The "final frontier" of pH in cellular structures

The Limits of Acidity

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Intuitively, everybody knows and understands the idea of acidity. We are all familiar with savory foods or drinks and have heard about "acid rain." Yet the chemical significance of the notion is in fact not really intuitive at all

Acidity (pH) is defined as the negative decimal logarithm of the concentration of hydrogen ions in a solution - but what does that really mean? Are there any limits to the scale? If so, where do they lie? Can we find some way to come to conceptual grips with this slippery notion?

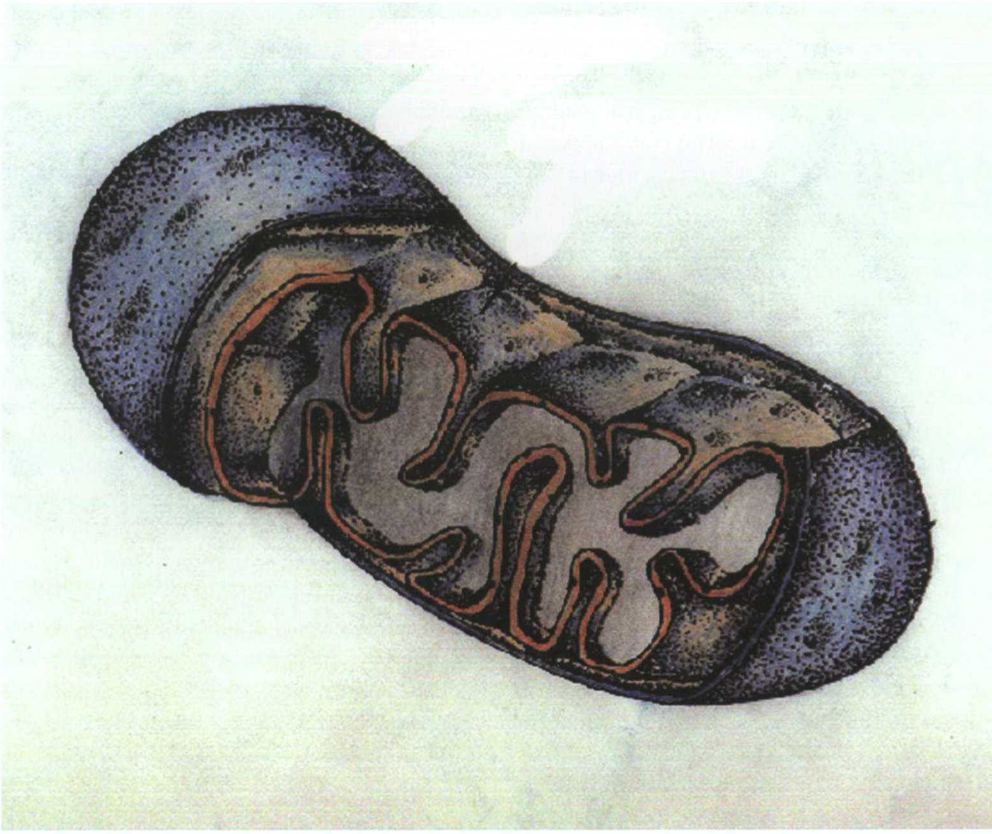
Moles and Molecules

We'll first start by explaining what we mean by "concentration." At its most basic level, a concentration is the amount of one substance mixed with or contained within another. There are many ways to express this, but two of the most popular used in the chemical realm are the percentage concentration and molar concentration. The percentage concentration is the mass of a substance A as a percentage share of the total mass of the solution including substance A plus a solvent. To take a real-life example, a 750 ml bottle of 12% red wine contains 90 ml of pure alcohol plus 660 ml of "solvent" (water together with all the chemical substances formed during the winemaking process).

The molar concentration, in turn, is perhaps less useful in everyday life but one can derive very interesting conclusions from it, bearing upon the very foundations of our scientific perception of the world. To explain it, we need to start by defining moles: one mole is a certain predefined number of things of a certain kind. The number is in fact a staggering one - 6.022×10^{23}. The unit is usually used to count molecules, although anything could theoretically also be counted using it, even people. If we assume that there are 7 billion (7×10^{9}) people living on the planet, we might say that the human race consists of 0.112 femtomoles of people (0.112×10^{-15} mole). In chemistry, one molar concentration (1M) is a mole of substance in a solution of a total volume of one liter - this fact will come in handy later on in the article.

Inseparable H^+ and OH^-

Now that we are more or less clear about what a "concentration" is, we can move on to pH. A pH number is a quantitative measure of the acidity of a given water solution, on a scale of 0 to 14. To put it simply, the pH number indicates whether a water solution contains more H+ ions (protons, i.e. hydrogen ions), in which case it is an acidic solution with a low pH number (e.g. 3), or more OH (hydroxyl) ions, in which case it is a basic solution with a high pH number (e.g. 10). If they are present in equal amounts, the solution is neutral (pH 7). Using the idea of molar concentration, one could say that pH depends on the ratio of H+ and OH ions in the solution. If the concentration of protons is higher than that of hydroxyl ions, the solution is acidic, and the higher the excess of protons, the more acidic the solution is (the lower the pH). However, it is very important to note where the H+ and OH ions come from: they are, at least in part, products of a process known as dissociation, in which water molecules (H_2O) are broken down into their component parts (H+ and OH ions). Since no other molecules take part in this process (in dilute solutions and under normal temperature and pressure),



