# Protein separation in Capillary Electrophoresis: the big move into the chromatographic world

Laurent Leclercq[1], Charly Renard[1], Michel Martin[2], Hervé Cottet[1,*]

[1] *IBMM, University of Montpellier, CNRS, ENSCM, Montpellier, France*
[2] *PMMH, CNRS, ESPCI Paris – PSL, Sorbonne Université, Université de Paris, France*

**Gold statement**
- How to quantify protein adsorption in CE ?
- What is the contribution of adsorption phenomenon to the peak broadening ?
- How the optimize CE separation in the presence of residual adsorption ?

**Introduction**
When the separation efficiency in CE is only controlled by axial diffusion, the theoretical plate number *N* is typically in the order of ~ $1\times10^6$ plates. However, experimentally, the observed plate numbers are much lower (~ $10^3$-$10^5$ plates). The origin of the main limiting factor which controls the separation efficiency of proteins in CE is still under debate, but the protein adsorption onto the capillary surface is known to strongly decrease the separation efficiency. This is therefore a need to quantify protein adsorption in CE.

**Body**
In this communication, we report a simple method allowing to quantify the adsorption of proteins onto the capillary surface based on the determination of the retention factor *k* using the theory of band broadening in open-tubular electrochromatography (CEC). This experimental approach is based on the determination of the protein peak broadening obtained at different separation voltages (i.e. different linear velocities). Very small residual adsorptions were determined on performant multi-polyelectrolytes capillary coatings (with typical *k* values ranging between 0.02 and 0.05) [1]. Despite the very low residual adsorption, the impact on the separation efficiency can be very limiting in practise. Another aspect of this study is that the presence of adsorption makes the CE move into the chromatographic world with the existence of an optimal velocity to optimize the separation efficiency [1]. These theoretical aspects will be discussed together with practical considerations to limit the impact of protein adsorption on the separation performances.

**Conclusion**
The quantification of residual protein adsorption onto capillary wall in CE is a key step if we want to control, improve and minimize it. This is especially true if we want to compare different coating having different electroosmotic mobility (i.e. with sometimes very different migration times). The objective of a million plates for protein separation, reachable in a repeatable manner, should be an accessible target in the future.

**References**
[1] L. Leclercq, C. Renard, M. Martin, H. Cottet. *Anal. Chem.* **92** (2020) 10743-10750.