a matrix to 50 mg, it was shown that the titre of a specific antibody to antibioticcefipime was high. Also the optimum concentration of glutaraldehyde in synthesis of a conjugate is defined.

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