Each question is worth ten points.

A. Electromosmosis and electroosmotic flow (EOF) plays an important role in capillary electrophoresis (CE).

1) What is the origin of EOF?

Capillary electrophoresis is almost always carried out in fused silica capillaries. The surface of fused silica capillaries is rich in silanol groups that ionize, producing a negatively charged surface in capillaries. This charge is known as the zeta potential. When filled with a buffer the capillary surface accumulates positively charged ions, forming a double layer. If a potential is applied across a capillary column, the positively charge double layer moves toward the negatively charge electrode at the end of the capillary. This movement of the positively charged double layer carries the rest of the liquid in the capillary with it in a phenomenon that is known as electroosmotic flow (EOF). The rate is proportional to the zeta potential and potential in V/cm.

2) Why is EOF important in capillary zone electrophoresis (CZE)?

EOF serves a very important function in CZE. Positively charged ions move toward the negative electrode with an electrophoretic velocity ($v_{wp}$) that is equal to the electrophoretic mobility (velocity at 1 volt/cm) times potential. Negatively charged ions move in the opposite directi on. But there is an EOF velocity of the liquid in the capillary as well. It turns out the EOF velocity is greater than that of either positive or negative ions. This means that all positive, negative, or neutral substances will be swept past the detector at the negative end of the capillary, irrespective of their charge. This allow you to get by with a single detector in CZE.

3) How can you alter EOF?

You can suppress ionization and the zeta potential with pH. At acidic pH there is far less ionization. You can also suppress and thus less EOF. At pH 2-3 there is almost none. You can also add high concentrations of buffer to the mobile phase to suppress zeta potential. Postively charge polymers suppress ionization as well by titrating the charge. Finally by increasing the viscosity of the mobile phase you decrease flow but this is not about reducing charge at the walls. It is simply harder to pump liquid at high viscosity.

B. Mass spectrometry is an important mode of detection in capillary electrophoresis (CE).

4) What are the issues and how would you interface a CE system with a mass spectrometer (MS)?

The interface functions by so-called electrospary ionization. The potential at the inlet to a mass spectrometer is opposite to that at outlet of the CZE capillary. Moreover, there is a large difference in potential across the gap between the end of the capillary and MS. Because the flow of liquid through a capillary is small an auxiliary flow of liquid or gas, or both is used to enhance droplet formation at the end of the capillary. This sweeping flow causes some dilution and diminishes the sensitivity of detection relative to that achieved in liquid chromatography.

5) Based on the fact that peaks are a second or less wide in CE and samples can have thousands of components, what type of MS would be best suited for identification and quantification in this case?
High scan rates are required with CE. Because the scan rate of a time-of-flight (TOF) MS is in the microsecond time frame, it is particularly good for CE. Put another way, any instrument having a scan rate of 1-10 milliseconds or less will work well. Triple quadrupole instruments also work but you have to know what compound you are looking for.

6) Describe the issues involved and some options for quantify analytes in CE-MS.

Electrospray ionization (ESI) requires the ionization of analytes in tiny droplets of mobile phase leaving the end of capillaries. When the MS is operated in the so-called positive ionization mode this means that analytes with a positive charge migrate to the surface of the droplet and are ionized. Because there is limited surface area at the surface of droplets there is competition for access to the droplet surface. Some ions compete more effectively than others. Those that compete very well suppress ionization of those that do not. The fact that there is ion suppressions means that some (many) analytes do not ionize quantitatively and there can be large differences in relative molar response of an MS that are variable from sample to sample. One must find some way to overcome this phenomenon. This is frequently achieved by addition of isotopically labeled internal standards at known concentration. Because the mass of the standard differs from the analyte the MS can discriminate between them and the concentration of the analyte determined by the isotope ratio.

C. There are multiple forms of capillary electrophoresis.

7) What is isoelectric focusing (IEF) and how are analytes resolved by IEF?
IEF is achieved by filling a capillary with an ampholine and forming a stable pH gradient across the length of the capillary through voltage application. When a sample containing a protein mixture is applied to the column and voltage applied, proteins will migrate to their isoelectric point (pI) and focus. IEF and the establishing of a stable pH gradient are enabled by the ampholine. An ampholine is a mixture of 1000 to 2000 species that are themselves amphoteric, each having its own pI. In the ideal gradient each ampholine specie is present in the same amount. An example of a synthetic ampholine is a polyethylene that has been reacted with acrylic acid.

8) How are molecules resolved according to the hydrodynamic radius in CE?
By gel electrophoresis, i.e. electrophoresis through a gel filled capillary. Electrophoretic mobility of macromolecules is described by the equation

\[ \mu_p \propto \frac{qz}{\pi \eta a} \]

where \( \mu_p \) is electrophoretic mobility, \( qz \) is the number of charges, \( n \) is viscosity, and \( a \) is hydrodynamic radius. The hydrodynamic radius term is greatly exaggerated by adding a linear polymer such a linear polyacrylamide (100 kD) to capillaries.

D. Sample introduction and band spreading are major issues in CE.

9) How are samples introduced into CE systems; what are the issues?

Sample introduction can be achieved in two ways. One is by electrokinetic injection. The capillary is simply placed in the sample and voltage applied across the capillary. Through a combination of EOF and electrophoresis analytes are transported into the capillary but the will be a sampling bias based on the electrophoretic mobility of analytes. The second mode of sample introduction is by placing the capillary inlet into the sample solution and applying a small pressure at the capillary inlet. Sample solution is transported into the capillary by hydraulic pressure. There is no sampling bias in this case.
10) What are the origins of band spreading in CE?

