УДК 57.083.24

SELECTION OF OPTIMAL CONDITIONS FOR THE PURIFICATION OF rAAV BY CATION EXCHANGE CHROMATOGRAPHY

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Introduction. Currently, gene therapy is a rapidly advancing field in biomedicine, focusing on the treatment of diseases by modifying the genetic system of the patient's somatic cells to rectify a faulty gene. [1]. Recombinant adeno-associated viral vectors (rAAV) are promising tools for delivering genetic material to specific cells. Therefore, scientists are currently working on developing universal and scalable technologies for producing rAAV-based drugs. One of the crucial steps in rAAV production involves separating and purifying viral particles. The goal of this process is to separate target viral vectors from impurities [2]. At the same time, the rAAV purification process is divided into two stages: the capture stage and the polishing stage. Cation exchange chromatography is a promising method for purification at the capture stage. This method has the advantage of not depending on the specific properties of the rAAV capsid, as it relies on separating molecules based on their charge. [3]. Therefore, cation exchange chromatography can be a versatile method for purifying rAAV.

Main part. The studies were carried out using a clarified cell lysate containing rAAV. At the first stage of the research, conditions for preparing a sample for load to a chromatography column were selected, as the efficiency of rAAV binding to the functional groups on the resin depends on these conditions. In this case, the binding efficiency depends on the degree of protonation of the viral capsids of the rAAV as well as the presence of competing protein impurities in the applied sample.

The required level of capsid protonation is achieved by selecting the pH and conductivity values of the sample. During the research, it was found that the optimal conditions for loading the rAAV to a cation exchange resin are pH 4.0 and conductivity X mS/cm. These conditions ensure an adsorption rate of 99% for rAAV.

To reduce the degree of protein impurities in the loading sample, which could limit the binding of rAAV to the resin, tangential flow filtration (TFF) was performed, selecting the optimal diafiltration factor. As a result of the research, it was determined that during TFF, the yield of rAAV relative to the initial clarified lysate was approximately 85%. The maximum level of the reduction in the content of impurity proteins was observed during the diafiltration process, which was carried out 32 times. The total reduction in protein content in the loading sample compared to the clarified lysate was 14 times.

During the final stage of the study, the total yield of the purification process was evaluated. Selected conditions were used to prepare a loading sample. The average yield of the process using cation exchange chromatography compared to clarified lysate was approximately 60%.

Conclusion. Our experiments led us to determine the optimal conditions for preparing the loading sample for rAAV purification using cation exchange chromatography. It has been discovered that the chosen conditions are optimal for the purification of rAAV, as the average yield of the purification process for this serotype exceeds 60%.

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