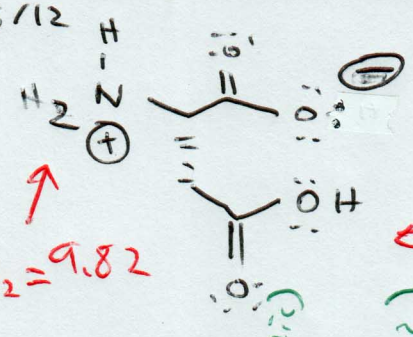
6/6/12

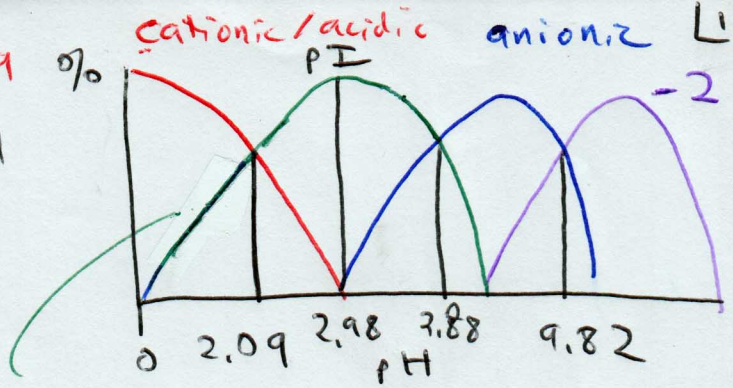


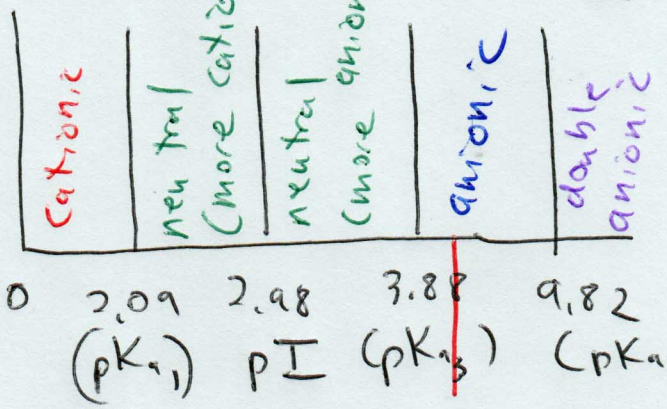
$pK_{a_1} = 2.09$

L-aspartic acid

$pK_{a_3} = 3.88$

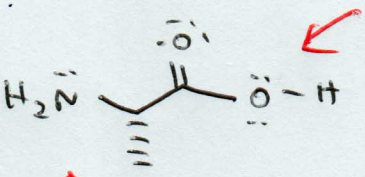
$pK_{a_2} = 9.82$





$$pI = \frac{pK_{a_1} + pK_{a_3}}{2} = 2.98$$

@ pH=4; anionic form predominates >

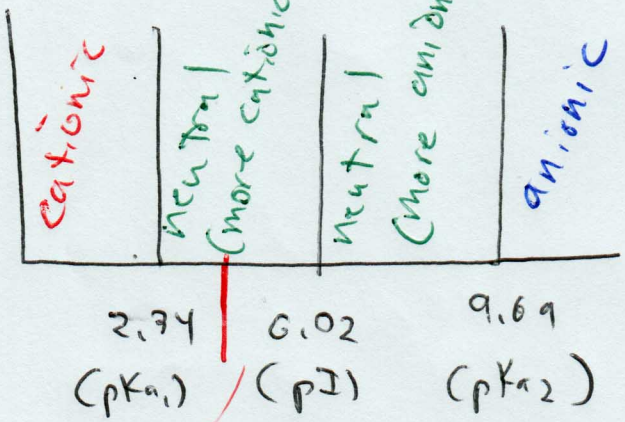


$pK_{a_1} = 2.34$

L-alanine

$pK_{a_2} = 9.69$

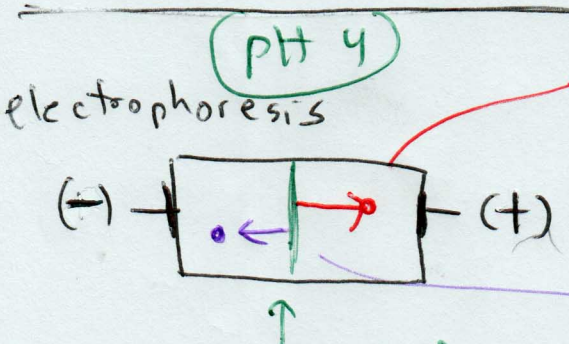
$$pI = \frac{pK_{a_1} + pK_{a_2}}{2} = 6.02$$



