Experiment No. 4

Ion Exchange Chromatography

Introduction:

Ion exchange chromatography is a separation technique used for purification or analysis of molecules based their charge. The method can be used to separate charged molecules from uncharged ones or it can separate molecules of different charge from one another.

Principle of the method:

Ionizable chemical groups are immobilized on a solid support such as cellulose or agarose. The support, or resin, is usually maintained in a column. Molecules of opposite charge can bind the column by electrostatic interaction while uncharged residues will pass through. Once bound to the column, molecules can be released with salt (NaCl is commonly used, but other salts can be used also). The salt ions compete for interaction for the column, and the molecule of interest is released. Hence the term "ion exchange".

Molecules having different charges can be separated from one another by gradually increasing the salt concentration. This is achieved with a gradient of increasing salt concentration in the solution being passed through the column. Lower charged groups are released at low salt concentrations because they are weakly bound. Highly charged molecules are more tightly bound and require higher salt concentration to release them. Thus molecules are released from the column according to the magnitude of their charge.

It should be noted that pH of the column buffers can have a profound effect on ion exchange chromatography. Both the ion exchange resin and the molecule binding to it are charged molecules with a defined pKa. If the pH is on one side of the pKa, the molecule will be uncharged, and it will be charged on the other. Also, the difference between the pH and the pKa will determine how much of the resin is ionized that in turn will determine how tightly other molecules will bind.

The charged resin can be of two types: cation exchangers and anion exchangers. The name of the resin refers to the molecules being exchanged, **not** the molecule bound to the resin. Cation exchangers bind positively charged molecules and anion exchangers bind negative ones.

In this experiment, you will separate adenosine 5'-monophosphate (AMP) and adenosine 5'-triphosphate (ATP). These compounds will be separated by chromatography on diethylaminoethyl (DEAE) cellulose. The mixture of compounds will be loaded onto the column, and eluted with a NH_4Cl/NH_3 gradient.

Reagents and Materials:
Compound mixture: AMP and ATP
Buffer: 0.25 M NH_4Cl/NH_3, pH 9.0

Experimental Procedure:

1. Prepare a column of DEAE-cellulose by placing a filter paper at the bottom of a column to serve as a plug. Add a slurry of DEAE-cellulose equilibrated in 0.05M buffer. The final height of DEAE-cellulose in the column should be between 7 to 8 cm.
2. Prepare 10ml solutions of eluting buffer from the stock 0.25M solution. The concentrations should range from 0.05M to 0.25M in increments of 0.05M.
3. Drain the column to just above the top of the resin (do not let resin go dry). Add 1 ml of the compound mixture and allow it to run into the column.
4. Cap the column, and start collecting effluent.
5. Have a test-tube rack ready with 20 numbered test-tubes. Collect ~3 ml of effluent in each tube.
6. Measure the absorbance of each tube at 260 nm.

Data Analysis:

1. Tabulate absorbance of column eluent at 260 nm vs fraction number.
2. Graph your data, plotting absorbance vs fraction number.
3. Draw the structural formulae of the predominant chemical species for the compounds separated in this experiment. Ionic charges in the species must be clearly labeled.