Recovery of histidine-tagged nucleocapsid protein of Newcastle disease virus using immobilised metal affinity chromatography

Abstract

An immobilised metal affinity packed bed adsorption chromatography (IMA-PBAC) for the purification of recombinant nucleocapsid protein (NP) of Newcastle disease virus (NDV) directly from clarified feedstock was developed. The XK 16/20 (i.d. = 16 mm) was used as a packed bed column and Streamline chelating adsorbent immobilised with Ni2+ ion was used as IMA adsorbent. This purification method has resulted in a 59% adsorption and 5.6% recovery of NP protein. Adsorbed NP proteins were successfully recovered using a two-step elution protocol which employed elution buffer 1 containing 50 mM imidazole to eliminate contaminating proteins and elution buffer 2 containing 350 mM imidazole to recover the NP protein at pH 8 with flow velocity of 10 cm h−1. About 70% of the adsorbed NP protein was eluted. The purity of the recovered NP protein was about 70% and the volume of processing fluid was reduced by a factor of 4. The antigenic features of purified NP proteins were confirmed by enzyme-linked immunosorbent assay (ELISA) analysis.

Keyword: NP protein, NDV, Escherichia coli, Affinity chromatography, IMA, PBA