Novel Column Designs for Improved Bioseparation of Complex Molecules using Counter Current Chromatography

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Despite major advances in detection methods, bioseparation limits molecular discoveries. Countercurrent chromatography (CCC) is a liquid-liquid partition chromatography that uses the Archimedean Screw principle to significantly decrease sample loss and denaturation by eliminating a solid support matrix. However, loss of stationary phase and consequently samples has not been eliminated. A novel flat spiral tube assembly was made based on the forces generated and calculated partition coefficients. Separation of mixtures of cytochrome c, hemoglobin, myoglobin, ovalbumin, and lysozyme proteins showed that partitioning efficiency was significantly enhanced with the new flat-tube compared with conventional column. A novel tube modifier was then used to generate columns with varying internal diameters and shapes. Column performance measured by theoretical plate number, peak resolution, stationary phase retention, and separation time showed significantly enhanced performance efficiency for our new flat-tube column. Tube integrity at high speeds was limiting the revolution speeds and resulted in longer run times. Improvement in separation efficiency and shorten run times, especially for large molecules was achieved with a thin layer CCC using a plastic disc. Modifications of CCC columns based on structural properties of the tubes and the forces generated results in enhanced bioseparation efficiency that has broad applications in science.