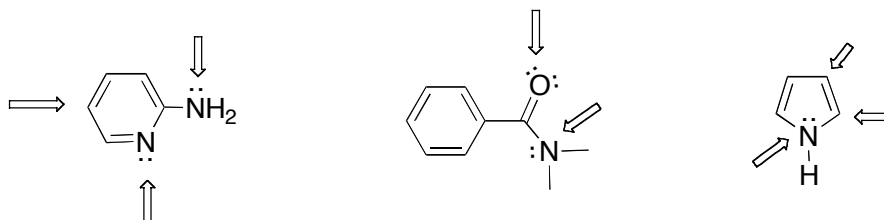


Problem Set: Predicting the Extent of Protonation and the Preferred Sites of Protonation in Organic Molecules

The protonation state of relevant functional groups is important in defining the types of noncovalent bonding interactions available to drugs and protein side chains. It is equally important to know *where* protonation occurs in drug molecules. This defines the true structure of the drug in physiological solution.

1. Predict the preferred site of protonation for the molecules shown below.

Approach: protonate the molecules at each of the indicated sites. Then draw all of the possible good resonance structures for each protonation state. The ability to draw a larger number of high quality resonance structures is typically taken as a sign of stability. So, if you can draw more good resonance structures for a particular protonation state (site of protonation)... protonation at this site is likely favored. Please see the review sheet on resonance structures on the following page. To calculate formal charges accurately it will help to always draw all lone pairs.



2. According to the table of amino acids, the lysine side chain (-CH₂CH₂CH₂CH₂NH₃⁺) has a pK_a of 10.8. What is the percent of protonated and unprotonated amine at pH 7 (-CH₂CH₂CH₂CH₂NH₃⁺ versus -CH₂CH₂CH₂CH₂NH₂). Please see the acid-base review handout on the following pages if you need guidance).

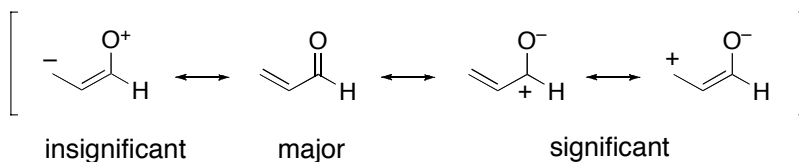
Resonance Structures

Rules and Guidelines for Drawing and Analyzing Resonance Structures

Fact: Resonance forms do not really exist as discrete structures, in equilibrium with one another. Rather, this is a method for predicting where partial charges exist in molecules.

1. Use a double-headed arrow between resonance structures; enclose structures in brackets.
2. *Atoms* don't move – the pi and nonbonded *electrons* do.
3. All structures must be appropriate Lewis structures. Octet around 2nd row elements is preferred.
4. All contributors must have the same number of paired and unpaired electrons.
5. The net charge on all contributors must be the same.
6. Not all resonance structures contribute equally to the "true" overall electron distribution in the molecule.
7. In major contributors, charges will be located on atoms of appropriate electronegativity.
8. Minimum charge separation is preferred.
9. Bond formation is (generally) thermodynamically favored. Thus, the number and strength of bonds present in each structure should be considered
10. Atoms participating in resonance must lie in the same plane. (Participating orbitals must be aligned – they cannot be orthogonal. One must consider 3D-molecular structure!)
11. Increased numbers of reasonable quality resonance structures is taken as a sign of increased stability for reactive intermediates.

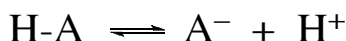
e.g.



Ionization of Functional Groups on Biomolecules and Drugs: The Use of pK_a Values

What is K_a and pK_a? Following our discussion of “weak binding forces” we can see that the ionization state of functional groups can be extremely important in drug-receptor interactions. In addition, ionization state is very important in drug solubility and transport. Charged drugs generally are more water soluble, but may not easily pass through lipid bilayers (membranes) to enter cells.

