

# Using gel filtration to study ligand protein interaction biology essay



The purpose of this experiment is to separate particles as complex and the other small ones by helping gel exclusion chromatography and calculate affinity as a percent.

## **THEORY**

Gel filtration chromatography is a method for separating proteins and peptides based on their size. The chromatographic matrix consists of porous beads, and the size of the bead pores. They can define the size of macromolecules that may be fractionated. Those proteins that are too large to enter the bead pores are 'excluded' and thus elute from the column first. Since large molecules do not enter the beads, they have less volume to pass through, which is why they are the first to elute from the column. Smaller macromolecules that enter some, but not all of the pores are retained slightly longer in the matrix and emerge from the column next. Finally, small molecules filter through most of the pores, and they elute from the column with an even larger elution volume. This method is also called gel permeation, molecular sieve, gel-exclusion, and size-exclusion chromatography. Since no binding is required and harsh elution conditions can be avoided, gel-filtration chromatography rarely inactivates enzymes, and often is used as an important step in peptide or protein purification. [1]

## **LIGAND**

Atom, or molecule attached to a central atom, usually of a transition element, in a coordination or complex compound. It is almost always the electron-pair donor in a covalent bond. Common ligands include the neutral molecules water (H<sub>2</sub>O), ammonia (NH<sub>3</sub>), and carbon monoxide (CO) and the anions cyanide (CN<sup>-</sup>), chloride (Cl<sup>-</sup>), and hydroxide (OH<sup>-</sup>). Rarely, ligands are

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cations and electron-pair acceptors (electrophiles). Organic ligands include EDTA (chelate) and nitrilotriacetic acid. Biological systems rely on ligands such as the porphyrin in hemoglobin and chlorophyll, and numerous cofactors are ligands. In chelates, the ligand attaches at more than one point, sharing more than one electron pair, and is called bidentate or polydentate having two or many "teeth." The ligands in a complex may be the same or different.

## **LIGAND -PROTEIN INTERACTIONS**

Ligand molecule can bind and make a complex with biomolecule for biological purpose. It binds those molecules with intermolecular forces such as ionic bonds, h-bonding and Van der Waals forces. Therefore; the reaction between Ligand and protein is reversible.

Ligand + Receptor  $\rightleftharpoons$  Ligand-Receptor

If Ligand and receptor makes covalent bond, the reaction becomes irreversible. This situation will block the biological processes.

Serum albumin is the most abundant plasma protein that is in humans and other mammals. It is required for maintaining the osmotic pressure that is needed for distribution of body fluids between intravascular compartments and body tissues. It is also a carrier protein by non-specifically binding several biomolecules.

## **APPARATUS**

Chromatography column

Spectrophotometer

Beaker

Clamps

Plastic cuvettes

Bovine Serum Albumin

Acetate buffer 0. 1M

Phenol red in acetate buffer

NaOH solution

Sephadex

## **PROCEDURE**

1. 0. 1g phenol red was dissolved in 10ml acetate buffer.
2. The sephadex gel was poured into it, after washing chromatography column with acetate buffer.
3. It was waited to polymerize gel.
4. 6 samples were prepared with different concentration of phenol red solution by mixing same volume of acetate buffer and albumin.
5. 250micromilli of first sample was put into the gel and added 50ml acetate buffer slowly.

6. The pourer of column was opened and filled cuvettes with 200micromilli 1M NaOH and 2mld H<sub>2</sub>O for each cuvette.

7. The absorbance of samples was measured at 520nm.

8. The steps were repeated for the other samples.

## **CALCULATIONS AND OBSERVATIONS**

Gel filtration was used to separate molecules. 6 samples were prepared to observe results and measure absorbance. My group was examined 5th and 6th samples.

20g BAS was put into 5th and 6th samples but phenol red and acetate buffer amount changes to observe that. 0. 60 ml acetate buffer and 0. 40 ml phenol red solution was put into 20g BSA. This was 5th sample in this experiment .

0. 40ml acetate buffer and 0. 60ml phenol red were put into 20g BSA. This was 6th sample in the experiment. In this experiment, our stationary phase was sephadex and mobile phase was acetate buffer. Before samples were put into column, the column was washed that means fixed with acetate buffer. The PH of acetate buffer is 4. 5 that is mobile phase. According the gel filtration principle, big molecules can be quicker than small ones.

Because there is less obstacle during movement compared with small ones.

Therefore we can separate by helping molecular weight difference. In this experiment, big molecules were BSA and phenol that was ligand. We observed color changes related to phenol red behaviour in different environment. In acidic conditions, phenol red is yellow and at 6. 8-8. 2 the color turns into purple. The first cuvette samples were lighter due to

complex and then darker due to only phenol red.

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## Reaction Number

## Reagent

1

2

3

4

5

6

BSA

(mg)

20

20

20

20

20

20

Acetate

Buffer(mL)

0, 95

0, 9

0, 8

0, 7

0, 6

0, 4

Phenol

(mL)

0, 05

0, 1

0, 2

0, 3

0, 4

0, 6

Affinity of sample 5:

$$100 \times \frac{x}{y} = 100 \times \frac{13}{31} = 41.935$$

Affinity of sample 6

$$100 \times \frac{10}{40} = 25$$

## RESULTS

In this experiment, 6 sample's absorbance were measured. 5th and 6th were measured by our group. We took sample from gel filtration and placed into cuvettes. And then added NaOH and d H<sub>2</sub>O. Cuvettes were placed into spectrophotometer and gained results. These results gave us two graphs that is shown in below.

figure sample 5

### **figure sample 6**

At the two graphs, distinct peak was observed. It means that is the point big molecules ended up then only small molecules phenol red observed.

## DISCUSSION AND CONCLUSION

In this experiment, we used gel filtration chromatography to detect interaction between ligand and just phenol red. First of all, we washed column with acetate buffer before put samples into column. The reason of that is to adjust column PH. Acetate buffer PH 4.5 is our mobile phase. It can affect protein connectivity with surface. And we used sephadex as a stationary phase. Because it adjusts pore size. Under these condition, we put sample into cuvette and measured at 520nm. The quicker ones the big ones, the lower one an small ones also. First observation that are lighter ones are big molecules. The big molecules are complex, ligand. Ligand is BSA and phenol red. The reason why is chosen albumin's solubility and binding site affinity are high.