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Analysis of Proteins and Peptides by Native and SDS Capillary Agarose Gel Electrophoresis Online Coupled to Electrospray Ionization Mass Spectrometry

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Summary

A novel coaxial sheath flow reactor interface (CSFRI) is introduced for CE-ESI-MS coupling, especially beneficial in CGE of protein and peptides, both in native- and SDS-gel electrophoresis separation modes. The major benefit of using the CSFRI approach is the continuous closed circuit-based transport of the separated analytes from the coaxial setting via the flow reactor tube to the ESI source, robustly stabilizing the electrospray process [1]. This arrangement also offers the option to conduct post-column reactions in the flow reactor section, e.g., to capture non-MS-friendly background electrolyte components, such as sodium dodecyl sulfate in SDS-CGE. In addition, this novel interface design allows safe and efficient decoupling of the electric circuit from the mass spectrometer, i.e., no current flow from the CE into the MS and vice versa, enabling stable electrospray formation independent of the capillary electrophoresis part of the system. Most importantly, the CSFRI connection does not require any microfabrication and specially modified (i.e., etched, sharpened, etc.) capillaries; a conventional blunt edge, rugged fused silica capillary with 30-50 µm i.d. and 365 µm o.d. can be simply and safely attached and detached to and from any commercial ESI sources originally developed and optimized for the actual mass spectrometer used with no modification requirements. In SDS capillary agarose gel electrophoresis mode, addition of γ-cyclodextrin to the sheath liquid efficiently removed the SDS content of the sample and the background electrolyte in the flow reactor section by inclusion complexation, while maintaining good separation efficiency and decreasing ion suppression. Optimization of the agarose based sieving matrix will be presented in detail [2] along with examples of the analysis of peptides and proteins in SDS-CGE-MS mode using the coaxial sheath flow reactor interface.

References

[1] D. Sarkozy, A. Guttman, Analysis of Peptides and Proteins by Native and SDS Capillary Gel Electrophoresis Coupled to Electrospray Ionization Mass Spectrometry via a Closed-Circuit Coaxial Sheath Flow Reactor Interface, Anal. Chem. 95 (2023) 7082–7086.

[2] D. Sarkozy, A. Guttman, Capillary Sodium Dodecyl Sulfate Agarose Gel Electrophoresis of Proteins, Gels 8 (2022) 67.