Laboratory 4 Worksheet

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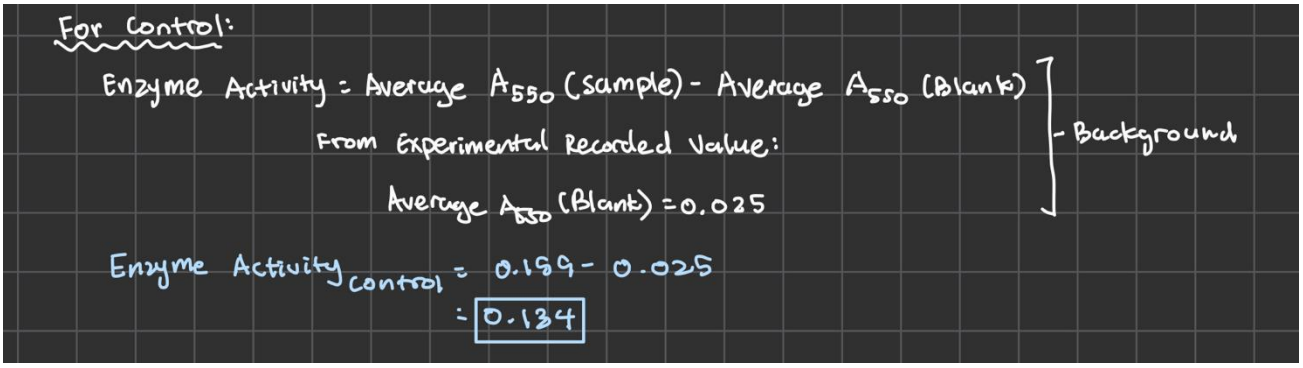
Q1a. Tabulate your results in the following table. **(5 pts)**

Media Type	Enzyme activity A_{550}	Enzyme activity A_{550}	Average A_{550}	Enzyme Activity (Abs Enzyme – Abs Blank)	% Recovery (Media/Control)* 100
Assay reagent Blank	0.024	0.026	0.025		
Control (No column media buffer only)	0.156	0.162	0.159	0.134	100%
Media UNOsphere S	0.125	0.169	0.147	0.122	91.04%
Media Nuvia Q	0.029	0.027	0.028	0.003	2.24%
Media Hydroxyapatite	0.033	0.035	0.034	0.009	6.72%

Q1b. Show a sample calculation on how you obtain the enzyme activity value. **(2 pts)**

(for control group)

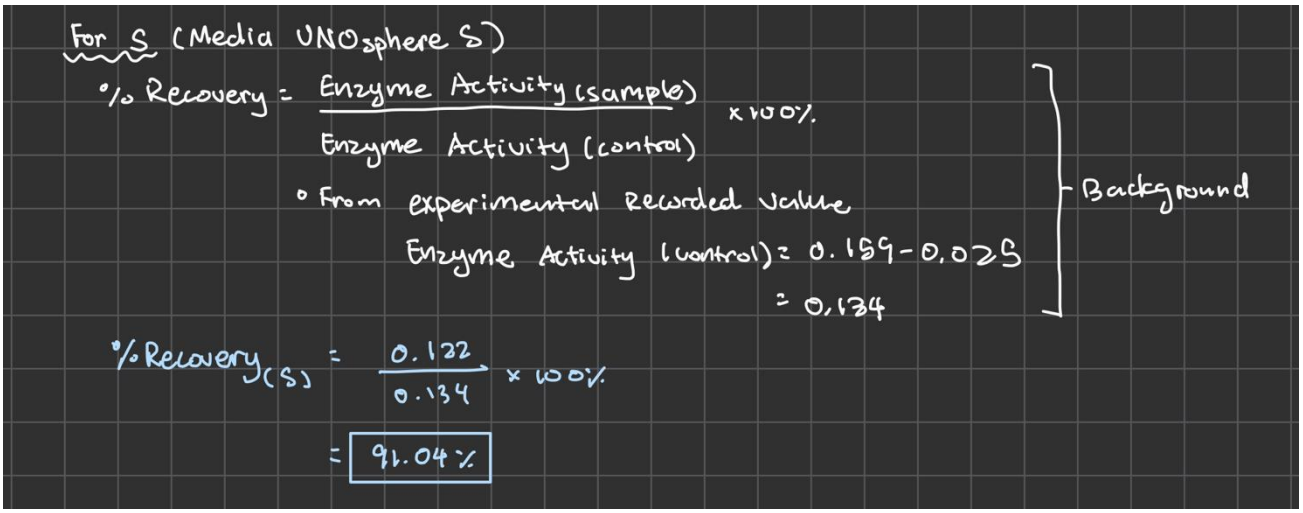


C = |enzyme| - |blank|

C = 0.159 - 0.025

= 0.134

Q1c. Show a sample calculation on how you obtain the % percentage recovery value. **(2 pts)**



