

Isoelectric Points On the Back of the Envelope

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The isoelectric “point” is a point on the pH scale. It is the pH at which the average charge of an amino acid or peptide is zero under the conditions of the experiment.

Although the *isoelectric pH* of a given protein will vary somewhat with the ionic composition of the solution, the isoelectric pH determined in the absence of ions other than hydronium or hydroxide, the *isoionic pH*, is a characteristic constant for a given amino acid or protein (1).

Calculation of the isoelectric point

While graphical methods (2) or spreadsheets (3) or web-based programs can be used to calculate the isoelectric pH, a quick estimate of this number, pI, with a “back-of-the-envelope” approach can be equally useful.

pI values for amino acids

For “neutral”, “acidic”, and “basic” amino acids we may not need the envelope.

Neutral amino acids

The pI of a “neutral” amino acid will be exactly half way between (the average of) the pK_a of the carboxyl group and the pK_a of the acidic form of the α-amino group.

If, for example, the carboxyl group were to have a pK_a of 2, and the ammonium group were to have a pK_a of 10 the pI of the amino acid would be 6. At this unique pH the carboxyl group would be negative for 99.99% of the time, and the ammonium group would be positive for 99.99% of the time. The average charge of the species at this pH would be zero.

Acidic amino acids

Again, as one can easily verify, the pI of an “acidic” amino acid, glutamic acid or aspartic acid, will be half way between (the average of) the pK_a values of the two carboxyl groups.

If the carboxyl groups were to have pK_a values of 2 and 4, the pI of the species would be 3. At this unique pH the more acidic carboxyl group would be negatively charged for 91% of the time, and the less acidic carboxyl group would be negatively charged for 9% of the time, while the ammonium group would be positively charged for 100% of the time. The average charge would therefore be zero at this pH.

Basic amino acids

A similar argument leads to the conclusion, which can be easily verified, that the pI values for lysine (9.74) and arginine (10.76) are half way between (the average of) the pK_a values for the acidic forms of the two basic groups of these two amino acids.

Small peptides

With small peptides the pI is also half way between (the average of) pK_a values for two of the acidic forms of groups of the peptide.

Example 1: Asp-Ile-Ala-Lys (DIAK) (Reference 3)

We start by listing (on the back of an envelope) the pK_a values (from Reference 3) of the acidic forms of groups in order of acidity along with the charge of the group at an intermediate pH, such as 5.

When we do this with the tetrapeptide Asp-Ile-Ala-Lys we get

Acidic Group	pK_a	Charge at pH = 5
Lys-α-COOH	3.1	-1
Asp-β-COOH	4.1	-1
Asp-α-NH ₃ ⁺	8	+1
Lys-ε-NH ₃ ⁺	10.8	+1

We see here that at an intermediate pH the negative charge of the Lys- α -carboxylate anion will essentially balance the positive charge of the Lys- ϵ -ammonium ion. These groups will not determine pI, and so we cross them out.

Acidic Group	pK _a	Charge at pH = 5
Lys-α-COOH	3.1	+1
Asp- β -COOH	4.1	-1
Asp- α -NH ₃ ⁺	8	+1
Lys-ϵ-NH₃⁺	10.8	+1
pI	6.05	

The isoelectric pH will be half way between (the average of) 4.1 and 8.

It is interesting to realize that so long as the pK_a value assumed for the carboxy terminal carboxylic acid is 2 or more pH units distant from 6.05 the value assigned to this carboxyl group will have essentially no effect on the pI calculated for the molecule.

On the other hand, the pK_a value assigned to the ammonium group of the amino terminal amino acid is one of the two that will determine pI. A 1.0 pH unit change in this value will change the estimated value for pI by 0.5 unit.

Three more examples

The next three examples, examples 2, 3, and 4, are from Reference 2, and the pK_a values used here are the same as those used in Reference 2.

Example 2: Gly-His-Val-Ile-Asp-Lys (Reference 2)

Listing the acidic groups of this hexapeptide in ascending order of pK_a with the charge at pH = 5 we get

Acidic Group	pK _a	Charge at pH = 5
Lys- α -COOH	2.15	-1
Asp- β -COOH	3.71	-1
His-Im ⁺	6.04	+1
Gly- α -NH ₃ ⁺	9.58	+1
Lys- ϵ -NH ₃ ⁺	10.67	+1

Expecting the pI value to be somewhere near the middle of the pH scale, we realize that the most acidic carboxyl group will surely be present “all” the time as its conjugate base, contributing a charge of -1, and that the amino group of lysine will surely be present “all” the time as its conjugate acid, contributing a charge of +1. We therefore see that their contributions to the average charge will cancel, and that they can be omitted from the estimate of the isoelectric point. We can then cross these two rows out of the table, thereby reducing this hexapeptide problem to the “basic” amino acid problem. When we do this we see immediately that the pI for this hexapeptide must be half way between the pK_a values of 6.04 and 9.58, or 7.81.

