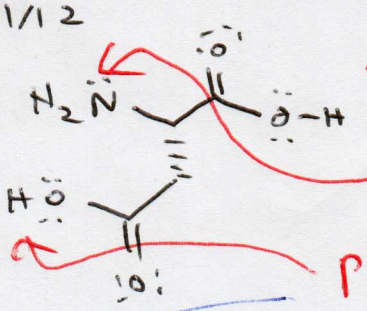
6/1/12



$pKa_1 = 2.09$ ← @ pH = 2.09

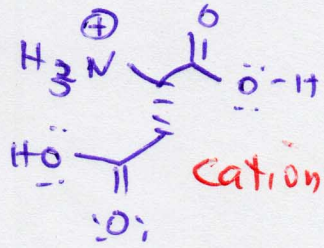
$pKa_2 = 9.82$

$pKa_3 = 3.86$

L-aspartic acid

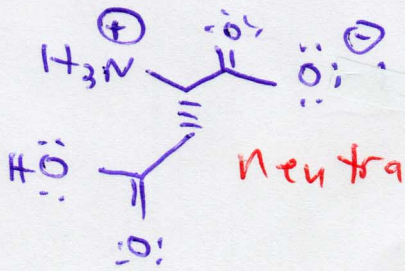
$pI = 2.98$

50/50



cationic

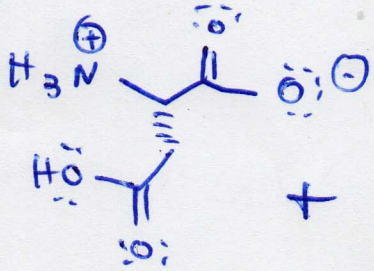
+



neutral

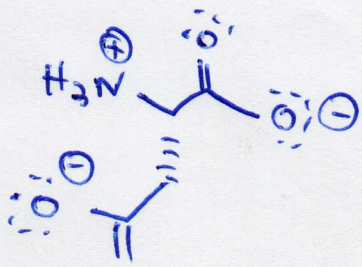
@ pH = 3.86

50/50



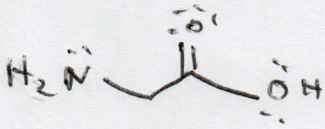
neutral

+



anionic

- ~~At~~ For L-aspartic acid, ~50% of the amino acid is in its neutral form @ pH = 2.09 and pH = 3.86. The maximum quantity of the neutral form will therefore exist between those two pH values → $pI = \frac{pKa_1 + pKa_3}{2}$



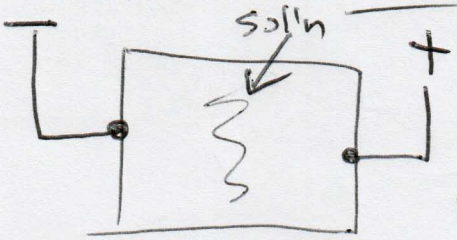
glycine

$pKa_1 = 2.34$

$pKa_2 = 9.60$

$pI = 5.97$

Imagine a soln contains both glycine + L-aspartic acid. @ pH 4, a larger proportion of L-aspartic acid will be in its anionic form, since the soln is more basic than the pI. Conversely, glycine will have a larger proportion of its cationic form, since the soln is more acidic than the pI.



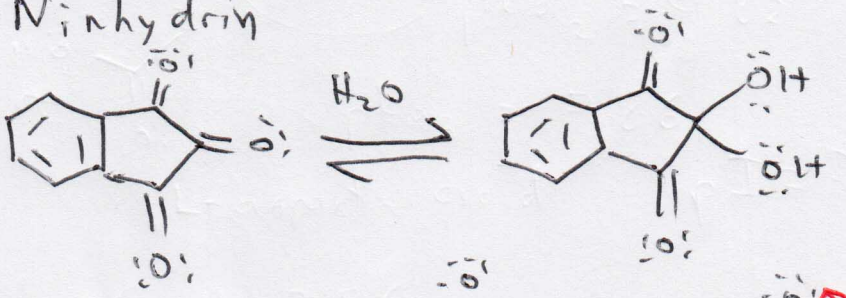
@ pH 4

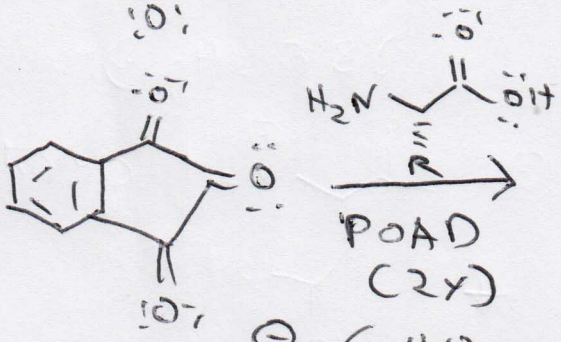
In this case, the amino acids can be separated by charge, since @ pH 4 one molecule will predominate in

its cationic form, the other in its anionic form.