(for S)

S = (0.122/0.134)*100%

= 91.04%

Q2. What media will you choose for a subsequent column chromatography purification? Use your data from Q1a to support your choice. **(5 pts)**

For purification, we will choose Nuvia Q for subsequent column chromatography because it has the lowest enzyme activity in the supernatant (0.003 A_{550}) and the lowest percent recovery of 2.24%. This means very little of the enzyme/protein was present in the unbound (flowthrough) fraction. As such, this suggests that most of the enzyme bound strongly to the Nuvia Q media.

Furthermore, Nuvia Q is a strong anion exchanger because it contains quaternary ammonium functional groups that are positively charged. These groups attract and bind negatively charged proteins through electrostatic interactions. The strong binding implies that the enzyme/protein likely has a net negative charge at the buffer pH, making Nuvia Q a good candidate for subsequent anion exchange purification.

As such, of these reasons, Nuvia Q is chosen. The ideal media is one that has most of the protein/enzyme binding to the resin (does not stay in the supernatant), low enzyme activity in the flowthrough (because it's being retained, or captured, on the column), and impurities are able to be washed out and later elute the target protein in a more purified form.

Compared to other media such as UNOsphere S which has a high enzyme activity in supernatant (0.122) and Hydroxyapatite which has a greater enzyme activity (0.009) than Nuvia Q (0.003), out of all the media types given in this lab experiment Nuvia Q is the best media to be chosen for subsequent column chromatography purification.

Q3. What type of elution protocol (stepwise vs. gradient, salt vs. pH) will you choose to use? What are the advantages of using such an elution protocol. **(4 pts)**

We will choose to use a gradient salt elution protocol. This involves gradually increasing the salt concentration in the buffer to elute proteins based on their strength of binding to the resin.

The main advantage of gradient elution is that it offers higher resolution, which is crucial for purifying a specific protein like ceruloplasmin from a complex biological mixture such as serum. Compared to stepwise elution, gradient elution allows for finer separation of proteins that have very similar charge properties because it gradually increases the salt concentration, giving proteins with only slight differences in binding strength more time and opportunity to elute at distinct points, rather than all coming off in a single step. A simpler explanation is that in stepwise elution, you go from low salt to high salt in big jumps (like going from 0.1 M to 0.5 M NaCl). This means both proteins may come off together in one step if they react similarly to that jump. In gradient elution however, the salt increases slowly and smoothly (like 0.1 → 0.15 → 0.2 M … and on). This gives proteins with slightly different binding affinities a chance to elute at slightly different times. Thus, a gradient elution allows for a better separation and cleaner fractions.

Although gradient elution requires more sophisticated equipment (such as a gradient mixer or automated FPLC system), this is practical in a university lab setting, and the benefit of improved resolution outweighs the increased complexity.

In addition, we chose salt instead of a pH elution. A pH elution is not preferred in this case because of the potential risk of denaturing the target protein.

Advantages of using a gradient salt elution protocol is firstly, it provides a higher resolution (compared to stepwise), and secondly, using salt instead of pH is more secure. If pH was used, changes can denature proteins, especially sensitive ones like enzymes or glycoproteins. As such, unless the exact pI and stability of the target protein is known, salt provides an advantage in that aspect.

All in all, we chose a gradient salt elution protocol for the above reasons and advantages.

Q4. Anion and cation chromatography leverage the charges of amino acids to separate them from a mixture. What types of amino acids will bind to anion and cation exchange columns? Provide one specific example for each. **(4 pts)**

Anion exchange columns, which are positively charged, bind to negatively charged amino acids (anions). For example, aspartic acid, which has a carboxyl (-COOH) group in its side chain, becomes negatively charged ($-COO^-$) at neutral pH (around pH 7). This is because the carboxyl group donates a proton (H^+) to the surrounding solution, leaving behind a negative charge. Since anion exchange resins are positively charged, they are designed to attract and bind negatively charged molecules like aspartic acid. Therefore, when a mixture of amino acids is passed through an anion exchange column, aspartic acid will be retained on the column due to this electrostatic attraction, while uncharged or positively charged amino acids will pass through.

Cation exchange columns are negatively charged, so they bind positively charged amino acids (cations). For example, lysine is an amino acid that has an amino group ($-NH3^+$) on its side chain. At neutral pH (around pH 7), this amino group carries a positive charge because it tends to pick up a proton (H^+). Because lysine is positively

charged under these conditions, it will interact strongly with a cation exchange column.