Most of the questions on this cume are related to the attached article “Capillary Electrophoretic Application of 1-Alkyl-3-methylimidazolium-Based Ionic Liquids,” by Stalcup and co-workers. Questions that pertain to specific passages are denoted in the margins of the article. Please consult point value in determining the required detail in your answers. Show your work clearly for full credit.

1. Consider Capillary Electrophoresis (CE), capillary electrokinetic chromatography (CEKC), wherein neutrals are separated using soluble running buffer additives, and capillary electrochromatography (CEC), wherein neutrals are separated using true stationary phases in answering the following.

   a) What factors influence (limit) efficiency in each technique (consider the usual sources of band dispersion of axial diffusion, resistance to mass transfer, etc. and state which are significant for each technique)? In your answer also order the techniques in terms of overall efficiency. (10pts)

<table>
<thead>
<tr>
<th>Source</th>
<th>CE</th>
<th>CEKC</th>
<th>CEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>eddy diffusion</td>
<td>no packing so no $H_e$</td>
<td>pseudo phase dispersed so no $H_e$</td>
<td>$H_e$ can be a factor</td>
</tr>
<tr>
<td>axial diffusion</td>
<td>always a factor, rapid</td>
<td>always a factor, rapid</td>
<td>always a factor, rapid</td>
</tr>
<tr>
<td>Res mass trans</td>
<td>small because of plug</td>
<td>small because of plug</td>
<td>small because of plug</td>
</tr>
<tr>
<td>mobile phase, $H_m$</td>
<td>EOF profile</td>
<td>EOF profile</td>
<td>EOF profile</td>
</tr>
<tr>
<td>Res mass trans</td>
<td>no stationary phase</td>
<td>pseudo phase nano</td>
<td>$H_s$ can be significant</td>
</tr>
<tr>
<td>stat phase, $H_s$</td>
<td>dimensioned so $H_s$ small</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   Most efficient CE and least efficient CEC

   b) Order the techniques in terms of versatility (ranges of compounds that are amenable to separations). What factors influence the versatility limits of these techniques? The authors work in this paper falls into which technique classification? (8pts)

   The most versatile is CEC while the least versatile is CE. The low versatility of CE is because it applies only to ions. CEKC is more versatile but is somewhat limited because both the running buffer additive and the sample component must be soluble in the Running buffer. For example MEKC is good for neutral separations but hydrophobic compound can exhibit solubility or separation problems. CEC does not have these problems (note EOF in solvents other than water is possible). The authors technique is CEKC type.

   c) Note that the authors use a simple rinsing technique between injections and employ a commercial UV absorbance detector. These experimental features are relevant to the main limitations of these capillary techniques when compared to HPLC; namely poor reproducibility and inadequate detection. Discuss the factors that contribute to these limitations. (8pts)

   EOF is a surface phenomena that depends on the zeta potential at the wall of the capillary. Running buffer and sample components can alter the condition of the capillary (and this potential) and this causes irregularly in flow which limit the qualitative capabilities (and even the quantitative if on-column detection is employed) of the techniques. Detection is usually performed on-column with these techniques because of the small volumes involved (e.g., column and band volumes are typically 1 $\mu$L and 50 nL, respectively, for
Performing detection on column in 50 µm id capillaries is very difficult. For example, the most common form of detection in liquid phase separations absorbance suffers from very short path lengths and stray light problems. Etc.....

2. List four other ionic additives (besides ionic liquids) that have been used in capillary electrokinetic separations of neutrals. (8pts)
alkyl ammonium salts, surfactant-micelles, cyclodextrins, calixarenes, crown ethers, etc.

3. Certain attributes of ionic liquids are listed by the authors. To what degree are these attribute significant in their work reported in this paper? (5pts)
good solvents - when I looked at the title of this paper I thought the authors were using running buffers that are ILs Since they are only used as dilute components in the running buffer this may not be particular significant.
thermally stable - not generally an issue in CE
non-volatile, non-flammable, environmentally benign - all are significant but because of the small amounts used in microscale separations not as much as with other applications of ILs.