Acidic Group	pK _a	Charge at pH = 5
Lys-α-COOH	2.15	+1
Asp-β-COOH	3.71	-1
His-Im ⁺	6.04	+1
Gly-α-NH ₃ ⁺	9.58	+1
Lys-ε-NH₃⁺	10.67	+1
pI	7.81	

Example 3: Gly-His-Ile-Asp-Arg-Lys (Reference 2)

Listing the acidic groups of this hexapeptide in order of pK_a with the charge at pH = 5 we get

Acidic Group	pK _a	Charge at pH = 5
Lys-α-COOH	2.15	-1
Asp-β-COOH	3.71	-1
His-Im ⁺	6.04	+1
Gly-α-NH ₃ ⁺	9.58	+1
Lys-ε-NH ₃ ⁺	10.67	+1
Arg-Guan ⁺	12.10	+1

Suspecting that pI will be on the basic side of 7, we add a column that indicates the charge of each group at a pH of 7.

Acidic Group	pK _a	Charge at pH = 5	Charge at pH = 7
Lys-α-COOH	2.15	-1	-1
Asp- β -COOH	3.71	-1	-1
His Im ⁺	6.04	+1	0
Gly- α -NH ₃ ⁺	9.58	+1	+1
Lys- ϵ -NH ₃ ⁺	10.67	+1	+1
Arg-Guan⁺	12.10	+1	+1
pI	10.13		

We see now that contributions to the average charge of the α -carboxyl group of lysine (-1) and the guanidium group of arginine (+1) will cancel, and that the contribution of the imidazole side chain of histidine will be 0. Crossing out these rows leaves, again, the pattern for a basic amino acid, and the isoelectric pH of this hexapeptide will be the average of the numbers in the boxes.

Example 4: Gly-Tyr-Ile-Asp-Lys (Reference 2)

In this last example from Reference 2 we again list the pK_a values of the acidic groups in ascending order, and indicate the contribution of each group to the overall charge at a pH of 5.

Acidic Group	pK _a	Charge at pH = 5
Lys- α -COOH	2.15	-1
Asp- β -COOH	3.71	-1
Gly- α -NH ₃ ⁺	9.58	+1
Tyr-Phenol	10.10	0
Lys- ϵ -NH ₃ ⁺	10.67	+1

Expecting that the contribution of the α -carboxyl of lysine will cancel the contribution of the δ -ammonium group, and that tyrosine will contribute 0 to the average charge we cross out these three rows and find the pattern for a neutral amino acid. The isoelectric pH will therefore be the average of the numbers in the boxes.

Acidic Group	pK _a	Charge at pH = 5
Lys- α -COOH	2.15	=+1
Asp- β -COOH	3.71	-1
Gly- α -NH ₃ ⁺	9.58	+1
Tyr-Phenol	10.10	0
Lys- ϵ -NH ₃ ⁺	10.67	+1
pI	6.65	

Extension to larger proteins

This approach to the estimation of the isoelectric pH of small peptides can be used to account in a general way for the isoelectric points of larger proteins.

Three proteins for which both the amino acid composition and the isoelectric point are available (Reference 1, pages 101 and 162) are β -lactoglobulin, bovine ribonuclease, and lysozyme.

β -Lactoglobulin

In β -Lactoglobulin the 27 aspartic acid and glutamic acid residues, 2 imidazoles, 4 tyrosines, 14 lysines and 3 arginines could be expected to contribute at a pH of 7 to a total charge of -10, as indicated in the table. We would therefore expect the isoelectric pH to be somewhat on the acidic side of 7 so that the imidazoles would be largely protonated, and the carboxylate groups would be protonated to a small degree. The reported isoelectric pH of 5.2 is consistent with this view.

Acidic Group	Number	pK _a	Charge at pH = 7
Asp/Glu-COOH	27	4	-27
His-Im ⁺	2	6	0
Tyr-Phenol	4	10	0
Lys-NH ₃ ⁺	14	11	+14
Arg-Guan ⁺	3	12	+3
pI		5.2	

Bovine ribonuclease

In bovine ribonuclease the 10 aspartic acid and glutamic acid residues, 4 imidazoles, 6 tyrosines, 10 lysines and 4 arginines could be expected to

contribute at a pH of 7 to a total charge of +4 as indicated in the table. We would therefore expect the isoelectric pH to be basic enough that the phenolic hydroxyls and ammonium groups of lysine would be somewhat deprotonated. The reported isoelectric pH of 9.6 is consistent with this view.

