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Journal of Chromatography & Separation Techniques

Perspective

Advancing Protein Enrichment with Novel Polyhedral Silsesquioxane Capillary Monolith in μ-IMAC

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DESCRIPTION

The isolation and purification of histidine-tagged proteins are crucial steps in biochemical research and biotechnological applications. Immobilized Metal Affinity Chromatography (IMAC) has emerged as a powerful technique for selective protein enrichment, particularly utilizing nickel ions (Ni(II)) as chelating agents. In this study, a novel approach is presented, involving the synthesis of a polyhedral silsesquioxane-based capillary monolith functionalized with Ni(II) for efficient isolation of histidine-tagged proteins *via* micro-IMAC (μ-IMAC). This overview outlines the synthesis protocol, functionalization process, and potential applications of this innovative capillary monolith in protein purification.

The synthesis of the capillary monolith initiates with the integration of Polyhedral Silsesquioxane (POSS) into the polymer matrix through thiol-methacrylate polymerization. This innovative technique provides numerous benefits, notably heightened mechanical stability, increased porosity, and expanded surface area, all of which collectively enhance chromatographic performance. The resulting monolith exhibits improved efficiency and resolution in analyte separation. Moreover, the synthesis protocol is characterized by its ease and efficiency, ensuring reproducibility and scalability of the process. These attributes render the methodology highly suitable for routine laboratory applications. Its straightforward implementation and robust performance make it a valuable tool for researchers seeking reliable and efficient chromatographic solutions in various analytical and biochemical settings, promising advancements in both research and industrial applications.

Following the synthesis of the capillary monolith, the next step involves the functionalization with Ni(II) ions to impart selective binding properties for histidine-tagged proteins. The direct incorporation of the chelating agent into the monolith matrix ensures uniform distribution and maximum accessibility of the immobilized Ni(II) ions, enhancing the efficiency of protein enrichment. This innovative functionalization strategy eliminates the need for post-synthesis modification steps, simplifying the overall process and reducing potential sample loss.

The synthesized and functionalized capillary monolith serves as a highly selective sorbent for the enrichment of histidine-tagged proteins *via* μ-IMAC. The specific interaction between the immobilized Ni(II) ions and the histidine residues allows for efficient capture and purification of the target proteins from complex biological samples. The μ-scale format offers several advantages, including reduced sample and reagent consumption, shorter analysis time, and increased sensitivity, making it ideal for high-throughput applications in proteomics research.

The proposed protocol for synthesizing and functionalizing the polyhedral silsesquioxane-based capillary monolith offers numerous advantages over conventional methods. The direct incorporation of the chelating agent into the monolith matrix streamlines the process, eliminating the need for additional modification steps and reducing sample loss. Additionally, the enhanced mechanical stability and surface area of the monolith result in improved chromatographic performance and increased protein binding capacity. These attributes make the synthesized monolith an attractive candidate for various applications in protein purification, proteomics research, and biotechnology.

In summary, the development of a novel polyhedral silsesquioxane-based capillary monolith functionalized with Ni(II) ions represents a significant advancement in the field of protein purification *via* μ-IMAC. The integration of thiol-methacrylate polymerization and direct chelating agent incorporation offers a facile and efficient approach for synthesizing selective sorbents for histidine-tagged protein enrichment. With its enhanced chromatographic performance, reproducibility, and scalability, the synthesized monolith holds great promise for applications in proteomics research, biotechnology, and biomedical sciences, paving the way for improved protein purification methodologies and enhanced understanding of biological systems.

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Received: 27-Feb-2024, Manuscript No. JCGST-24-30577; **Editor assigned:** 01-Mar-2024, PreQC No. JCGST-24-30577 (PQ); **Reviewed:** 15-Mar-2024, QC No. JCGST-24-30577; **Revised:** 22-Mar-2024, Manuscript No. JCGST-24-30577 (R); **Published:** 29-Mar-2024, DOI:10.35248/2161-0940.24.15.564

Citation: Gelfand E (2024) Advancing Protein Enrichment with Novel Polyhedral Silsesquioxane Capillary Monolith in μ-IMAC. J Chromatogr Sep Tech. 15:564.