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Mitochondria – the "power plants" of the cell – have a volume of approx. 1 fL (one femtoliter). Does the pH scale still actually make sense on such a small scale?

for water the process is known as self-ionization or auto-disassociation. The reaction is extremely rare, and relatively few of the many water molecules in a solution undergo the process. This is how we obtain the ionic product of water, forming the basis of the definition of pH:

$$[H^+] \times [OH^-] = 1 \times 10^{-14} \quad \text{(Equation 1)}$$

The ionic product of water combines the concentration of protons and hydroxyl ions in a single equation, and its value is constant under given conditions (1×10^{-14} at 25°C and constant atmospheric pressure). This means that a decrease in one coefficient forces an increase in the other, so the equation continues to hold. It is also the reason why the pH scale ranges between 0 and 14 ($-\log_{10}0 = 0$, and $-\log_{10}10^{-14} = 14$). Changes in pH, caused by the addition of acid or base, are a result of the interplay of protons and hydroxyl ions originating from the self-ionization process of water, with protons or hydroxyl ions added to the solution. A pH value cannot be defined solely in terms of either one of the ions, H+ or OH. They must always be considered together, since it is their ratio that forms the basis of pH.

Below the macro scale

As long as we remain in the macro realm of liters and milliliters, it seems reasonable to respond to all this by asking: "All right, but so what?" However, as we have shown in our paper "The Final Frontier of pH and the Undiscovered Country Beyond" published this year in *PLOS One*, things get far more complicated when we consider the miniscule size of cells and their component organelles. A typical mammalian cell has a volume of 1.2 picoliters (or 1.2×10^{-12} L) and its organelles are generally from a thousand to a million times smaller (e.g. mitochondria – the cells "power plants" – have a volume of about 1 femtoliter, or 1×10^{-15} L). Coated vesicles can be as tiny as 30-800 zeptoliters ($30\text{-}800\times10^{-21}$ L). When we recall that concentration is a function of volume, things really start to get interesting. The smallest possible concentration of a substance is a single molecule within a certain volume. The smaller the total volume, the larger the concentration that single molecule represents. For example, a single molecule of a given substance contained in just 4.2×10^{-16} L (420 aL, or attoliters) - the typical volume of water in an *E. coli* bacterial cell - makes a concentration of 4.0×10^{-9} moles/liter (4 nM, or nanomole). Smaller volumes yield still higher minimal concentrations: the lowest possible concentration within a lysosome (3.0×10^{-17} L) is 8.0×10^{-8} mole/litre (80 nM), while a single molecule within a coated vesicle (containing 3×10^{-20} L of water) represents a minimum concentration of 8.0×10^{-5} M (80 μM).

All this might be interesting, but what has it got to do with pH and acidity? Quite a lot, in fact. Remember that pH is about the relative concentrations of H+ and OH ions, which are inseparably bound by Equation 1, whereby their ratio always has to be constant. The smallest possible volume of a solution of pH 7 (containing a single H+ and a single OH ion) is 1.66×10^{-17} L (or 16.6 aL) - this is a biologically relevant volume, on the order of certain subcellular structures! What is more, any deviation from this pH, either up or down, will require a larger minimal volume! The consequences of this fact are quite startling: in small but physiologically relevant volumes, the concept of pH no longer validly applies, even though the notion is widely used to describe the conditions and reactions taking place in cells and their organelles! Another consequence is that in those small volumes water is not sufficiently dissociated to act as a source of protons for the acid-base chemistry taking place within cells and organelles.

A new paradigm?

This fact seems to run counter to our current general understanding of intra- and sub-cellular chemistry. It forces us to ask: What really occurs within cells? How do living organisms actually function, if everything we have so far thought about such processes turns out to rest on false premises? This "crisis" in our understanding of pH is somewhat reminiscent of certain problems faced by quantum physicists. A some very, very small level (the atomic level), classical physics breaks down, and the observed phenomena need a new theory to describe and explain them. This is where quantum physics comes to the rescue, even though at first glance it seems to stand in direct contradiction to classical (macroscopic) physics. Perhaps a similar method can be applied to pH on the intercellular level. The "classical" theory that works for large volumes apparently fails to capture the kind of chemistry that takes place below a certain volume limit.

These simple observations, based on pen and paper calculations, lead to very surprising conclusions that need to be verified. The next step, of course, is to devise and carry out appropriate experiments to uncover what happens when biological volumes become too small to properly obey the currently known laws. This is an extremely delicate situation: the answers to these exceptionally important questions are by no means obvious, and the potential results may likely invalidate the current paradigm. This paradigm holds that what we see on the macro scale of a test tube is basically the same as what happens on the micro scale of cells and their organelles. Yet as our research has shown, "it ain't necessarily so." ■

For technical reasons the English version of this text is being published without having yet received final acceptance from the author.

Further reading:

W. Bal, E. Kurowska, W. Maret (2012). The Final Frontier of pH and the Undiscovered Country Beyond, *PLOS One*, e45832.

The pH scale is a quantitative measure of the acidity of a given water solution on a scale of 0 to 14; it is measured using litmus paper

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