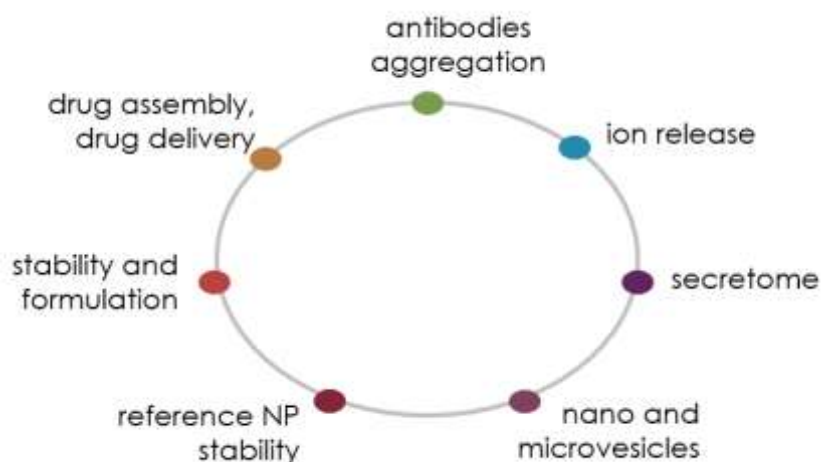# *A look into Flow Field Flow Fractionation-multidetection: native separation, purification and characterization of nanostructured, biological samples for clinical and pharmaceutical applications*

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Flow Field-Flow Fractionation (F4) is a flow-assisted separation technique ideally suited to separate in liquid mobile phase nanostructures of any origin, with a gentle separation mechanism and broad range of operating conditions. The operational flexibility and the ability to preserve the native state of the investigated species, united to the chance of up/downscaling, is appealing both for early-stage development of materials and semipreparative purposes. Vaccines, nano-vectors for drug- or nucleic acid- delivery, antibodies, vesicles, are just a few examples of newly emerging materials of overwhelming importance that benefited F4 analysis. Indeed, R&D, certification, validation, industrialization, and large-scale production of nano-bio pharma products require strict QC procedures based on methodologies specifically tailored to handle such analytes in the most native and representative conditions. F4 can be on-line hyphenated to various concentration and size detectors, facilitating the analysis of the multifunctionality of nanomaterials, their stability, aggregation state, and response to formulation; in this regard, the use of F4 is recommended by the FDA for the characterization of antibody formulations. Despite this potential, the practical information obtained by F4 and its possible applications are still rather unfamiliar to the scientific community, among which pharmaceutical scientists. After a brief introduction on the technique theory, features and configurations, most recent contributions for the analysis and characterization of macromolecules and nano particles of clinical/bio/pharmaceutical interest will be revised.