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Short Communication

Impact of Size Exclusion Chromatography in Drug Discovery and Development

Erwin Gelfand*

Department of Chemistry, University of Assiut, El Fateh, Assiut Governorate, Egypt

ABOUT THE STUDY

Size Exclusion Chromatography (SEC), also known as gel filtration chromatography, is a powerful technique for the separation and purification of biological macromolecules, such as proteins, nucleic acids, and polysaccharides, based on their size and shape. The principle of SEC is based on the differential migration of molecules through a porous gel matrix, where the larger molecules elute first, followed by the smaller ones [1]. SEC is a popular method for the isolation and analysis of biomolecules due to its high resolution, reproducibility, and compatibility with a variety of biological samples. The principle of SEC is based on the exclusion of molecules from the pores of the gel matrix based on their size and shape. The gel matrix is typically composed of cross-linked polymers, such as agarose or polyacrylamide that form a network of pores of different sizes [2]. The sample is applied to the top of the gel column, and the molecules are separated as they migrate through the gel matrix under the influence of the mobile phase, which is usually a buffer solution. The larger molecules are excluded from the pores and move through the interstitial spaces, while the smaller molecules enter the pores and are retained for longer periods. Thus, the elution order of the molecules is inverse to their molecular weight, with the larger molecules eluting first and the smaller ones eluting later [3].

One of the main advantages of SEC is its high resolution and reproducibility. The technique can separate molecules of similar molecular weight but different size and shape, which is difficult to achieve with other chromatographic techniques, such as ion exchange chromatography or affinity chromatography. Additionally, SEC can separate mixtures of biomolecules without altering their conformation or activity, making it a useful tool for protein purification and analysis. Moreover, SEC is compatible with a variety of biological samples, including crude cell extracts, culture media, and body fluids, which makes it a popular choice in the field of biotechnology [4].

However, SEC also has some limitations that need to be considered. The main limitation is the requirement for a calibration curve to estimate the molecular weight of the molecules eluted from the column. The calibration curve is generated using standard proteins of known molecular weight, and the elution volumes of the standard proteins are plotted against their logarithmic molecular weight to obtain a calibration curve.

However, the accuracy of the molecular weight estimation depends on the quality and purity of the standards used and the conditions of the chromatography, such as temperature and flow rate. Another limitation of SEC is the choice of the gel matrix and the mobile phase. The gel matrix should be chosen based on the size range of the molecules to be separated and the stability of the biomolecules under the chromatographic conditions. Additionally, the mobile phase should be chosen based on the solubility and stability of the biomolecules and the compatibility with the gel matrix. The choice of the buffer system, pH, and ionic strength can affect the resolution and reproducibility of the chromatography [5].

CONCLUSION

In conclusion, SEC is a powerful technique for the separation and purification of biological macromolecules based on their size and shape. The technique offers high resolution, reproducibility, and compatibility with a variety of biological samples, making it a popular choice in the field of biotechnology. However, the accuracy of the molecular weight estimation depends on the quality and purity of the standards used and the conditions of the chromatography, and the choice of the gel matrix and mobile phase can affect the resolution and reproducibility of the chromatography. With its wide range of applications and potential for further development, SEC will continue to play a vital role in biomolecule analysis and research in the future.

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Correspondence to: Erwin Gelfand, Department of Chemistry, University of Assiut, El Fateh, Assiut Governorate, Egypt, E-mail: Gelfand36@gmail.com

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