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## Introduction

Chromatography is a technique that is used to separate different solutions or mixtures into their different components by running the mixture through a separation column. Different types of chromatography are based on varying physical properties of the components, such as polarity, charged residues, or differences in size. Size-exclusion and ion-exchange chromatography were performed in this laboratory to separate four known proteins from a protein mixture. The experiments were conducted to understand the theory behind chromatography by applying the classroom knowledge in a practical setting. And, the UV-Vis spectrographs of the elution were taken and analysed to quantify the strength of separation. The results from a protein solution containing mixtures of four proteins were plotted and evaluated to test the accuracy of the conducted chromatographies.

## 2 Body

### 2.1 Theory

Size-exclusion (SE) chromatography separates components based on their sizes. A porous, hydrophilic support is used to reduce the potential of intermolecular bonding. This support is usually cross-linked between dextrans and cellulose<sup>[1]</sup> and is transported throughout the column as means of inserting a flow of a suitable mobile phase. As the protein mixture travels down the column, the smaller components can fit more easily into the pores of the support. As a result, smaller proteins are maintained in the stationary phase for longer and elute out of the column more slowly than larger molecules. Accordingly, the first peaks of the chromatogram show the largest proteins in the solutions.

Ion-exchange (IE) chromatography takes advantage of the difference of charges between the regions of a molecule and a charged-group on the beaded resin (stationary phase)<sup>[1]</sup>. Each protein has an iso-electric point (pI). This is the pH at which the overall charge on the surface of the protein is 0<sup>[2]</sup>. When the protein is on the mobile phase that has a pH greater than the isoelectric point, it would be negatively charged. Conversely, if the pH of the solution is lower than the isoelectric point of the protein, its surface is positively charged<sup>[1]</sup>. As the mobile phase, along with the analyte, flows through the column, the components that are most reactive with the resin are retained longer, leaving the column lastly.

A spectrometer measures absorbances at different wavelengths and plots them against the times or fraction number.

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