Out-of-Class Questions


1. This abstract is a good example of how to present a large amount of quantitative data concisely in an abstract. Using the abstract and the body of the article as needed, report the following values for these experiments, and compare them to typical values for HPLC and CE from your text, class notes, or lab data. Don’t forget units where needed!

<table>
<thead>
<tr>
<th>Parameter</th>
<th>This Work</th>
<th>Typical HPLC</th>
<th>Typical CE</th>
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2. Using a form of the resolution equation, the authors remind the reader that there are two contributors to peak resolution.
   a. What are they?

   b. Copy equation (1) below, then circle and label the terms that correspond to each of the contributors to \( R_s \) that you listed above.

3. Why do the authors choose to target plate height as a means to improve resolution? What other parameters could they have targeted?
4. Based on the final paragraph of the introduction, what was the objective of this work?

5. Why do the authors use an electric field, rather than pressure, to drive these separations?

6. The authors use silanes to polymerize the packing material and to form what is likely to be a very thin layer of short carbon chains on the surface (i.e., \( d_f \) is extremely low). If the stationary phase is so thin, how do the authors know that chromatography, rather than just electrophoresis, is occurring?

7. Estimate the value of \( H \) for the lysozyme peak in Figure 2. Show your work to receive credit. Does your estimate match the authors’?
8. Why is the plate height lower for these separations than for previous separations of dyes using the same packing material?

9. Why does heterogeneous packing increase the plate height? What term(s) in the van Deemter equation is/are affected by packing inhomogeneity like that seen in Figure 3?

10. In Figure 4C, why does peak variance increase linearly with time? In other words, what process causes this peak broadening?
In-Class Questions


1. When characterizing plate height for lysozyme in Figures 4 and 5, the authors determine the width of the peak in space rather than in time. They are able to do this because they are using a camera as a detector, but why do they need to do this? How does the width of the peak in space relate to the width of the peak in time? How does the detector contribute to plate height in these experiments?

2. In discussing Figure 6, the authors assert that the A and C terms of the van Deemter equation are negligible for their separations. In Figure 7 and the latter part of the Results & Discussion, the authors address whether the extremely low plate heights observed could be due to focusing rather than to the achievement of a diffusion-limited separation. Why would the A and C terms be negligible under the conditions used in this work? What evidence supports the authors’ assertion that the efficiency of their separations is limited only by diffusion?

3. The authors specifically state that their goal for this work was not to achieve a practical method for protein separations. That would have been outside the scope of this paper because many practical considerations would need to be addressed before this type of packing could be made available in commercial columns. Imagine that an instrument manufacturer wants to use columns like these in a commercial HPLC instrument. What changes to the instrument and practical improvements in the column would be needed?