Let’s review a few facts about acid-base ionization reactions. The acidity for a given functional group is described by equilibria such as the one shown below:

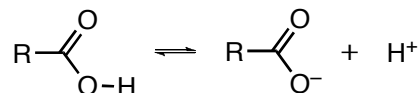


The equilibrium constant for this type of reaction is called K_a. Defined by the equation:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

A useful value related to the K_a is pK_a. Where: $\text{pK}_a = -\log K_a$

Using pK_a Values. Consider the acid-base ionization of a common functional group found in drugs, the carboxylic acid group. The carboxylic acid group can exist in the protonated (neutral) state or in an ionized (charged) form, as shown:



Let’s ask the medically-relevant question: “at physiological pH (pH = 7), does the carboxylic acid group exist as the neutral protonated form or in the ionized, negatively charged form?”

For an average carboxylic acid functional group $K_a = 1 \times 10^{-5}$; thus, the $\text{pK}_a = 5$ (Such values are found in tables and are taken as a “given” in a problem like this)

Without doing any calculations we can quickly estimate: First let us note that when $\text{pH} = \text{pK}_a$, the ratio of $\text{RCO}_2\text{H}:\text{RCO}_2^- = 50:50$, or “1”. Now, let’s take one step up to pH 6. At pH 6, the solution is less acidic. One log unit less acidic means 10-fold lower in H^+ concentration and a corresponding 10-fold change in the ratio $\text{RCO}_2\text{H}:\text{RCO}_2^-$ (to 0.1). Moving from pH 5→6, we decrease the proton concentration and the equilibrium shown above will “lean” (or be “pulled”) toward the right (in accord with LeChatlier’s Principle) yielding more of the carboxylate form $[\text{A}^-]$. At pH 6 the ratio of $\text{RCO}_2\text{H}:\text{RCO}_2^- \sim 10:90$. At pH 7 H^+ concentration drops by a factor of 10 again, pulling the equilibrium even further to the right. At pH 7 the ratio of $\text{RCO}_2\text{H}:\text{RCO}_2^- \sim 1:99$

For some functional groups the equilibrium looks like the one shown below (different charge states than those shown above). This changes the identity of the “players” but does not change the methods of analysis.



Such quick estimates provided above are often all we need to know in order to determine whether a given functional group exists predominantly in a neutral, anionic, or cationic form at pH 7.

For more accurate calculations, we can use the Henderson-Hasselbalch equation to calculate these values:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

When $\text{pH} = 7$ and $\text{pK}_a = 5$, then $2 = \log \frac{[\text{A}^-]}{[\text{HA}]}$ and $100 = \frac{[\text{A}^-]}{[\text{HA}]}$
Then solve for percent A^- and HA as shown below.

Or, we can just start with the equilibrium acidity expression to obtain exact answers to the question “at pH 7, what percentage of carboxylic acid exists in the ionized form?” Remember that:

$$K_{\text{eq}} = \frac{[\text{H}^+][\text{RCO}_2^-]}{[\text{RCO}_2\text{H}]}$$

Multiply both sides by $[\text{H}^+]$ to get:
 $K_{\text{eq}}/[\text{H}^+] = [\text{RCO}_2^-]/[\text{RCO}_2\text{H}]$

The values for K_{eq} and $[\text{H}^+]$ are known:
 $1 \times 10^{-5}/1 \times 10^{-7} = [\text{RCO}_2^-]/[\text{RCO}_2\text{H}] = 100$ (100 times more RCO_2^- than RCO_2H)!

To calculate the percent RCO_2^- we write that $\text{RCO}_2^- + \text{RCO}_2\text{H} = 100\%$
thus, $\text{RCO}_2\text{H} = 100 - \text{RCO}_2^-$. So $[\text{RCO}_2^-]/[100 - \text{RCO}_2^-] = K_{\text{eq}}/[\text{H}^+] = 100$ solve this to find that:

At pH 7, 99.01% exists as RCO_2^- and 0.99% exists as RCO_2H .