Chromatographic characterization of liposome-encapsulated proteins

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Encapsulation of proteins inside hydrophilic core space of liposome is an important issue in such applications of targeted delivery of therapeutic proteins, immobilized reactions of industrial enzymes, and bionano sensing of diagnostic proteins. The encapsulation yield is generally quite low and needs to be improved for industrially viable applications. While a few literatures reported the methods of liposomal encapsulation of protein for high yield, basic characteristics of protein encapsulation was not fully elucidated. In this study, by fixing the phospholipid as DPPC, we evaluated the effects of protein size (i.e., trypsin, 15 kDa; horseradish peroxidase, 44 kDa; hyaluronidase, 80 kDa; enterokinase, 250 kDa), liposome size (nominal 100, 200, 400 nm), phopholipid concentration, buffer pH, and salt concentration on encapsulation yield. Size exclusion chromatography technique was applied to separate liposome and free proteins. We found that encapsulation yield was mainly driven by electrostatic interaction between phospholipids and protein, and the bioactivity was significantly affected by buffer pH and salt concentration. This approach can be used to scout the optimal encapsulation condition for high yield and bioactivity.