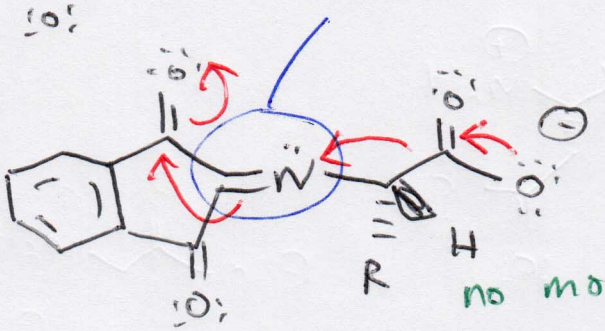
→ Electrophoresis

Ninhydrin

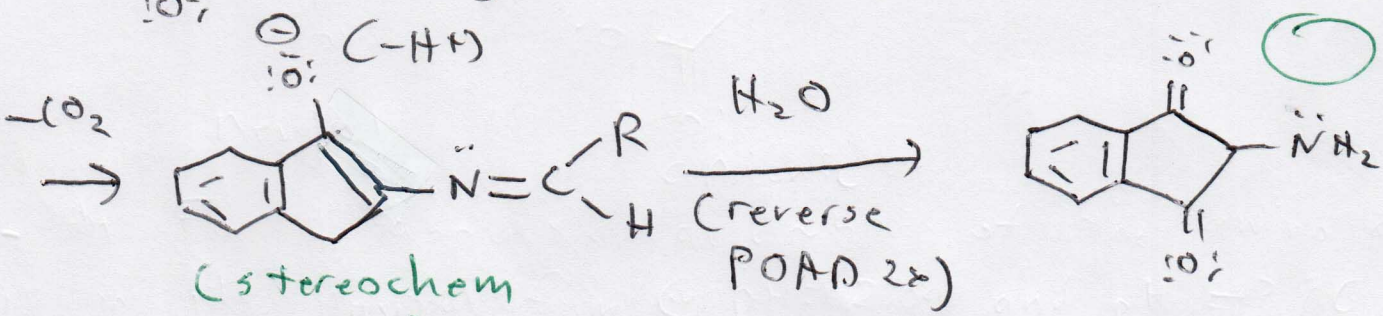


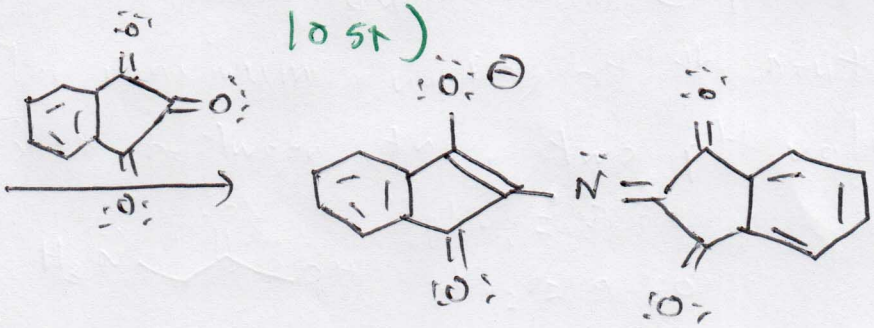


imine



no more R groups!

