

Compared Document

#### Introduction

Chromatography is a technique that is used to separate different solutions or mixtures into their different componen running the mixture through a separation -column. Different types of chromatography are based on varying physica separation due to varying polarity, charged residues, or differences in size. Size-exclusion and ion-exchange chrom

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performing were performed in this laboratory to separate four known proteins from a protein mixture. The experiments are were conducted to understand the theory behind chromatography: by applying the classroom knowledge in a practical setting. And, the The 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.

# 2Two Body

## 2.1 Theory

Size-exclusion (SE) chromatography separates components based on their Sizes Ansizes. A porous, hydrophilic support is used to reduce the potential of intermolecular bonding, this This support is usually cross-linked between dextrans and cellulose [1], [1], 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 could ancan fit more easily into the poorspores of the support. As a result, smaller protein proteins are maintined maintained in the stationary phase for longer, and elute out of the column more slowly than larger molecules. Accordingly, the 1 first peaks of the chromatogram show the largest proteins in the solutions.

Ion-exchange (IE) chromatography take advantage advantage of the difference of charges between the regions of a molecule; and a charged-group on the beaded resin (stationary phase)[1]. [1] Each protein has san iso-electric point (p[], the). This is the pH at which the overall charge on the surface of the protein is 0<sup>[2]</sup> [2]. When the protein is onin 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 negatively charged[1]. 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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**Nancy Conner, Matthew MacDonald** 

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