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Chromatography Purification of Human Serum Albumin from Serum

Human serum mainly contains proteins, lipids, salts and other molecules. The protein concentration of serum is around 60-80 mg/ml and comprise mainly albumins (50-60%) and globulins (40%) [1]. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Human Serum albumin (HSA) functions as a carrier protein for steroids, fatty acids, and thyroid hormones, and is vital in regulating the colloidal osmotic pressures of blood. Albumin is also seen to bind to exogenous substances, particularly drugs (e.g., ibuprofen, warfarin), and strongly influence their pharmacokinetics.[2]

HSA is a valuable biomarker of many diseases, including cancer, rheumatoid arthritis, ischemia, post-menopausal obesity, severe acute graft-versus-host disease, and diseases that need monitoring of the glycemic control. Moreover, HSA is widely used clinically to treat several diseases, including hypovolemia, shock, burns, surgical blood loss, trauma, hemorrhage, cardiopulmonary bypass, acute respiratory distress syndrome, hemodialysis, acute liver failure, chronic liver disease, nutrition support, resuscitation, and hypoalbuminemia [3].

Blood serum is one of the largest sources of biomarkers for diagnostics or therapeutics. Purification of HSA is complicated by the presence of hundreds of other proteins, lipids and salts. In this study we report the purification of HSA by Chromatography using the resin for separation of human serum albumin from human serum under two different chromatographic conditions:

1. Briefly an appropriate resin was suspended in water and packed into a 5 ml (1.5 x 7.0 cm) chromatography cartridge. The column was equilibrated with 10 column volumes of appropriate buffer. Normal human serum (Millipore Sigma, St. Louis, MO) was dialyzed against the buffer and diluted 1:1 in the buffer. Any precipitate was removed by centrifugation and loaded on to the column. The resin was washed with buffer and collected 5 ml fractions. Elution was carried out with proper buffer concentrations and 5 ml fractions were collected. Absorbance of all the fractions were measured at 280nm and the purity was analyzed by SDS-PAGE and densitometry.

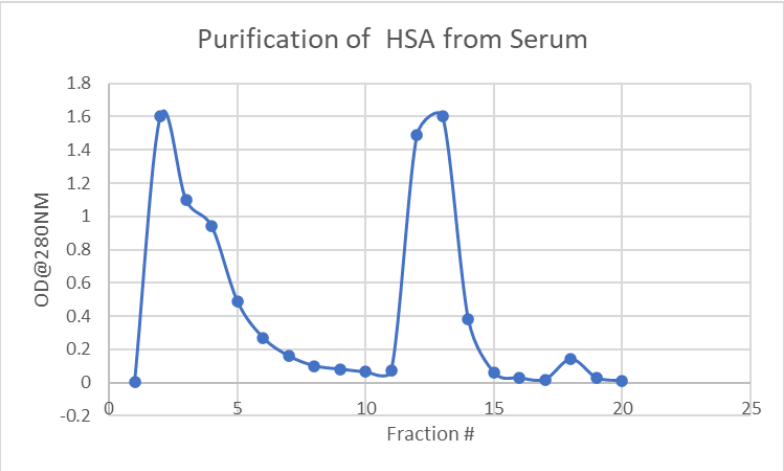

Fig.1: Absorption of chromatographic fractions at 280nm.

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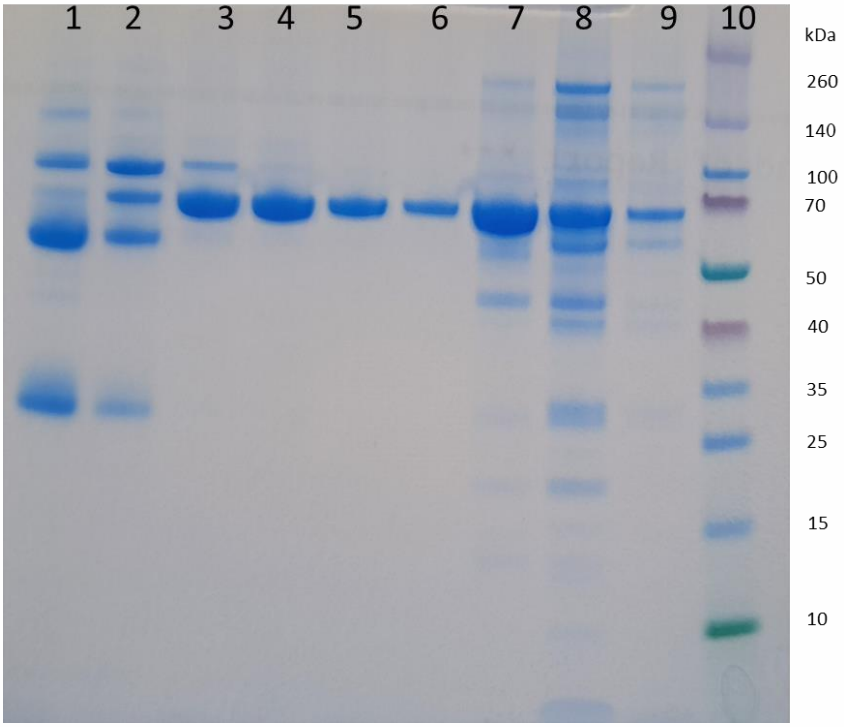

Fig.2 SDS-PAGE Analysis of Chromatographic fractions under reduced conditions: Lanes 1-6: Wash fractions; Lanes 7-9: Eluted fractions and Lane 10: Molecular Weight Markers.

