

Purification of rabbit polyclonal immunoglobulin G with ammonium sulphate precipitation and mixed-mode chromatography

ABSTRACT

Immunoglobulins G (IgG) against hepatitis B core antigen (HBcAg) was successfully purified using a purification scheme comprising ammonium sulphate precipitation and SepFast™ MM AH-1 column chromatography. Ammonium sulphate precipitation performed at 40% saturation was optimum in terms of the recovered polyclonal IgG concentration (7.8 mg/ml) and the removal of albumin (72%). The yield, purity and purification factor achieved from this simple purification method were 99%, 94% and 7.8, respectively. The IgG recovered from ammonium sulphate precipitation was subjected to SepFast™ MM AH-1 column chromatography and the purity of IgG was further increased to 98%, corresponding to a purification factor of 8.1. Protein aggregation was also reduced significantly in the purified IgG sample. Furthermore, the salt content in the purified sample was reduced by 75% and therefore the need of desalting final product was eliminated. Enzyme-linked immunosorbent assay (ELISA) showed that the antigenicity of anti-HBcAg IgG obtained after these purification processes was maintained.

Keyword: Purification; Immunoglobulin G; Hepatitis B core antigen; Ammonium sulphate precipitation; Mixed-mode chromatography