

Immunology

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Discussion (In terms of quantity yield and quality of samples) Section The fractions eluted from IEX are shown in the first section of the results. The presence of protein in F2 and F3 fractions due to the formation of antigen-antibody complex is confirmed by the development of precipitin lines. The line is also forming for the F5 fraction.

Section 2:

The results of IgG purification through ammonium sulphate precipitation are shown in section 2 of the results. The aggregation of protein increases with increase in the concentration of ammonium sulphate. At the end, high protein concentrations are achieved. This is seen for fractions F1, F2 and F3. The fourth fraction, F4, has a low concentration of protein. This is because in this fraction, serum was not diluted. Therefore, aggregation of proteins could not occur because of lack of hydration of protein. Protein concentration of F1 was 5.90mg. This increased till 14.56mg protein concentration for F3. Fraction F5, which is a mixture of F2 and F3 had a protein concentration of 16.00mg – the highest. Purification by ammonium sulphate precipitation is useful for large protein samples. It is a cheaper process. However, the resulting protein obtained is not very pure. Moreover, this technique requires additional purification steps.

Section 3:

The results of purification through ion exchange chromatography (IEX) are shown in section 3 of the results. The purification of IgG was performed. IgG accounts for only 10% of the serum proteins. Yet, the concentrations of IgG in fraction F2, F3 and F5 were 2.08, 1.42 and 2.80 mg/ml respectively, which are relatively high. IEX is a cost effective process and results in higher protein recovery. It has high resolving power.

Section 4:

The percentage yield of protein for all fractions is given in section 4. A higher yield of IgG is seen in fractions F2 and F5 compared to fraction F3. A machine error may be the reason behind this. This is because the reading for F2/F3 and F5 was taken on different spectrophotometers.

Section 5:

The results of the Single Radial Immunodiffusion assay and precipitin arcs are shown in section 5. In figure 1, purple precipitin arcs confirm the presence of IgG. Multiple arcs for F3 and F4 indicate the presence of contamination in these protein fractions. In figure 2, the precipitation ring is observed. It is seen that as the diameter of the ring increases, there is a decrease in the protein (antigen) concentration. Moving further away from the center, IgG concentration decreases.