Finally, a User-Friendly Way of Computing and Presenting Individual Group Contributions to Polyprotic Ionization of Drugs

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ABSTRACT

It is tempting to "assign" the macroscopic ionization constants (apparent pK_a's obtained from titration experiments) of molecules to specific ionizable groups; however, this is strictly appropriate only in the case of monoprotic molecules. An extreme example at the other end of the spectrum is mellitic (benzene hexacarboxylic) acid [1] where the six measured apparent pK_a's range from 1.4 to 7.0 – a span of 5.6 orders of magnitude. Assigning each apparent pK_a to a specific group is clearly absurd in this case, since the six carboxylic acid groups are completely equivalent. This potentially confusing situation is clarified by considering microscopic ionization equilibria, yielding a precise thermodynamic picture, replacing the inaccurate and misleading "one group = one pK_a" paradigm. We have explored microequilibria theory in detail and have developed novel concepts: the pH-dependent Average Single Proton Acidity (ASPA), and the pH-dependent Average Site Protonation profiles (ASP). The ionization midpoint of the latter – the pK_{50} - is pH-independent and closely related to concepts from the physical chemistry of proteins. We show that the pK_{50}, unlike macroscopic pK_a, is a transferable property of an individual ionizable group, illustrating its inherent acidity in the absence of intergroup interactions. For example, we calculate a chemically realistic pK_{50} = 3.92 for each carboxyl group in the mellitic acid. In the case of monoprotic molecules as well as molecules with well-separated ionization patterns, the pK_{50}'s correspond to macroscopic pK_a's exactly and approximately, respectively. An added bonus is a direct determination of individual site occupancies from the calculated AAPP at any pH of interest, which eliminates the need to deduce these quantities from pK_a.

BACKGROUND

Polyprotic ionization is complex.

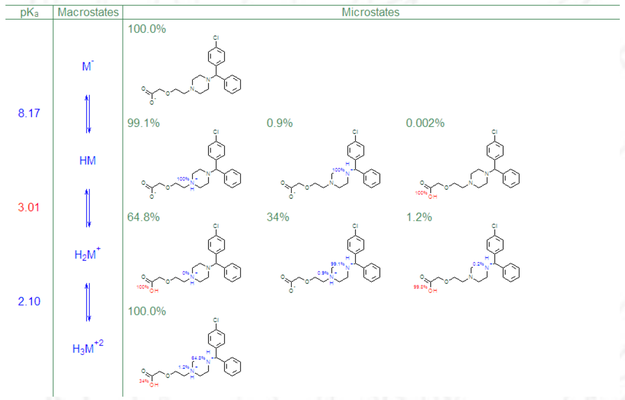

Figure 1. Experimental macro- and microscopic ionization equilibria measured for cetirizine. [2]

Ionization microconstants are individual group properties, but they strongly depend on the protonation state of other groups and are tricky to obtain.

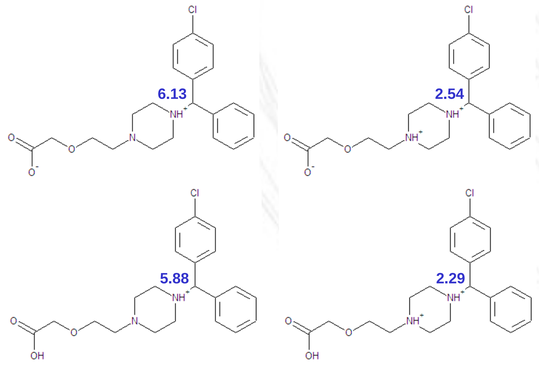

Figure 2. Blue numbers are micro-pK_a (microconstants) for the indicated tertiary amine in the selected 4 microstates where this group is protonated. All microconstant values were obtained experimentally. [2]

BACKGROUND

Apparent macroscopic pK_a measured by titration experiments reflect only the global charge state, **NOT** ionization of individual groups!

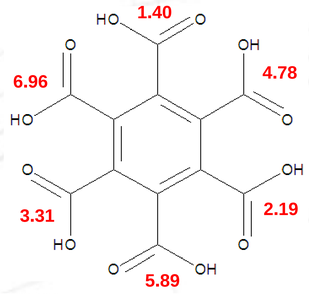

Figure 3. A clear example of misleadingly naïve "pK_a assignments" for mellitic acid reported by many software packages – equivalent groups receive values differing by as much as 5.6 orders of magnitude. Red numbers are experimental macroscopic pK_a taken from Ref. [1] placed at groups suggested by one unnamed software specializing in *in silico* pK_a predictions.

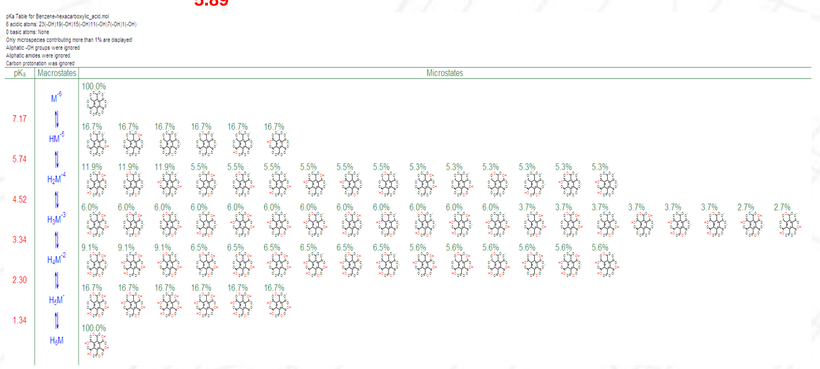

Figure 4. Ionization patterns of mellitic acid, predicted by ADMET Predictor 6.0, involve 64 microstates, none of which can be neglected.

