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Commentary

Special Techniques of Chromatography-Hydrophobic Interaction and Hydrodynamic Chromatography

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DESCRIPTION

Hydrophobic Interaction Chromatography (HIC) is a purification and analysis technique that separates an analyte based on the hydrophobic interaction between the analyte, such as a protein, and the chromatography matrix. It can provide a non-denaturing orthogonal approach to reverse phase separation while preserving its original structure and possibly protein activity. In hydrophobic interaction chromatography, matrix materials are easily replaced with hydrophobic groups. These groups can be in the range of methyl, ethyl, propyl, butyl, octyl, or phenyl groups. At high salinity, the non-polar side chains "change" on the surface of proteins with hydrophobic groups. That is, both types of groups are excluded by protic solvents. The sample is applied to the column in a highly polar buffer. This binds the hydrophobic spots of the analyzed object to the stationary phase. The eluent is usually an aqueous buffer that lowers the salt concentration, increases the surfactant concentration, and changes the pH.

Separability can be improved by adding organic solvents or other less polar components. Hydrophobic interaction chromatography (HIC) is generally useful when the sample is sensitive to changes in pH and the harsh solvents commonly used in other types of chromatography, but not to high salt concentrations. The amount of salt in the buffer usually varies. In 2012, Muller and Franzreb described the effect of temperature on HIC using bovine serum albumin (BSA), which contains four hydrophobic resins. In this study, the temperature was changed to affect the binding affinity of BSA to the matrix. It was concluded that a cycle temperature of 50-10 degrees was not enough to effectively clean all BSA from the matrix, but it was very effective when the column was used only a few times. There may be. By changing the temperature, the laboratory can reduce the cost of purchasing salt and save money.

Hydrodynamic chromatography (HDC) derives from the observed phenomenon that large droplets move faster than small droplets. For columns, this happens because the overall size is so large that the center of gravity of the large droplets cannot approach the sides of the column as it does for the small droplets. Large droplets first elute from the center of the column, small droplets attach to the sides of the column and finally elute. This form of chromatography, when used in combination with light scattering detectors, viscometers, and refract meters, helps separate analytes by molecular weight, size, shape, and structure. The two main types of HDC are open tubes and packed columns. Open Tube shortens the separation time of small particles, while HDC with a packed column can increase the resolution and is suitable for particles with an average molecular weight of more than 105 Daltons. HDC differs from other types of chromatography in that the separation is only done in the intrusive volume, that is, the volume around and between the particles in the packed column.

CONCLUSION

The elution order of HDC is the same as Size Exclusion Chromatography (SEC), but the two methods still differ in many ways. In a study comparing the two separations, both Isenberg, Brewer, Cote, and Striegel used methods to characterize polysaccharides, with more HDC associated with Multi Angle Light Scattering (MALS) compared to offline MALS. We conclude that we have achieved an accurate molar mass distribution. This is significantly shorter time than SEC. This is mainly because the SEC is a more destructive method because the pores of the column tend to decompose the object to be analyzed during separation and affect the mass distribution. However, the main drawback of HDC is the poor resolution of peaks on the analyzed object. This makes the SEC a more viable option when used with chemicals that are not easy to decompose and where rapid elution is not important. HDC plays a particularly important role in the field of microfluidics.

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