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Commentary

Performance of a Flow Reactor and its Use in Micro Column High-performance Liquid Chromatography (HPLC)

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DESCRIPTION

The post-column derivatization reaction can be used to improve the sensitivity or selectivity of the detector. Having a suitable post-column reactor for your micro chromatography system can enhance the benefits of capillary chromatography for microanalysis. However, post-column derivatization is challenge due to the small peak volume associated with the capillary column.

Chemical reactions can be combined with chromatography to improve detection sensitivity or selectivity. For example, the lower limit of detection can be realized by converting the sample component to a product that is more sensitive to the detector. You can improve the selectivity of the detector and avoid interference from substances that cannot be sufficiently separated by chromatographic separation. Chemical reactions can be performed in pre-column, post-column, online or offline. Pre-column formation is easy, but it can change the chromatographic properties of the compound. In addition, because the reaction takes place in a sample matrix, it is more likely that pre-column derivatization artifacts will be generated. Therefore, post-column derivatization is often preferred. Post-column chemical reactions can be performed offline by collecting the column eluate in fractions, adding derivatization agent, and then measuring the reaction products. Or you can run it online which requires a flow reactor.

When using a flow reactor to produce reaction products, the following criteria must be met: Reagents and solvents must not interfere with detection; The reaction kinetics must be fairly fast in order to avoid large capacity reactors causing large peak broadening; The reaction yield must be high so that a low detection limit can be achieved; The reaction product must be stable on the experimental time scale.

Open tubular, packed bed, and segmented flow reactors have all been used for post-column derivatization. A number of papers have addressed the design and performance of these types of chemical reactors for chromatography. The most common is the open tube reactor, which consists of a narrow tube through which the effluent–reagent mixture flows. The biggest concern when implementing a post-column reactor of any kind is the additional peak broadening that occurs within the reactor itself. In a properly designed post-column reactor, the additional peak broadening should be minimal to reduce the inevitable loss in resolution. This is especially difficult when designing post-column reactors for use in micro-column HPLC columns, as these analyzes are associated with small peak volumes.

The two liquid streams must be mixed before the reaction occurs between the column eluent and the derivatization reagent. Fortunately, research surrounding liquid mixing in micro channels has expanded in recent years due to efforts focused on microfluidic systems. As a result, a major emphasis has been placed on developing hybrid designs that are compatible with lithography-based micro-fabrication technology. In these microfluidic channels, the flow is characterized by a low Reynolds number (Re) value. Microfluidic devices that require mixing usually rely on diffusion mixing when the fluid flow is combined into a single channel, as the viscous coupling to the wall dominates the flow at low Re. Much of the driving force of micro flow devices is to achieve high speed separation in very small quantities. The drive of faster separation in small quantities is inconsistent with the need to allow molecular diffusion to mix reagents and analytes in large channels (cross-sectional dimensions ~ 100 μm) after separation. Therefore, clever micro-fabrication channel designs have been used to enhance diffusion mixing.

The manufactured device has to be tested using a post-column derivatization technique based on Biuret chemistry developed in laboratory. In particular, the peptide binds to copper (II) *via* the amide backbone, allowing reversible electrochemical detection at moderate potentials. It is possible to form a copper (II)-peptide complex in front of the column, but it alter the retention time of the peptide. For this reason, post-column derivatization is most commonly performed.

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Post-column flow reactors used in capillary chromatography systems differ from micro fabricated mixers commonly used in rapid capillary electrophoresis in that the wide peaks require longer mixing lengths. However, the volume of the reactor should be small compared to the peak volume in order to minimize the contribution of band spreading. There are no published reports of flow reactors for post-column derivatization when capillary columns are used for chromatographic separation.