Technical Note

Permanent methylcellulose (MC) coated cartridges achieves iCIEF free from polymers as dynamic coating for straight forward characterization of protein drugs

Demerits of MC as dynamically coating additive in iCIEF

- Polymers such as methylcellulose (MC) usually serve as an additive of dynamical coatings in the sample solution to improve the peak shape and resolution during the iCIEF separation, especially for complex proteins. However, it tends to result in tedious operation and capillary blockage.
- MC is not compatible with mass spectrometry (MS) and easily produces the contamination of MS ion source when carrying out iCIEF-MS direct coupling.

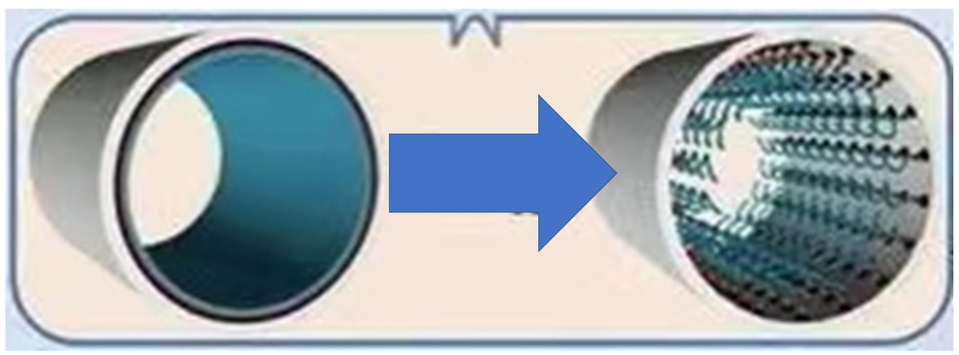

Figure 1. Diagram of permanent MC coated capillary

Merits of MC-free iCIEF on permanent MC coated capillary cartridge

- Recently, the MC-free iCIEF method was developed employing an MC-coated capillary cartridge, to avoid the addition of MC in the whole process of analysis. The established iCIEF method employing protein drugs demonstrated high repeatability, coating stability, separation efficiency, and excellent pI differentiation. In addition, the iCIEF separation in the MC coated capillary can be compared with that in the routine coated capillary such as fluorocarbon (FC), to ensure consistency in drug discovery.
- The MC coated capillary cartridge was applied to iCIEF-MS for characterizing protein charged variants with reliable identification of MS after iCIEF separation, which can greatly simply the operation steps and prevent the contamination of MS ESI resulted from using routinely coated capillary that usually needs the pre-rinse with MC solution before sample running. This is a breakthrough in iCIEF-MS to greatly improve the compatibility with MS.

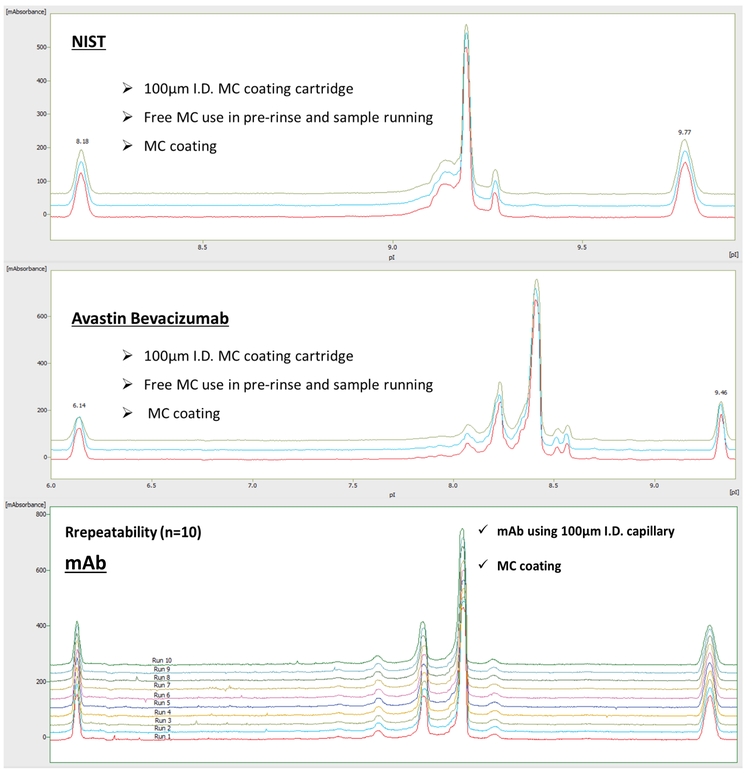

Figure 2. Excellent resolution and repeatability of iCIEF separation of mAbs on the MC cartridge.

