Asymmetric field-flow fractionation as a potential analysis tool for characterising lipid nanoparticles – method development and verification

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With the continued growth in the range of applications for lipid nanoparticles (LNPs), there is a corresponding need for suitable characterisation techniques to support research, production quality, production reproducibility and quality control. The measurand of interest strongly dictates what techniques can be used for characterisation, with common measurands including particle size distribution, encapsulation efficiency, particle number concentration, zeta-potential and particle morphology. One of the more popular LNP applications is RNA LNPs, driven by a slew of vaccines (including those from Onpattro, Moderna and Pfizer developed during the COVID-19 pandemic). For these applications, encapsulation efficiency, RNA copy number concentration and RNA integrity are also of great interest.

Here we discuss the development of a measurement protocol for asymmetric flow-field flow fractionation (AF4) that is suitable for LNPs using polystyrene (PSL) particles of similar diameter as the control. We explore how different instrument settings affect the separation profile of LNPs, as well as for multi-modal dispersions of model PSL particles. Figure 1 illustrates the difference in elution time for four different settings of flow parameters (P1 to P4). To fully evaluate the method, we apply the protocol to monomodal and multimodal samples and investigate the light scattering in combination with ultraviolet-visible spectroscopy and refractive index detection to assess the eluting particles and determine the encapsulation efficiency of real LNP formulations.

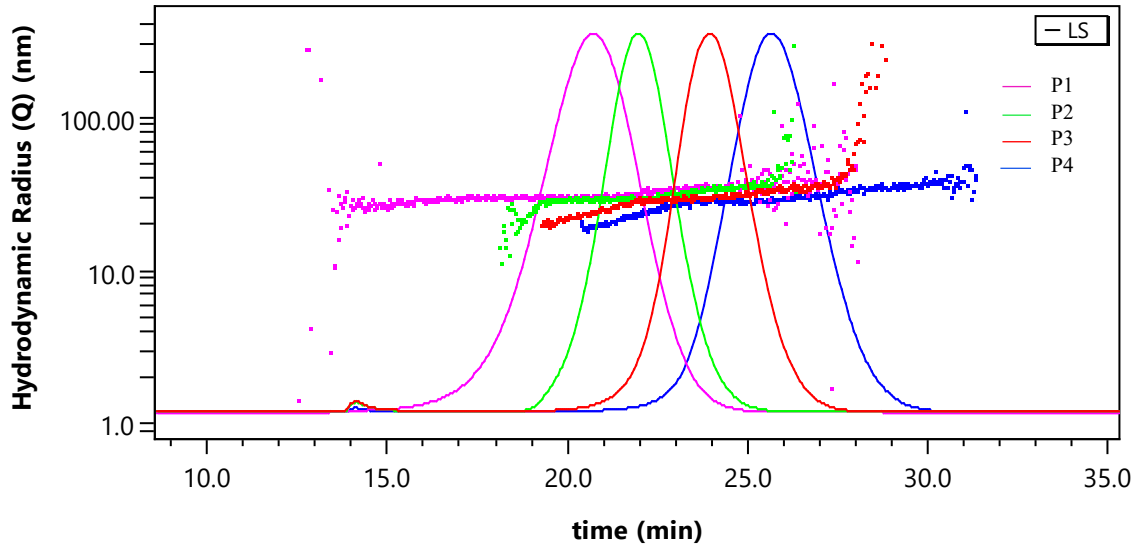

Figure 1: AF4 fractograms resulting from different methods with varying focus flow and cross-flow (P1 to P4) applied to nominally 60 nm polystyrene particles. The graph shows the normalised peaks from the light scattering signal for the eluting particles, and the corresponding hydrodynamic radius for each sample versus elution time.