MULTIPLE CHOICE (2 pts each). Circle the letter corresponding to the correct choice.

1. The basis of the technique of chromatography for separating components of a mixture is
   A. the differing movement of particles of different mass in an electrical field
   B. the interaction of the components with a stationary and a mobile phases
   C. the absorption of infrared radiation by the components.
   D. the deflection of charged particles in a magnetic field.

2. Analytical techniques used in forensic chemistry should ideally be non-destructive, that is, the sample should not be destroyed during the analysis. Which of the following detectors used in either GC or HPLC is correctly identified as either destructive or non-destructive?
   A. FID detector in GC is destructive
   B. Refractive Index (RI) detector in HPLC is destructive.
   C. The Atomic Emission Detector (AED) used in GC is non-destructive
   D. The absorbance detectors (UV-VIS, Fluorescence, IR) used in HPLC are destructive.

3. In gas and liquid chromatography, the concentration of a substance can be determined by
   A. comparison of the retention time (Rt) of the substance with that of the standard
   B. measurement of the height of the peak produced by the substance
   C. calculation of the selectivity factor, α of the substance
   D. comparison of the peak areas of the substance vs. that of an internal standard.

4. Which of the following is the most suitable gas to use as a carrier gas in a gas chromatography?
   A. CO
   B. He
   C. CH₄
   D. NO

5. A student runs a sample of n-propanol through a gas chromatogram at 95°C. The n-propanol produces a peak after 4.2 minutes. The student then injects a mixture of unknown organic substances into the same column at the same temperature. There are peaks after 3.1, 4.2 and 7.4 minutes. From this information, it can be concluded that
   A. the mixture has at least three components, one of which must be n-propanol.
   B. the mixture has three components, but n-propanol is not one of them.
   C. the mixture has three components, one of which must be n-propanol.
   D. the mixture has at least three components, one of which might be n-propanol.

6. A new youth drink contains sugar, salt, alcohol and vitamin C. A GC could be used to determine the ...
   A. Concentration of all the ingredients in the drink
   B. Alcohol content only
   C. Alcohol and sugar content only
   D. Alcohol, sugar and vitamin C content of the drink.

7. A mixture of polyacrylic acid (PAA, MW = 4500) and polymaleic acid (PMA, MW = 16000) is approximately 90% PMA and 10% PAA. The mixture is passed through a gel permeation column with a cut-off MW of 10,000. The chromatogram obtained is likely to show that, compared to PMA, polyacrylic acid has
   A. longer retention time and greater peak area
   B. longer retention time lesser peak area
   C. shorter retention time and greater peak area
   D. shorter retention time and lesser peak area
8. Of the following compounds, which would you expect to elute first from a gas chromatography column?
   A. Methanol
   B. Ethanol
   C. n-propanol
   D. n-butanol

9. Of the following compounds, which would you expect to elute last from a reverse-phase liquid chromatography column?
   A. Methanol
   B. Ethanol
   C. n-propanol
   D. n-butanol

10. Which of the following is not a common detector for gas chromatography?
    A. Refractive Index Detector
    B. Flame Ionization Detector
    C. Thermal Conductivity Detector
    D. Mass Spectrometry
    E. All of these are common detectors for GC

11. In gas chromatography, if two solutes with short retention times co-elute (i.e. are not resolved), what is the simplest way to attempt to resolve the peaks?
    A. Use a longer column
    B. Use a higher column temperature
    C. Use a lower column temperature (at lower T elution times are longer)
    D. Use a more polar solvent
    E. Use a different carrier gas

12. In normal phase HPLC,
    A. the mobile phase is polar and the stationary phase is nonpolar.
    B. the mobile phase is nonpolar and the stationary phase is polar.
    C. eluent strength is increased by adding a more polar solvent to the mobile phase.
    D. both the mobile and the stationary phase are non-polar
    E. eluent strength is increased by adding a less polar solvent to the mobile phase.

13. The type of chromatography wherein the analyte ions in the mobile phase is attracted to the counter ions in the stationary phase is
    A. Ion-pair chromatography
    B. Ion exclusion chromatography
    C. Ion-Exchange chromatography
    D. Affinity chromatography
    E. Size exclusion chromatography
14. Examine closely the two chromatograms in the previous page. Which of the following is likely true for chromatograms A and B above?

A. A and B are GC chromatograms  
B. A and B are HPLC chromatograms  
C. A is a GC chromatogram while B is an HPLC chromatogram  
D. A is an HPLC chromatogram while B is a GC chromatogram

SHORT ANSWERS: (47 pts)

1. There are three terms in the van Deemter equation: the A term, the B term, and the C term, each describing a different type of contribution to the band broadening. Explain the type of band broadening described by 2 of the 3 terms. (6 pts)

A Term: Multiple path term—solute molecules can take different paths through the stationary phase, so some take longer to go through column than others. This is term is independent of flow rate.