NEW CONCEPTS

Averaged Single Proton Acidity (ASPA) – a property of an individual ionizable group (G). Defined analogously to an ionization constant:

$$ASPA(G) = \frac{[\text{sum of microstates where G is deprotonated}]\,[H^+]}{[\text{sum of microstates where G is protonated}]}$$

Averaged Site Protonation (ASP) – a property of an individual ionizable group (G). Defined analogously to a fraction ionized:

$$ASP(G) = \frac{[\text{sum of microstates where G is protonated}]}{[\text{sum of all microstates}]}$$

It can be shown that:

$$ASP(G) = \frac{[H^+]}{[H^+] + ASPA(G)}$$

Single Proton Midpoint (pK_{50}) – a property of an individual ionizable group (G). Defined analogously to a monoprotic pK_a:

$$pK_{50}(G) = pH_{midpoint} \quad \text{where } ASP(G) = 50\%$$

REFERENCES

1. Maxwell W & Partington J. Trans Farad Soc. **31**:922 (1935).
2. Marosi A, Kovacs Z, Beni S, Kokosi J and Noszal B. Eur J Pharm Sci. **37**:321 (2009).

RESULTS

Averaged Single Proton Acidity (ASPA) continuously follows a group's acidity changes affected by protonation of other groups as a function of pH. ASPA can be thus interpreted as a population-averaged "micro pK_a".

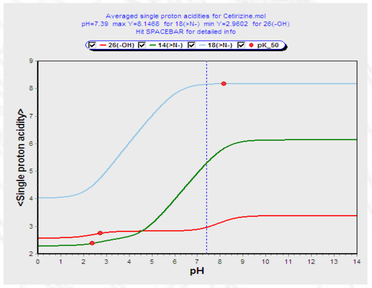

Figure 5. ASPA profiles for cetirizine calculated from data in [2]. Green curve corresponds to the tertiary amine indicated in Figure 2.

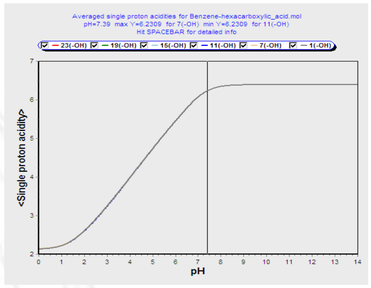

Figure 6. Predicted ASPA profiles for mellitic acid. As expected, the curves for all six carboxyl groups overlap perfectly.

Averaged Site Protonation (ASP) focuses on a single group occupation as a function of pH and resembles titration curves. The midpoint of these curves is pK_{50}.

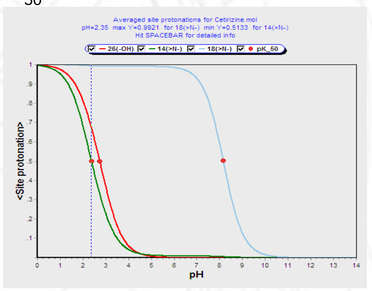

Figure 7. ASP profiles for cetirizine calculated from data in [2]. Green curve corresponds to the tertiary amine indicated in Figure 2.

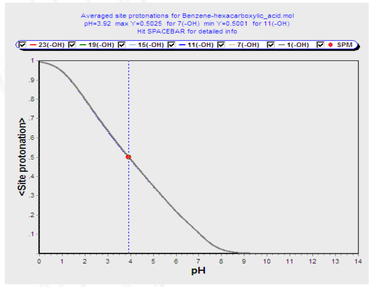

Figure 8. Predicted ASP profiles for mellitic acid. As expected, the curves for all six carboxyl groups overlap perfectly.

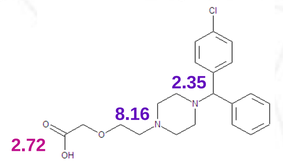

Figure 9. pK_{50} values for cetirizine calculated from data in [2].

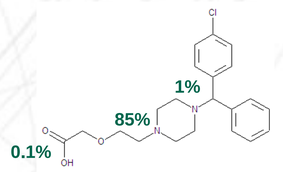

Figure 10. ASP values (as percent protonated) at pH=7.4 calculated from data in [2] for individual groups in cetirizine. Please note that the molecule's charge state at this pH can be obtained directly.

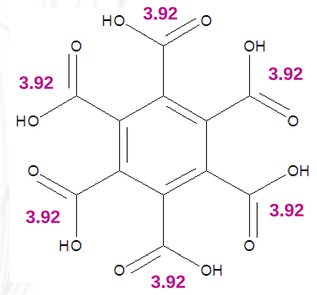

Figure 11. Predicted pK_{50} values for mellitic acid. As expected, the group equivalence is preserved. Also, 3.92 is in the expected range for carboxyl groups.

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