Effect of Temperature, pH and Ionic Strength on Stability of Cytochrome C-Conjugated Citrate-Silver Nanoparticles (Cyto c-AgNP) Using Asymmetrical Flow Field-Flow Fractionation (AsFlFFF)

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***Abstract*:** *Surface modification of nanoparticles with materials such as protein is used as an approach to stabilize the nanoparticles. Surface modification with properly selected materials prevents particle aggregation, and enhances the bioactivity and biocompatibility of the nanoparticles. Cytochrome c (Cyto c) is a protein that has been reported to show strong affinity to inorganic nanoparticles, and serves as a useful agent to protect the nanoparticles by preventing nanoparticles from agglomeration. Cyto c is a well characterized protein that may undergoes a conformational change in unstable conditions. It is known that the HEME group of cyto c is sensitive to changes in various parameters including temperature and solvent composition (pH, ionic strength). These parameters also affect the size and size distribution of Cyto c-conjugated nanoparticles. In this study, 40 nm citrate-silver nanoparticles (AgNPs) was synthesized using citrate reduction method. Then the surface of the nanoparticles was modified by attaching Cyto c at various molar ratios of Cyto c to the AgNPs. AsFlFFF was then used for separation and simultaneous size characterization of Cyto c-conjugated AgNPs obtained at various conditions of temperature, pH and ionic strength. Also the conformational changes of Cyto c that are caused by adsorption on colloidal AgNPs have been investigated by dynamic light scattering (DLS) and UV/VIS spectroscopy.*

Keywords: *Aggregation, Silver nanoparticles (AgNPs), Field-flow fractionation (FFF), Cytochrome C*