Bandspreading arises in several ways during migration of an analyte through a capillary. One is by simple longitudinal diffusion according to the Einstein equation in which the width of an analyte band is defined in terms of the standard deviation ($\sigma$), $D$ is diffusion coefficient and $t$ is time.

$$\sigma^2 = 2Dt$$

A second, but smaller contributor to bandspreading is heat generated by CE. Thermal gradients across the capillary radius will cause the viscosity and thus the electrophoretic mobility to greater in the center of the capillary than at the walls, causing band spreading. A third contributor would be electrostatic adsorption. This process causes peaks to skew, increasing band width.
CRIB Biochemistry course (September 2011)

1. DNA melting curves are determined to assess the stability of double-stranded DNA. DNA stability depends on several factors including the nature of the solvent. In aqueous environment, DNA stability is influenced by pH and concentration of monovalent and divalent ions.

   Earlier studies assessed DNA stability by determining absorbance (A260) of a DNA-containing solution as the function of temperature.

   ![DNA Melting Curve Diagram]

   The resulting curves are known as DNA melting curves. The melting temperature (Tm) corresponds to the temperature at which half of the increase in absorbance is obtained. DNA stability depends on GC and AT content of DNA. DNA molecules with high AT content melt at a lower temperature (red plot) than those that are GC-rich (blue plot).

2. Since DNA melting profiles are often broad, it might be difficult to accurately determine the midpoint of a melting transition. For that reason, researchers often construct a differential melting curve for determining Tm.

   To obtain a differential melting, A260 is determined at two temperatures: A1 at T1 and A2 at T2. Usually T2 - T1 (ΔT) is about 3°C. To construct a differential melting curve, A2-A1 is determined.

   Subsequently, (ΔA)/(ΔT) is plotted as the function of Temperature: T = (T2+T1)/2
For EthBr containing samples, after UV excitation, fluorescence is determined at two temperatures: F1 at T1 and F2 at T2. To construct a differential melting curve, \((\Delta F)/(\Delta T)\) is plotted as the function of Temperature: \(T = (T2 + T1)/2\)

3. Strategies for determining DNA stability includes use of Ethidium Bromide (EthBr). EthBr has a planar structure. It intercalates between two adjacent Watson-Crick base pairs in double-stranded DNA. EthBr interacts poorly with single-stranded DNA.

When exposed to ultraviolet light, EthBr will fluoresce producing an orange glow.

When EthBr is bound to double-stranded DNA, its fluorescence intensity increases nearly 20 folds. Fluorescence intensity does not significantly increase when EthBr is bound to single-stranded DNA. Consequently, researchers can use EthBr to follow the stability of double-stranded DNA as the function of temperature.

Since EthBr interacts strongly with double-stranded DNA but poorly with single-stranded DNA, for a homogenous DNA sample one could expect the melting curve shown below.

![DNA melting curve of a homogenous double-stranded DNA sample in the presence of EthBr](image)

4. In the sequence shown below, a * marks the position of methylated C residues in a short DNA fragments:

---------------AT*CGGTCCCT*CGTTAAAA--------------

The sequence obtained after sulfonation and desulfonation reactions:

---------------AT*CGGTUUUT*CGTTAAAA--------------

5. The sequence after several rounds of PCR amplification:

---------------ATCGGTTTTTTCGTTAAAA--------------

2
6. The reason for the difference in the observed differential melting curves of two DNA samples:

In sample 1 (0% methylated DNA), all Cytosine residues were converted to U after sulfonation and desulfonation reactions.

In sample 2 (100% CpG-methylated DNA), methylated C residues were not converted to U.

After PCR reactions, the DNA in sample 2 will be more GC-rich than the DNA in sample 2. Consequently, the DNA in sample 2 melts at a higher temperature.
Question 1 (21 points)
During the recent BP oil spill in the Gulf of Mexico, many people were saying that Nature has organisms that can eat and degrade the oil. For the most part, these organisms are bacteria. Although there may be many oil eating bacteria and enzymes within, only two have been characterized in appreciable detail. Both enzymes use inorganic chemistry to catalyze the breakdown of oil.
A) What are these bioinorganic, oil eating enzymes called?
B) What metals are used by the enzymes? Answer for both enzymes.
C) Draw a very rough picture of each enzyme active site. You need only show the relative orientation of the metals and a general idea of how they are bound by the protein.

Question 2 (18 points)
The infrared stretching frequency of CO gas is 2143 cm\(^{-1}\). The CO stretching frequency is provided for each of the following compounds.
\[
\begin{align*}
[Mn(CO)]^+ &= 2090 \text{ cm}^{-1} \\
Cr(CO)_6 &= 2000 \text{ cm}^{-1} \\
[V(CO)_6]^+ &= 1866 \text{ cm}^{-1} \\
[Ti(CO)_6]^2- &= 1750 \text{ cm}^{-1}
\end{align*}
\]
A) Why do these stretching frequencies differ from that of CO gas?
B) Explain the trend.

Question 3 (21 points)
Think about gold mining.
A) What is the classic method used to extract gold from the ore dug out of the ground?
B) Why is it bad?
C) Propose an alternative.

Question 4 (20 points)
\[
\begin{array}{c}
\text{H}_3\text{N} \quad \text{Pt} \quad \text{Cl} \\
\text{H}_3\text{N} \quad \text{Cl}
\end{array}
\]
A) What is the claim to fame of this molecule? What is it called most often?
B) Give a brief explanation of the mechanism by which this compound does what it is famous for. One paragraph maximum for your explanation.

Question 5 (20 points)
The color for each titanium halide is provided.
\[
\text{TiF}_4 \quad \text{colorless/white}
\]
Organic crib is exam itself
September 24, 2011
Written by Professor Chmielewski
Ph# 40135

No Physical crib available
September 24, 2011
Written by Professor Wasserman
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