25. Please answer the following questions True or False (2 points each).

a) Gradient elution liquid chromatography refers to systems where the flow rate of the mobile phase is changed (either as gradual increase or in steps) during the course of the separation.

b) To perform sensitive anion exchange chromatography using conductivity detection, the suppressor or stripper component of such a system would normally be made with a high capacity cation exchange packing or membrane.

c) Thermal conductivity detectors are always more sensitive than flame ionization detectors because in the latter, the carrier gas/solute mixture must be diluted with hydrogen gas.

d) Normal phase liquid chromatography would involve using a mobile phase that is very non-polar, and a stationary phase that is also very non-polar.

e) At low mobile phase flow rates, the efficiency of a GC system will always increase to a greater degree than in an LC system because of the enhanced diffusion coefficients of solutes in the gas vs. liquid mobile phases.

f) In temperature programming for GC analysis, the temperature of the column is usually lowered during the separation to reduce the retention time of late eluting solutes.

g) For ion exchange chromatography of cations, one would expect that Cs^+ would elute later than K^+ because cesium ions have a larger ionic radius.

h) A photomultiplier tube will always yield in decrease in current as the intensity of light striking it increases.

i) Diode array detector-based spectrophotometers generally have poorer spectral resolution (higher bandwidths) than instruments that scan a diffraction grating type monochromator to impinge radiation of a given bandwidth on a single channel detector (e.g. PMT).

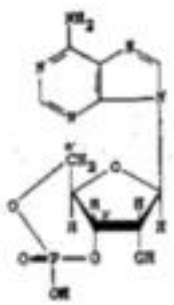
j) In size exclusion chromatography, solutes with the largest size and molecular weight will always have the longest retention time, since they move down the column very slowly owing to their large size.

26. Sketch (on the same figure) the three van Deemter plots you would expect to obtain for an HPLC experiment in which the following different columns were use (10) :

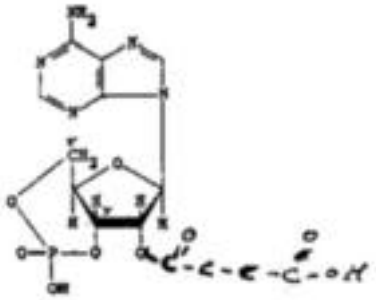
a) a 25 cm long column packed with 5 µm C-18 reversed phase silica
b) a 15 cm long column packed with 5 µm C-18 reversed phase silica
c) a 25 cm long column packed with 10 µm C-18 reversed phase silica

27. Explain via text and a figure, how a thermal conductivity detector (TCD) works to measure levels of eluting organic solutes in gas chromatography, and briefly discuss its advantages and disadvantages vs. other GC detectors. (10)

28. Some time ago, an energetic graduate student in our department attempted to separate two nucleotide compounds by reversed phase HPLC. The two compounds were cyclic-AMP (1) and the $O^{2'}$-monosuccinyl of cyclic AMP (2). The structures of these two compounds are shown below:



(1)



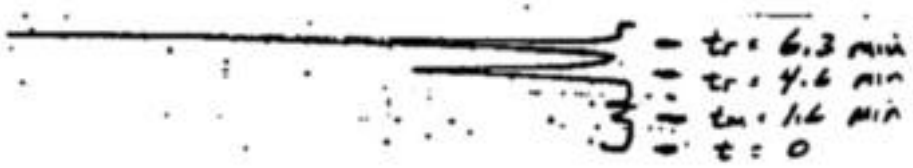
(2)

The pKa of the cyclic phosphate in both compounds is approximately 8.0, while the pKa for the carboxyl group of the succinyl derivative is approximately 5.2. The graduate student ran two different isocratic chromatograms with the following mobile phases: (a) 12 % methanol, 88% phosphate buffer, pH 5.5; and (b) 12% methanol, 88% phosphate buffer, pH 6.0. Both chromatograms were obtained with the same flow rate on the same reversed phase column. The injected sample contained approximately a 50/50 mixture of the two compounds. Based on the resulting chromatograms shown below, please answer the following questions:

a) Identify which component eluted first and explain via general chromatographic theory why the resolution between the two components was so much improved when the mobile phase was changed to the pH 6.0 buffer system. Make sure you cite chemical reasons for the improvement in resolution. (8)

b) calculate the selectivity factor (α) for the separation of the two solutes in each chromatogram. (4)

(a)



(b)