NovaSheet

Technology: Flow FFF - UV/Vis
Application: PlasmidDNA and DNA Complex

Analysis Conditions / System Set-up

Sample:	PlasmidDNA and DNA Complexes
Separation System:	Postnova Flow FFF System
Detector 1:	UV/Vis Detector

Researchers have tried to use cationic lipid particles to deliver DNA plasmids to target cells. Unfortunately, the heterogeneity of these lipid–DNA complexes appears to interfere with their transfection efficiency. Thus, the researchers used Flow FFF to isolate and subsequently characterize the complexes. The researchers found that the FFF migration of the plasmid DNA depended on the nature of the molecules with which it interacted. DNA complexed with cationic cobalt was 22% smaller (migrated farther) than free DNA (Figure 1). This is due to the compaction of the DNA molecules as the negative charges of the phosphodiester backbones are neutralized by the cation. DNA mixed with the cationic lipid lipofectamine was 21% larger than naked DNA, indicating the formation of lipid–DNA complexes. The researchers also noted that complexes incubated for long periods appear to aggregate with time, which might help to explain the concomitant decrease in transfection efficiency.

Self-assembled cationic lipid-DNA complexes have shown an ability to facilitate the delivery of heterologous DNA across outer cell membranes and nuclear membranes (transfection) for gene therapy applications. While the size of the complex and the surface charge are important factors that determine transfection efficiency, lipid-DNA complex preparations are heterogeneous with respect to particle size and charge. This heterogeneity contributes to the low transfection efficiency and instability of cationic lipid-DNA vectors. Efforts to define structure-activity relations and stable vector populations have been hampered by the lack of analytical techniques that can separate this type of particle and analyze both the physical characteristics and biological activity of the resulting fractions. In this study, we investigated the feasibility of Flow FFF to separate cationic lipid-DNA complexes prepared at various lipid-DNA ratios. The compatibility of the lipid-DNA particles with several combinations of FFF carrier liquids and channel membranes was assessed. In addition, changes in elution profiles (or size distributions) were monitored as a function of time using on-line ultraviolet, multiangle light scattering, and refractive index detectors. Multiangle light scattering detected the formation of particle aggregates during storage, which were not observed with the other detectors. In comparison to population-averaged techniques, such as photon correlation spectroscopy, Flow FFF allows a detailed examination of subtle changes in the physical properties of nonviral vectors and provides a basis for the definition of structure-activity relations for this novel class of pharmaceutical agents.

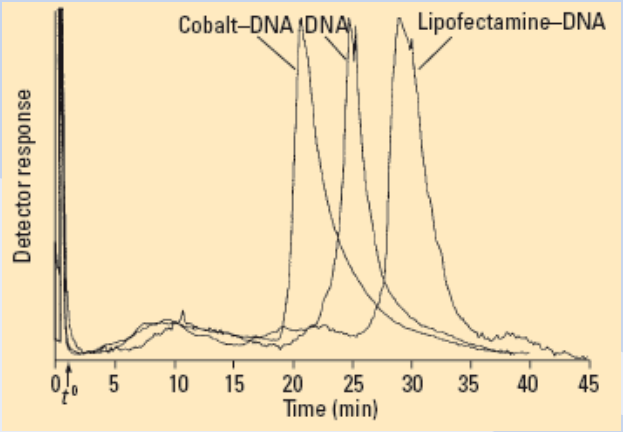

Fig 1. Characterize Plasmid DNA and DNA complexes. (With permission from Lee, H.; et al.)

Anal Chem. 2001 Feb 15;73(4):837-43.
Analysis of self-assembled cationic lipid-DNA gene carrier complexes using flow field-flow fractionation and light scattering.
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