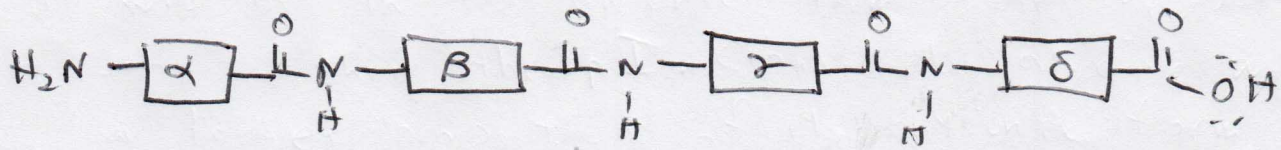




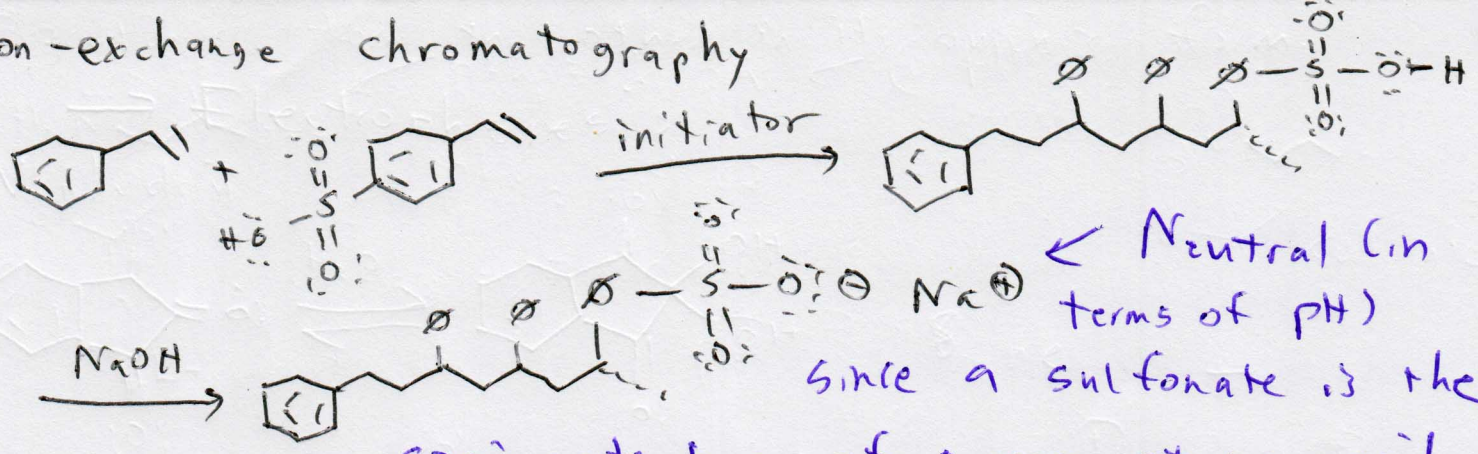
Ruhemann's Purple

Sequencing - determining the order and kind of amino acids in a protein, the tetrapeptide αβγδ

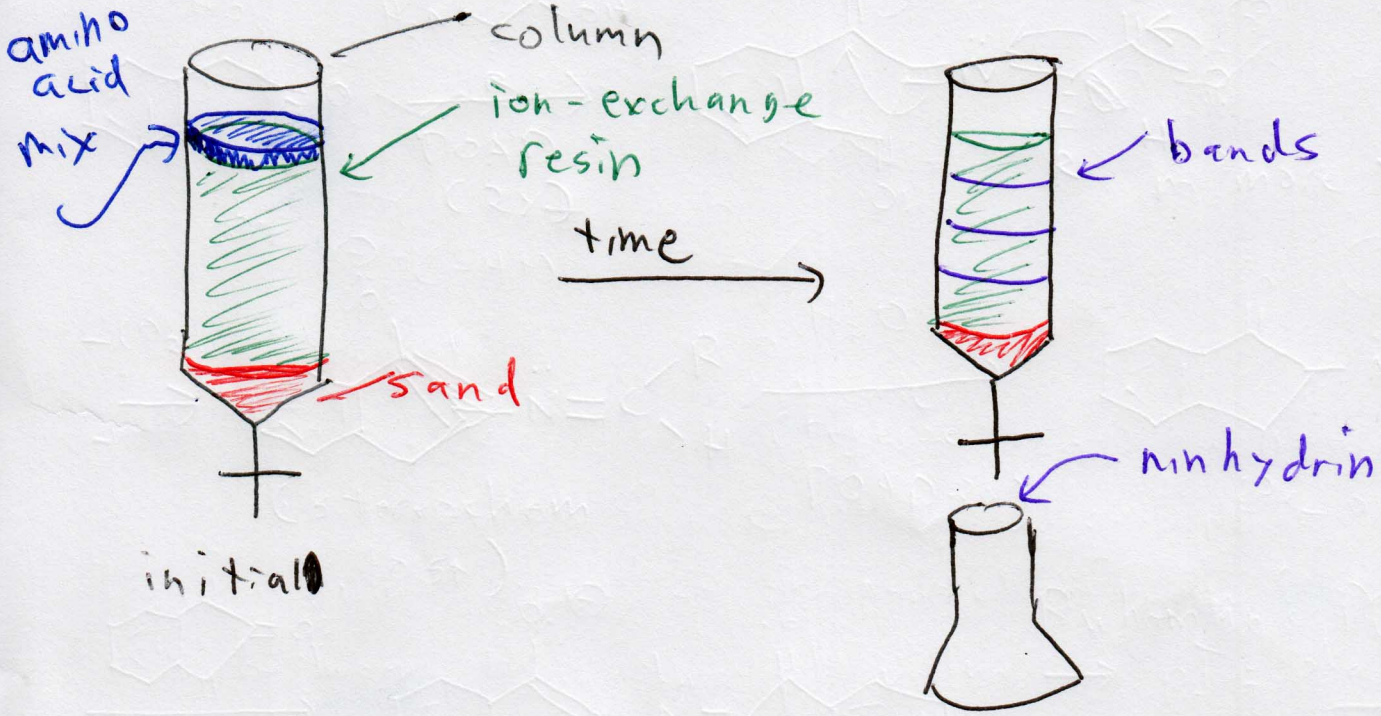
protein ⇌ polypeptide



Ion-exchange chromatography



← Neutral (in terms of pH) since a sulfonate is the conjugate base of a very strong acid.



Once a polypeptide has been fully hydrolyzed, the amino acid "soup" is added to the top of an ion-exchange column. A solvent is forced through the column, some of the amino acids travel more slowly because they are found in greater proportion in their cationic form, so they interact heavily with the ion exchange resin. A protocol has been established to identify an amino acid by the amount of time it takes to elute (come off the column) → retention time or elution time