

Removal of endotoxins from plasmid DNA: analysis of aggregative interaction of mobile divalent metal cations with endotoxins and plasmid DNA

Abstract

Endotoxin lipopolysaccharide removal from plasmid DNA-based vaccine remains a very challenging task for bioprocess engineers. This paper examined the potential use and advantages of divalent cation ($Zn(2+)$, $Ca(2+)$, $Mg(2+)$) induced aggregation as a plasmid DNA purification method for lipopolysaccharide removal. Analysis of zeta potential, hydrodynamic size, percentage of aggregation; UV-Vis spectroscopy and electron microscopy were performed to determine the optimal cation for preferential aggregation of lipopolysaccharide over plasmid DNA. The results from the hydrodynamic size analysis showed that the addition of $Zn(2+)$ resulted in the maximum theoretical number of lipopolysaccharide molecules per aggregate particle. Dynamic light scattering analysis showed that plasmid DNA aggregates formed a larger maximum hydrodynamic size when it was treated with $Ca(2+)$ than the other two cations. The $K(m)$ value for lipopolysaccharide- $Zn(2+)$ was substantially low (0.28 M) and considerably large (>2 M) for plasmid DNA- $Zn(2+)$. Scatchard plots for plasmid DNA cations showed positive slopes indicating that there was a minimum concentration of plasmid DNA or cations before a significant aggregation occurred. This work concluded that $Zn(2+)$ had the most preferential aggregative interaction with lipopolysaccharide compared to $Mg(2+)$ and $Ca(2+)$.