Ampholytes, isoelectric point, biochemical examples

Ampholytes, Isoelectric Point and Biochemical Examples

Amphoteric and Amphoprotic

- Definition of “amphoteric”: An „amphoteric species is a molecule or ion that can react as an acid as well as a base”. The word roots in greek language meaning “both”. Amphoteric substances are called ampholytes.
- Definition of “amphoprotic”: An „amphiprotic molecule (or ion) can either donate or accept a proton, thus acting either as an acid or a base”.

Thus: All amphiprotic (proton needed!) substances are amphoteric (substances must act as acid/base – no proton required), but not vice versa!
In biochemistry are widely spread example of amphoteric molecules are proteinogenic amino acids, since they have at least an acidic carboxylic group and an alkaline amino group. The amino group can accept a proton and acts as base, while the carboxylic group can donate one and acts as acid (this depends on the pH value).

pI Value

Each of those groups has its own pK value. To find the isoelectric point, you add the two pK values and divide them by two.

pI = (pK1+pK2)/2

At the pH that equals the isoelectric point (pI), the amino acids are so-called “Zwitterions”, meaning, they are electrically neutral with groups in the form of $-NH_3^+$ and $–COO^-$. The pI is for some amino acids around the physiological pH of around 7 (for Glycin e.g. pI = 6,0), but some other proteins have a pI of larger than 10 (histone proteins) or below 1 (Pepsin).

If you put amino acids (for easier understanding assume a neutral side chain) in an acidic environment (coming from neutral pH), an additional proton will be added to the amino acid:

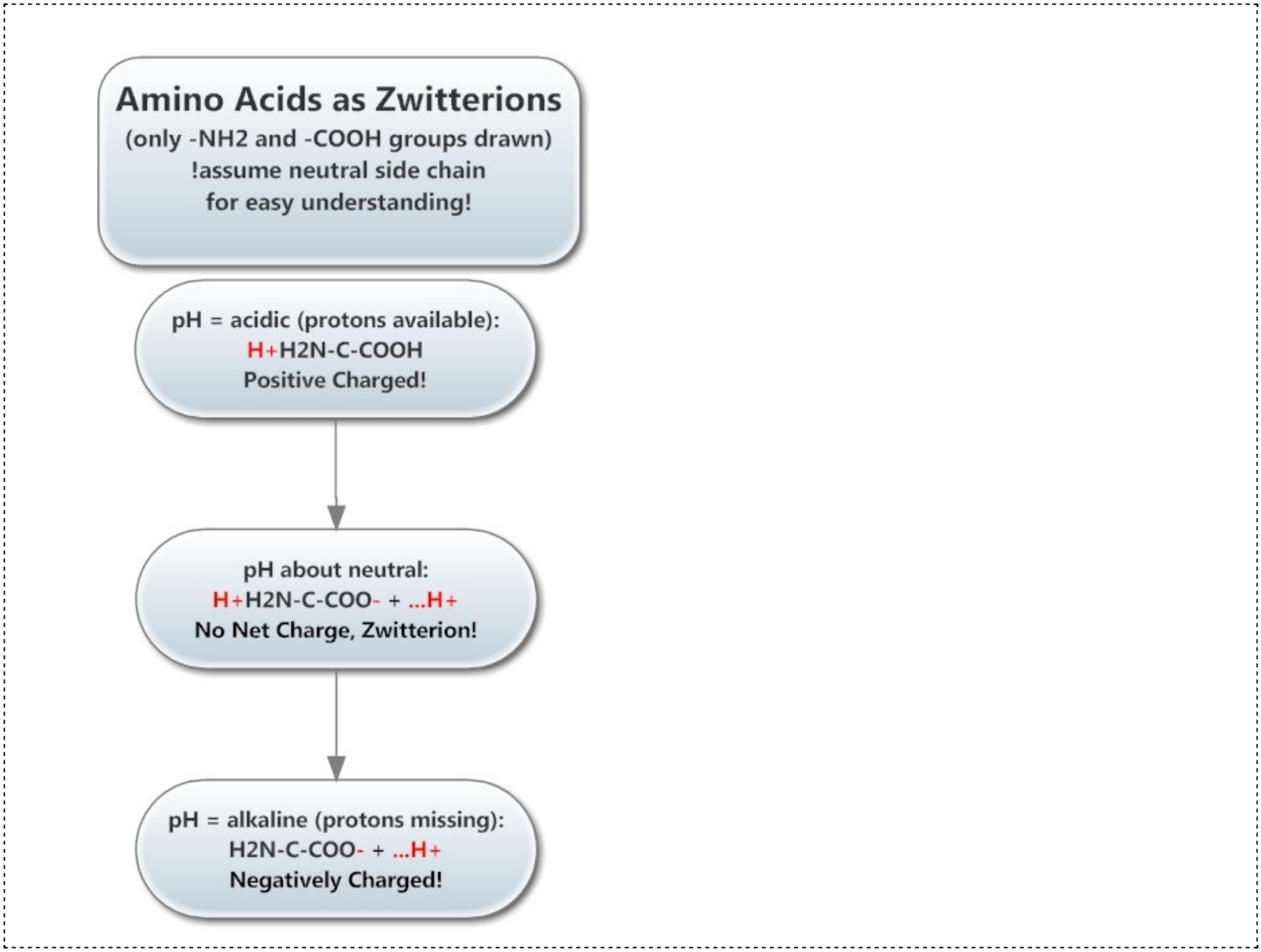
- -NH2 becomes –NH3+
- -COOH is already fully protonated and does change
- => The amino acid becomes positively charged in low pH.

If you put the same amino acid in an alkaline environment, protons will be taken away from the amino acid:

- –NH3+ becomes –NH2
- -COOH becomes –COO-
- => The amino acid is negatively charged in alkaline solutions.

There´s an in-between-state, where there is still one proton attached to the amino group and already one proton missing at the carboxylgroup. This is the case at pI, where the negative (carboxyl group) and positive (amino group) charge cancel out. The Zwitterion has a net charge of 0.

Here´s the order of protonation/deprotonation depending in which direction the pH moves:



Separation by Isoelectric Focusing

Net charge of proteins is dependent of pH. At isoelectric point, the molecule doesn´t move in an electric field (for it´s net charge 0). To separate the proteins you have to create a pH gradient as stationary material (synthethized polyelectrolytes with a lot of positive and negative charges with different pK values).
Then a mixture of proteins will be applied to the electrophorese gel and a voltage applied. The charged molecules start migrating, until they reach a region, where the pH equals their pI (making them electrically neutral). There they will stay. If the molecule diffuses somewhere (e.g. for temperature reasons), it is recharged and will migrate back to it´s pI region. This leads to bands of high resolution.

Positively charged molecules migrate towards the minus pole (cathode) and vice versa.

You can do this 2-dimensional, having the dimensions a) pH and b) molecular weight. This means combining isoelectric focusing (depends on pH) with SDS-Page (depends on molecular weight). First you do isoelectric focusing, which leaves you with a kind of “line” (first dimension) with separated proteins according to isoelectric points. Then you apply this to SDS gel and again use voltage to let the molecules migrate into it (second dimension). Use coloring or marking to detect “protein spots” on an area.

Examples of biochemical important ampholytes

- Amino acids (see above), which can as well have more acidic (Glutamic Acid and Aspartic Acid) or basic groups (Histidin, Arginine, Lysine) influencing the pI
- as well as other proteins like histone proteins or Pepsin or peptides

Sources: Biochemie, Werner Müller-Esterl, 2nd edition, Spektrum Akademischer Verlag (german issue)
http://en.wikipedia.org/wiki/Amphoterism