@ pH=4; more neutral form

~~or~~ + cationic form

~~predominant~~ present

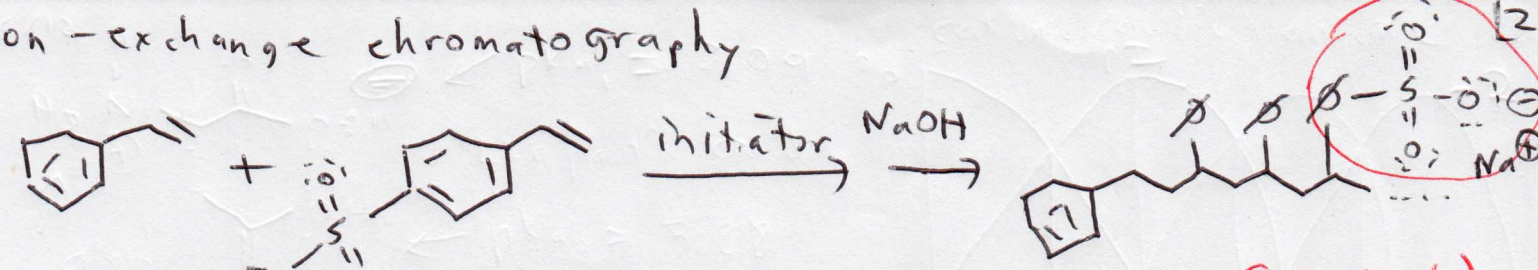


mixture of L-aspartic acid + L-alanine

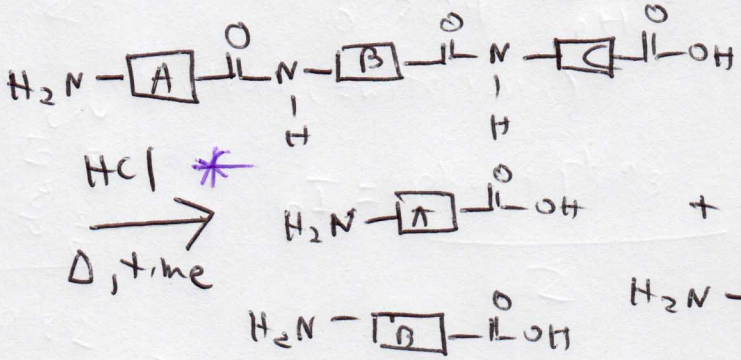
aspartic acid migrates towards the (+) terminal because the anionic (-) form predominates

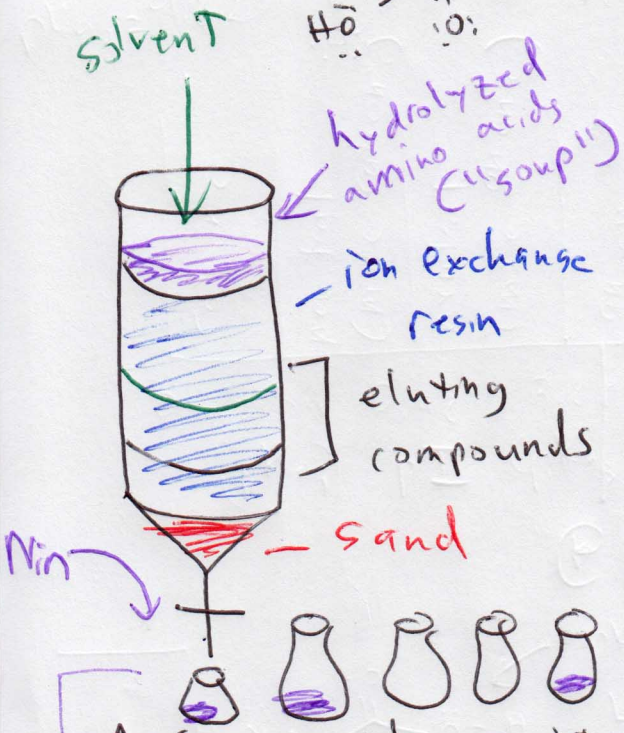
alanine migrates towards (-) terminal since it is slightly biased towards the cationic (+) form

Ion-exchange chromatography



Sulfonate salt (neutral)

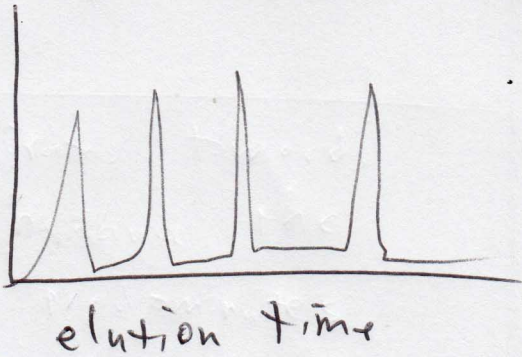




* Any information about the polypeptide sequence is lost when it is hydrolyzed.

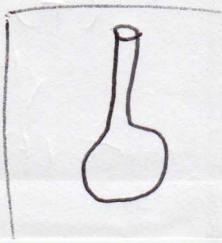
Amino acids exist in different proportions of their various ionic forms depending on sol'n pH. Based on their charge, amino acids will interact w/ an ion exchange resin to different degrees. If a specific protocol is consistently used, the identity of an amino acid can be determine by the time it takes for the amino acid to pass through the column (retention time / elution time)

Fractions – small portions of collected sol'n



Beer's Law: $A = \varepsilon c \ell$

absorbance — extinction coefficient — concentration — path length



The concentration of a sol'n of ninhydrin derivative (Ruhman's Purple) can be determined by spectroscopy. Since the volume of the sol'n can be measured, the moles of amino acid that led to the ninhydrin derivative can be determined ($n = M \cdot V$). This means the mole fraction of each amino acid in a polypeptide can be determined.