

Additional Questions:

1. The primary advantage of using size exclusion chromatography over SDS page is that size exclusion chromatography can be performed on proteins in their native conformations, whereas SDS page can only be used on proteins that have been denatured. Although both methods separate based on migration, size exclusion chromatography has the advantage because the native forms of a protein gives more insight into the original structure of the protein, specifically if protein subunits separate out.
2. It is possible to determine an unknown molar ratio between methylene blue and Ponceau S red dye using only a UV-Vis spectrophotometer and a Sephacryl S200-HR resin packed column. In this experiment it is imperative that the column length is fairly long. This is because even though both methylene blue and Ponceau S red dye are below the fractionation range, Ponceau red is twice the weight and significantly larger than methylene blue. Therefore, Ponceau red must spend significantly less time traveling through the pores and thus elute prior to methylene blue. A long column will help increase the resolution of these two peaks, which would otherwise be very close to each other. After running the column and collecting the eluant, the fractions can be measured in the spectrophotometer. The measured absorbances can be related to the known molar extinction coefficients in order to find the concentrations. Integrating a gaussian curve of the peaks would result in the total concentration of methylene blue and Ponceau red such that the total moles and molar ratio can be determined.