As seen in figure 2 most of the contaminant proteins (IgG and others) elute in the early wash fractions and later wash fractions. The wash fractions (Lanes 3-6) have essentially pure HSA protein. About 40% of HSA comes out in these wash fractions with purity >92% (by densitometry) and the remaining 40% comes out with some contaminant proteins upon elution with appropriate buffer conditions. This method eliminates the need to remove IgG by rather expensive affinity chromatography using Protein A. For large scale purification, SMB (Simulated Moving Bed) Chromatography would be very beneficial to continuously produce pure HSA and the eluted impure HSA (fractions 10-20) can be recycled following dialysis to achieve high yield of the final purified product.

2. In this chromatographic separation was performed as described above with modified buffer conditions.

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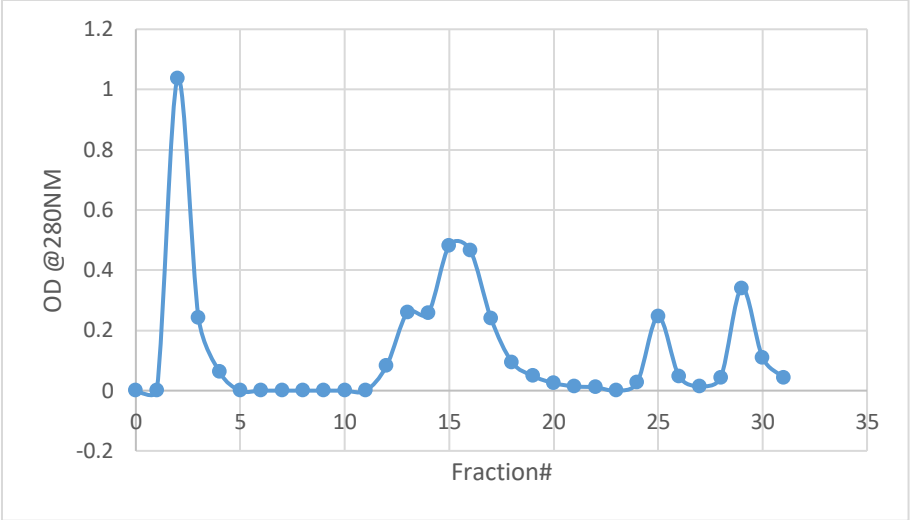

Fig.3: Absorption of chromatographic fractions at 280nm.

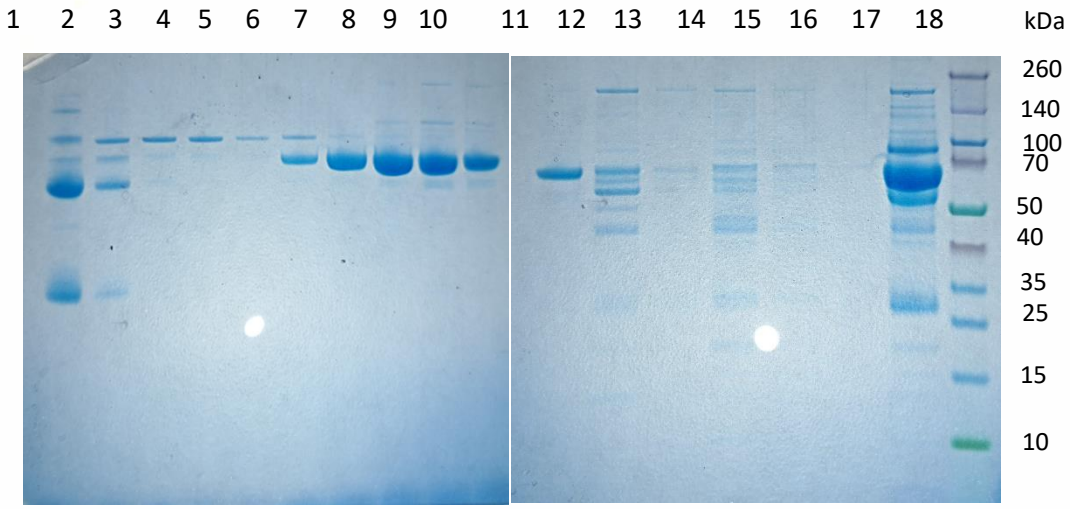

Fig.4 SDS-PAGE Analysis of Chromatographic fractions under reduced conditions: Lane: Flowthrough; Lanes 2-5: Wash fractions; Lanes 6-11: Eluted fractions under appropriate buffer conditions; Lanes 12-16: Eluted fractions under appropriate buffer conditions; Lane 17: Serum load and Lane 18: Molecular Weight Markers

As seen in figure 4 most of the contaminant proteins (IgG and others) elute in the early wash fractions. The eluted fractions (Lanes 6-11) contain predominantly HSA protein. About 90% of HSA comes out in these fractions with purity ~85% (densitometry). This method eliminates the need to remove IgG by rather expensive affinity chromatography using Protein A. For large scale purification, SMB (Simulated Moving Bed) Chromatography would be very beneficial to continuously produce HSA.

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References

[1] Barrett KE, Brooks H, Boitano S and Barman SM. (2010) *Ganong's review of medical physiology.* 23. New York: McGraw-Hill Medical.

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[3] Fanali G, Masi AD, Trezza V, Marino M, Fasano M and Ascenzi P. (2012) Human serum albumin: from bench to bedside. *Mol Aspects Med.* Jun;33(3):209-90.