

Proteins and their properties: always exciting, and always a challenge

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The characterization of therapeutic proteins by electrophoresis and chromatography is an ongoing success story. More and more proteins are considered as pharmaceuticals, with very different properties. Therefore, the existing experience for protein separations needs to be systematically structured and mined to achieve optimal separations for all protein classes, including enzymes, intrinsically disordered proteins, structural proteins and viruses, often in complex mixtures. Using a number of generic methods, most of the proteins can be well characterized, employing the best-suited capillary coatings and rinsing procedures.

In particular, we present a 2-dimensional separation using strong anion exchange high performance liquid chromatography (SAX-HPLC, using an Agilent PL-SAX 1000 Å 8 µm 50 x 5.7 mm column, and a Tris buffer pH 8.5, 20 mM, combined with an NaCl gradient) and microchip CE-SDS (Labchip GX II touch HT, with HT Protein Express 200 assay), offering comparable performance to traditional two-dimensional gel electrophoresis (2-DE). Today 2-DE is still a key technique to validate LC-MS results. We present this microchip-based approach which only requires approximately one hour per separation, compared to several days for traditional 2-DE. This concept is discussed in the light of three successful applications.

Collagen is a very important and highly abundant protein but hard to analyse. As a structural protein, its nature is to be insoluble, but as analyte it needs to be investigated in a liquid environment. In order to maintain its biological function, we milled and suspended collagen in a phosphate buffer pH 7.4, 25 mM using a dual centrifuge (ZentriMix 380R) to obtain particles with a size below 5 µm. Using these small particles, electrophoretic investigations became possible including affinity CE (ACE) for binding studies. In addition, a CE-based collagen quantitation assay was developed employing a polybrene- dextran sulfate- polybrene triple layer as semipermanent capillary coating, and validated using the Sirius red assay as reference.

The data quality of reported binding parameters, such as dissociation constants, is far from being satisfactory. The mean error between two reported pK_i- values was found to be 0.44 [1]. The overall reason for this low quality is multifactorial. Some of the reasons, for example the diversity of biomaterials, are naturally unalterable. Fortunately, others can be optimized. We investigated the influence of the maximum response range, the design space and the analytical design on the precision and trueness of binding parameters. The precision can be maximized by increasing the maximum response range and choosing an optimal design. The highest influence has the design space itself, e.g. the apparent hill coefficient has a strong influence on the precision of the other binding parameters. In favorable cases the measurement uncertainty of specific binding parameters can be reduced by factors higher than 10. All of these design considerations can be chiefly transferred to most of the common ligand binding assays.

[1] C. Kramer, T. Kalliokoski, P. Gedeck, A. Vulpetti, The experimental uncertainty of heterogeneous public k_i data, *Journal of Medicinal Chemistry* 55 (2012) 5165–5173.