CADET Homework

You have been hired by a small biopharmaceuticals company alongside a team of skilled chromatographers. For one of your first tasks, you have been given a mixture of a protein-based drug product along with two highly toxic impurities. You are asked to develop a process to isolate the protein from its impurities. After performing some first-pass experiments, your colleague informs you of a column modeling software called CADET. This software can be used to accurately predict behavior on chromatographic systems. You decide to use this software to better inform the development of the purification process in question.

In the sample you are given, the concentration of the product greatly exceeds the concentrations of both impurities—the concentration (in mg/ml) of the protein is 5x greater than impurity A, and 10x greater than impurity B. To meet the drug product quality attributes, the purity of the processed sample must exceed 96%. For the process to remain economical, the yield of the product must exceed 85% and the length of the elution sequence (not including the loading step) must be less than 40 CV. Due to complications with mixing in the chromatographic system, it is not permissible to use linear salt gradients and only salt steps may be used.

a) Initially, you wish to model this separation under low-loading conditions (where the amount of protein added refers to the linear region of the isotherm). The intended column loading of the low loading operation is 5 mg/ml of total protein (including the product and impurities).

b) To optimize the amount of protein you can purify in this process, you decide to model this separation under high-loading conditions (where the amount of protein added refers to the nonlinear region of the isotherm. The intended column loading of the high loading operation is 45 mg/ml of total protein (including the product and impurities).

Use the following information about the feed solution and chromatography column. The residence time for all steps in the process is 3 minutes.

| | **Impurity A** | **Product** | **Impurity B** |
|---|---|---|---|
| **MW** | 36 | 20 | 9 |
| **concentration [g/L]** | 0.4 | 2 | 0.2 |
| **Keq** | 0.0329 | 0.0037 | 0.016 |
| **steric factor** | 25.5 | 15.2 | 17 |
| **characteristic charge** | 5.2 | 5.6 | 3.3 |

| **Column parameter** | **Value** |
|---|---|
| **column ID [cm]** | 1 |
| **column length [cm]** | 20 |
| **Ee** | 0.4 |
| **Ep** | 0.9 |
| **particle diameter [um]** | 85 |
| **pore radius [nm]** | 32 |
| **ionic capacity [mol / L column]** | 91 |

Note: You must calculate the length of the loading step using the intended column loading and given information. Parameters you can change to achieve your goal include the salt concentrations, step lengths, and fraction collection cutoff concentration. All other parameters should match those given in the problem. To be successful, the purity and yield of component two from step 2 alone should be >96% and >85%)

Please submit the salt concentration, step lengths, and fraction cutoffs that you found for part A and B along with screenshots of your 2 chromatogram plots and 2 output results tables.