B Term: Longitudinal diffusion term—the longer time a solute stays on the column the more time it has to diffuse, therefore spreading out its concentration. This term is inversely proportional to flow rate (i.e. longer time on column with slower flow rate and vice versa)

C Term: Mass transfer term—this arises from slow equilibrium for solutes partitioning between the mobile and stationary phases. If the equilibrium is slow, while a solute molecule is in the stationary phase other molecules will have traveled down the column a certain distance. The overall result is band spreading that is proportional to the flow rate.

2. A. What is the order in which acetone, acetamide and 1,2-dichloroethane would be eluted from an HPLC column packed with C\textsubscript{18} hydrocarbon bonded to the siloxane backbone material? (4 pts)

   Acetamide first  
   Acetone  
   1,2 dichloroethane last to elute

B. Give an example of a mobile phase solvent/solvent system that can be used with the column above. (3 pts)

   Any polar solvent or mixtures of those: water, methanol, acetonitrile, etc.

C. Roughly sketch a chromatogram (label the peaks) that would be obtained if the mixture in A) is run through a column with –C\textsubscript{2}H\textsubscript{4}CN functional group attached to the siloxane backbone and n-Hexane as the mobile phase. (4 pts)

   A chromatogram showing 1, 2 dichloroethane eluting first then acetone and last is acetamide.
3. In GC, the flame ionization detector is probably the most common detector in use while in HPLC systems, the most common detector is a UV-VIS spectrophotometer.

A. Explain the difference in the detector systems suited for GC and HPLC. (4 pts)

B. In HPLC, how is the detector different from the “normal” UV-VIS spectrophotometer? In other words, what modifications had to be made for its use in HPLC? (a diagram can be useful) (4 pts)

4. Using and example, describe what is gradient elution and when is it advantageous to use gradient elution instead of isocratic elution in HPLC? (4 pts)

5. Outline the reaction in the preparation of a C18 bonded packing material for HPLC from silica and the appropriate bonding agent. (4 pts)

6. What is a guard column and why is it used in HPLC but not in GC systems? (4 pts)
7. Sketch a diagram for an HPLC system that is capable of an isocratic elution. Label each part. Specify a detector that you can use for your system. (10 pts)

PROBLEM SOLVING: (25 pts) Show your solution. Circle final answer.

1. A chromatograph of a two component mixture is shown next page. The LC column is 25-cm long. The flow rate was 0.40 mL/min. Using the chromatograph determine/calculate the following:
   A. The time components A and B spends on the stationary phase. (4 pts)
   \[ t_{RA} = 30 \text{ min} \]
   \[ t_{RB} = 50 \text{ min} \]

   B. The retention factor for components A and B. (4 pts)
   \[ k_A' = \frac{t_{RA} - t_m}{t_m} = \frac{30 - 5}{5} = 5 \]
   \[ k_B' = \frac{t_{RB} - t_m}{t_m} = \frac{50 - 5}{5} = 9 \]

   C. The resolution between the two peaks (4 pts)
   \[ R_s = 2 \left( \frac{t_{RB} - t_{RA}}{w_A + w_B} \right) = 2 \left( \frac{50 - 30}{18 + 22} \right) = \frac{2 (50 - 30)}{40} \]
   \[ w_A = 19 \]
   \[ w_B = 22 \]
   \[ R_s = 1.0 \]

   D. What column length would be required to achieve a resolution of 1.75? (5 pts)
   \[ N_A = 16 \left( \frac{t_{RB}}{w_A} \right)^2 = 16 \left( \frac{30}{19} \right)^2 = 44 \]
   \[ N_B = 16 \left( \frac{t_{RA}}{w_B} \right)^2 = 16 \left( \frac{50}{22} \right)^2 = 82 \]
   \[ N_{AV} = 63 \]
   \[ H = \frac{25 \text{ cm}}{63} = 0.40 \]
   \[ L = 19.3 \times 0.40 = 7.7 \text{ cm} \]
2. A GC separation was conducted on a sample containing a pesticide analyte, Pymetozine. This sample was treated with an internal standard of chlorobenzene, giving a concentration of 15.0 ppm. A 1.0 μL injection onto the GC gave an FID response of 1012 for chlorobenzene and 3411 for Pymetozine. A 1.0 μL standard solution of 30.0 ppm chlorobenzene with 15.0 ppm of Pymetozine was injected giving a response of 899 and 2791 respectively. What is the concentration of Pymetozine in the sample? (8 pts)

In Standard Solution

\[
\frac{A_p}{C_p} = \frac{RF}{C_c} \times \frac{A_c}{C_c} \\
\]

\[
\frac{2791}{2301} = RF \left( \frac{899}{30} \right) \quad RF = 6.21
\]

In Sample:

\[
\frac{3411}{C_p} = (6.21) \left( \frac{1012}{15} \right)
\]

\[
C_p = 8.16 \text{ ppm}
\]

--- END OF EXAM ---