Acidic Group	Number	pK_a	Charge at pH = 7
Asp/Glu-COOH	10	4	-10
His-Im ⁺	4	6	0
Tyr-Phenol	6	10	0
Lys-NH ₃ ⁺	10	11	+10
Arg-Guan ⁺	4	12	+4
pI		9.6	

Lysozyme

In lysozyme the 10 aspartic acid and glutamic acid residues, 1 imidazole, 3 tyrosines, 6 lysines and 11 arginines could be expected to contribute at a pH of 7 to a total charge of +7 as indicated in the table. We would therefore expect the isoelectric pH to be somewhat on the basic side of 10 so that the phenolic hydroxyls and ammonium groups of lysine would be substantially deprotonated. The reported isoelectric pH of 11.0 is consistent with this view.

Acidic Group	Number	pK_a	Charge at pH = 7
Asp/Glu-COOH	10	4	-10
His-Im ⁺	1	6	0
Tyr-Phenol	3	10	0
Lys-NH ₃ ⁺	6	11	+6
Arg-Guan ⁺	11	12	+11
pI		11.0	

Hemoglobin

The α and β chains of normal human hemoglobin are shown in Reference 1 on page 111. The amino acid compositions are similar but not identical, and so preceding as above we get similar but not identical tables for the two forms of hemoglobin.

α -hemoglobin

Acidic Group	Number	pK_a	Charge at pH = 7
Asp/Glu-COOH	12	4	-12
His-Im ⁺	10	6	0
Tyr-Phenol	3	10	0
Lys-NH ₃ ⁺	11	11	+11
Arg-Guan ⁺	3	12	+3
pI		< 7	+2

 β -hemoglobin

Acidic Group	Number	pK_a	Charge at pH = 7
Asp/Glu-COOH	16	4	-16
His-Im ⁺	9	6	0
Tyr-Phenol	3	10	0
Lys-NH ₃ ⁺	11	11	+11
Arg-Guan ⁺	3	12	+3
pI		> 7	-2

We can estimate that the salt-free isoelectric pH, or isoionic pI for both forms should be near 7, with the slight variation from exactly 7 being accounted for by the fraction of the imidazole side chains of histidine in the positively charged form.

We did not need to consider either the C-terminal carboxyl group or the N-terminal amino group because each would be present in its charged form at a pH near 7, and the opposite charges would cancel.

General conclusion for large proteins

In general, if the total of Asp plus Glu is greater than the total of Lys plus Arg, we might expect pI to be on the acidic side of 7; if the opposite is true, we could expect pI to be on the basic side of 7.

Summary*Amino acids*

The isoelectric pH of an amino acid is half way between (the average of) the pK_a values of the acidic forms of a pair of ionizable groups in the molecule.

For “neutral” amino acids, the pK_a values are those of the α -carboxyl group and the acidic form of the α -amino group.

For “acidic” amino acids, the pK_a values are those of the α -carboxyl group and the side chain carboxyl group.

For “basic” amino acids, the pK_a values are those of the acidic form of the α -amino group and the acidic form of the basic group in the side chain.

Small peptides

Just as is true for amino acids, the isoelectric pH of a small peptide is determined by the pK_a values of the acidic forms of *only two of the ionizable groups* in the molecule. Furthermore, just as is true for amino acids, the isoelectric pH is half way between the two pK_a values. We have seen a simple strategy, the “back of the envelope” approach for finding the two ionizable groups that determine pI.

Larger proteins

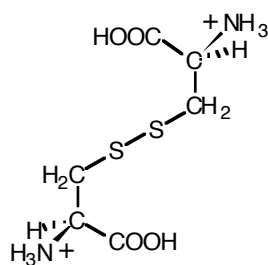
In general, if the total of acidic groups is greater than the total of basic groups, we expect the isoelectric pH to be on the acidic side of 7. If the opposite is true, we could expect the isoelectric pH to be on the basic side of 7.

Pedagogical implications

Discovering the relationship between structure and properties is one of the main goals of chemistry. Sometimes the relationship is easy to find, but sometimes, as in the case of the isoelectric pH values of amino acids and peptides, obtaining the insight that makes the connection requires a little more effort. The final simplicity of the connection is the reward.

Question

The four pK_a values of Cystine, the S-S dimer of Cysteine, are 1.65, 2.26, 7.85, and 9.85. What is the isoelectric pH of this substance? You should not need the envelope.



Cystine, at pH = 0

References

1. Lehninger, A. L. *Biochemistry*, 2nd ed., Worth Publishers; New York, New York, 1975; p 162.
2. D'Andrea, Gabriele, and Di Nicolantonio, Giuseppe *J. Chem. Educ.* **2002**, *79*, 972-975.
3. Sims, P. A. *J. Chem. Educ.* **2010**, *87*, 803-808