4. Determine the molar absorptivity of catechin at 230 nm. (6pts)
\[ A = \varepsilon b C \]
Absorbance appears to be ~ 0.3; C is given as 20 µM; b is given as 1 cm
\[ \varepsilon = \frac{0.3}{(1 \text{ cm} \times 2 \times 10^{-5} \text{ M})} = 15,000 \text{ M}^{-1} \text{ cm}^{-1} \]

5. EOF is fundamental to capillary electrophoretic separations.
   a) Describe the phenomena including a diagram in your answer. (10pts)
   My drawing capabilities are not great so please consult virtually any text on CE for answer to this one.
   b) Give one significant advantage and one significant disadvantage of EOF in CE separations (not related to efficiency). (6pts)
   One advantage - all components (+, -, neutral) move toward one end of the column facilitating detection.
   One disadvantage - often this creates system retention problems that limit resolution.
   Other answers possible.
   c) What effect does viscosity of the running buffer have on the magnitude of EOF? (4pts)
The viscous drag of the running buffer counteracts EOF. High viscosity - low flow.
   d) Do you agree with the authors regarding their attributing differences between the EOF observed with 1E-3MI-TFB and TEA-TFB to differences in the dissociation constants for these salts? Explain. (6pts)
   Their explanation seems reasonable. If the anion (TFB) and the IL cation associate at the wall then the cation is less able to carry the EOF. I will accept logical explanations that oppose this.
   e) Notice that as the concentration of the IL (the running buffer additive that causes increased retention in the system) is increased in Figure 6, migration times increase. How is this possible? (6pts)
Increasing the RB IL additive will increase the net association with the IL cation that migrates to the cathode and away from the detector. Hence, one would expect the migration time to increase. However, the increase in EOF with increasing [IL] counters this effect and migration times actually decrease. Since the question is misstated I gave full credit to all.
6. Derive the equation on page 3842 starting with \( \mu_p = f_c \mu_c + f_f \mu_f \) where \( f_c \) and \( f_f \) are the fractional amounts of complexed and free analyte. This will require use of the equilibrium binding constant expression, \( K = [A-IL] / ([A] [IL]) \), and will include an intermediate expression that takes the form of the common binding isotherm; i.e.,

\[
\mu_p = \mu_f + \mu_c K[IL] \\
1 + K[IL]
\]

Show and comment on the steps in your derivation very clearly. Also comment on any assumptions that are made in coming up with the equation on page 3842 and state what is meant by “corrected mobility.” (15pts)

\[
\mu_p = f_c \mu_c + f_f \mu_f = \frac{[A-IL] \mu_c}{[A] + [A-IL]} + \frac{[A] \mu_f}{[A] + [A-IL]} = \frac{1}{1 + K[IL]} \mu_c + \frac{1}{1 + K[IL]} \mu_f + \frac{\mu_c + \mu_f K[IL]}{1 + K[IL]}
\]

Substrating \( \mu_i \) from each term & some rearrangement

\[
(\mu_p - \mu_f) = \frac{\mu_c (\mu_c - \mu_f) K[IL]}{1 + K[IL]} = \frac{\mu_c - \mu_f}{1 + K[IL]} K[IL]
\]

Inverting and a little more rearrangement yields the equation on page 3842

Assumptions include that 1:1 complexes are involved and concentrations are small enough to consider concentration = activity.

Corrected mobility is mobility corrected for EOF

7. Is the free energy sentence (underlined on page 3842) made by the authors accurately stated (if not how would you change it)? Also, does the entropy change associated with binding add to or subtract from the observed binding constant value. (8pts)

The authors got a bit careless. It is free energy change that is related to the equilibrium constant. Since they are clearly not at standard state conditions the use of the word apparent is probably appropriate. “The apparent Gibbs free energy changes associated with binding ....” Note - the binding will increase system order, decrease entropy, increase DG (make it less negative) and decrease or subtract from the value of the binding constant.