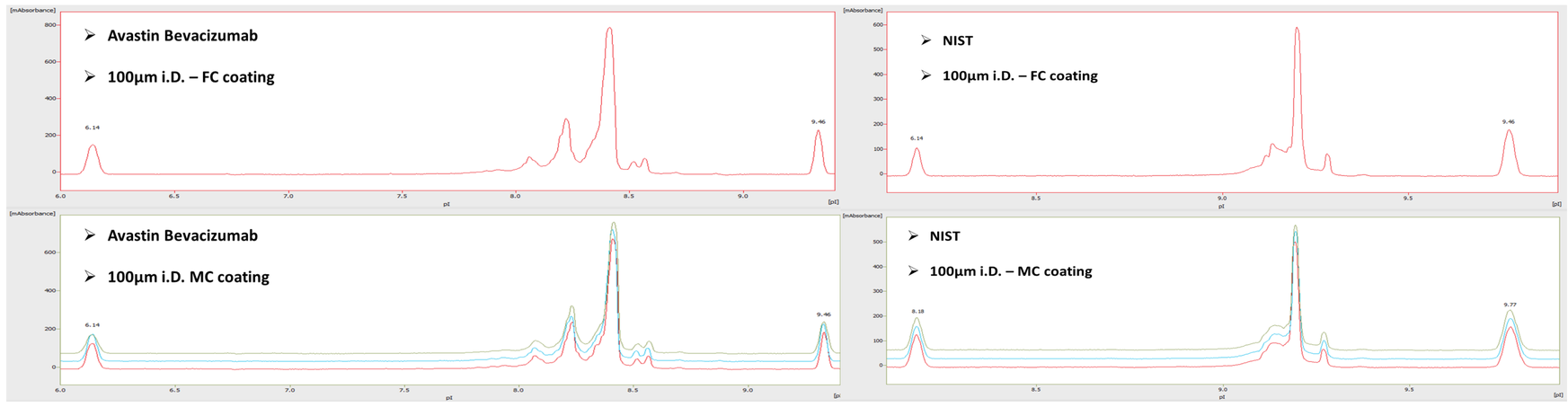

Figure 3. Comparable iCIEF on the FC by and MC cartridges.

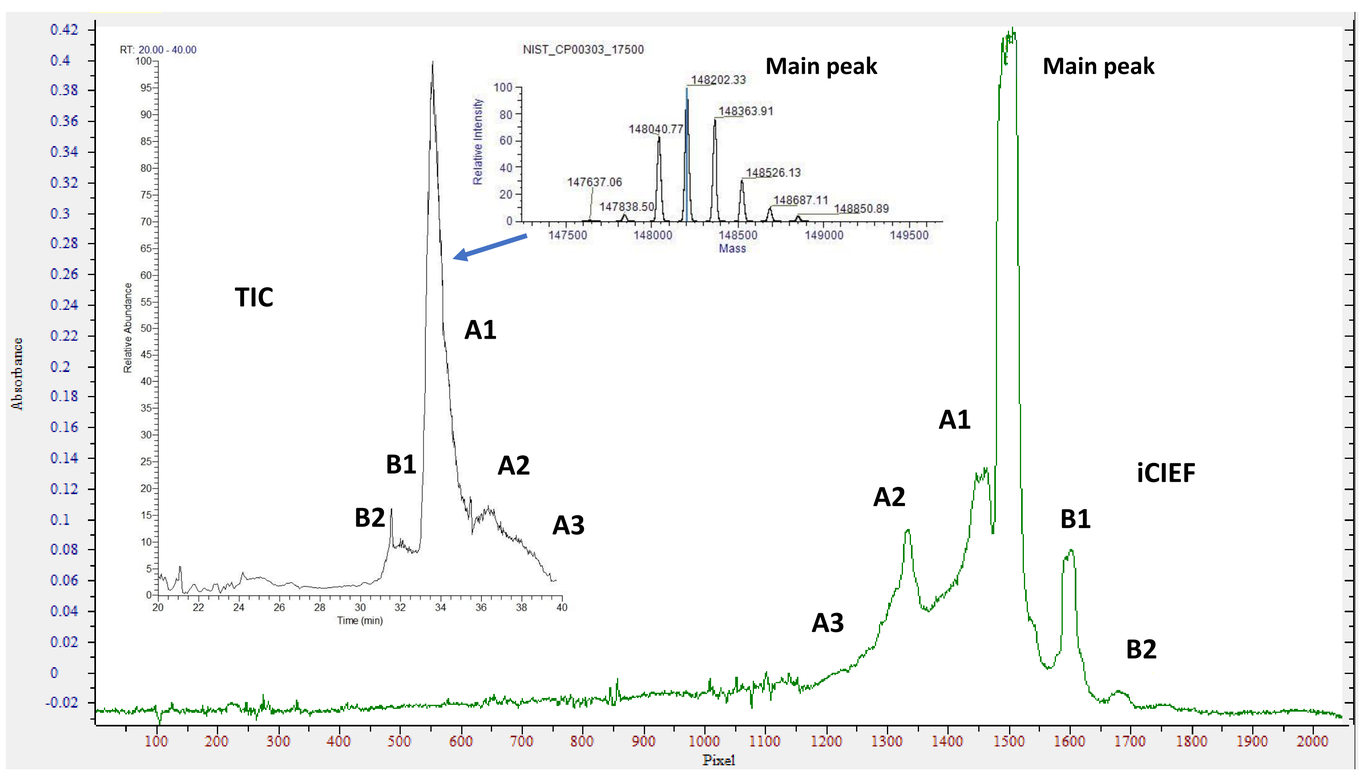

Figure 4. NISTmAb iCIEF-MS using MC coated cartridge (MC-free during analysis).

Highlights:

1. Permanent MC coating, with the same performance as traditional FC plus MC dynamic coating.
2. Hydrophilic, omit the use of MC wash and eliminate the MC in the master solution.
3. Superior stability. Mass spec friendly.
4. Analytical iCIEF to preparative iCIEF to iCIEF-MS method seamlessly transfer thanks to the same coating.

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