Determination of Properties of Individual Liposomes by Capillary Electrophoresis with Postcolumn Laser-Induced Fluorescence Detection

Ciara F. Duffy, Sheik Gafoor, Dawn P. Richards, Hossein Admadzadeh, Richard O’Kennedy, and Edgar A Arriaga*

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Individual liposome measurements by capillary electrophoresis with postcolumn laser-induced fluorescence detection facilitated the determination of liposome property distributions, two-dimensional plots, and an improved characterization of a liposomal preparation. This advancement in liposome analysis was feasible by using a high-sensitivity postcolumn laser-induced fluorescence detector wired for millisecond response. For each individual liposome containing fluorescein, peak height and migration time were determined. From these measurements, the individual entrapped volumes and electrophoretic mobilities were determined. Distribution analysis of these properties facilitated comparison of various liposome dilutions and indicated that the method is reproducible and unaffected by the density of liposomes (10^7–10^9 liposomes/mL) in the suspension. Furthermore, liposomes showed entrapped volumes that vary from 0.3 to 13 fl with apparent radius varying from 370 nm to 1.8 μm. Two-dimensional plots of reduced mobility versus kR (Debye parameter × liposome radius) revealed that the liposomes resuspended from a dried film of phospholipids are heterogeneous in regard to the surface charge density of individual liposomes. The described method has the potential of becoming a new tool for characterization of commercial liposomal preparations and theoretical studies.

Liposome suspensions are widely used in therapeutic treatments, in the cosmetics industry, as food ingredients, and to transfer genetic material to cells. In addition, they are an excellent model to study biological membrane properties and to evaluate methods for organelle analysis. In these suspensions, the liposomes, artificial vesicles that have one or more continuous phospholipid bilayer membranes enclosing an aqueous interior, are capable of encapsulating drugs, chemicals, or water-soluble molecules. For the above-mentioned applications, homogeneous liposomes would be ideal. In practice, liposomes in a suspension are typically heterogeneous. They may exhibit different size, lamellarity (number of bilayer membranes within the liposome), or membrane composition. Therefore, in parallel to the appearance of new methods to prepare more homogeneous liposome suspensions, developing novel techniques for liposome characterization are essential.

Techniques such as light scattering, gel filtration, microscopy, field flow fractionation, and capillary electrophoresis have been used to characterize size distributions of liposomes prepared from resuspension of dried films of phospholipids. Among these techniques, only light scattering and microscopy directly determine size distribution through the measurement of individual liposome size. The rest of the techniques determine distributions affected by the intrinsic resolution of the technique that normally impedes the measurement of individual liposome properties. Thus, techniques that measure properties of individual liposomes would provide better options for describing liposome property distributions and improving the characterization of liposome suspensions. A few investigators have used capillary electrophoresis to characterize liposome suspensions through electrophoretic mobility determinations. This property is a function of liposome size, zeta potential, membrane composition, and ionic strength of the separation buffer. Despite the complicated interrelationship among these parameters, relationships among these properties and electrophoretic mobility have been modeled and experimentally confirmed. By taking into account the electric field-induced

---