# pH in Action

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## SUMMARY

**Based on fundamental chemical relations, well-established in chemical engineering and chemical technology over almost a century, the effects of pH in food and agricultural products will be deduced for different situations and processes. Based on simple equilibria and dissociation of water, salts, acids and bases, and considering the reaction of hydrogen ions in these food-related systems, simple and reliable formulations are developed capable of describing surprisingly well the discoloration of blanched vegetables and the activity of enzymes in different buffered and unbuffered systems. The line of reasoning is generic and can be applied repeatedly to different problems, situations and processes.**

## INTRODUCTION

In chemical engineering and chemical technology, the effects of pH on rates and occurrence of reactions are well established. In many situations appropriate models are developed based on the theories of kinetics, equilibria and physical chemistry, as described in textbooks on chemistry and biochemistry (Chang 1981, Fersht 1984, Whitaker 1994) and in specialised journals and recent books on modelling in the food area (van Boekel and Tijskens 2001). The theory of pH behaviour in buffered and unbuffered solutions is well established and well understood for more then a century and is consistently applied in chemical engineering, where the systems present and the occurring processes are more or less clearly defined.. The situation is completely different in food technology and food research, but especially in predictive microbiology. The effect of pH on behaviour of all kinds of food and agricultural products (e.g. microbial safety) is still modelled in a very empirical way. All kinds of equations, mostly polynomials of a second or higher order are used to describe the effect of pH (Zwietering 1993, Wijtzes 1996). The high complexity of our food, in terms of composition and of occurring processes, apparently prevents the consistent application of the fundamental rules of chemistry and physics. Interactions with external conditions like the paramount temperature and storage atmosphere composition become very rapidly so confusing that the occurring processes are hard to discern and impossible to describe based on these fundamental rules.

It should, however, be possible to model, describe and predict the effect of pH on many processes occurring in and applied to our food in a more fundamental manner. In many cases pH can be kept or considered constant. As a consequence, pH is not often included in conceptual and mathematical models. However, when pH is important and can not be considered constant, we cannot neglect its effects.

In this paper will be shown that in spite of the complexity of the food matrix, fundamental reasoning can be applied to develop models based on simplified and sometimes oversimplified assumptions. Based on simple, plausible and fundamental assumptions, we have developed a model for the activity of enzymes at different but

constant temperatures and pH values (Tijskens et al. 2001a, Seyhan et al. 2001). A similar approach was applied to the colour degradation in blanched broccoli as affected by pH in acid based salad dressings (Tijskens et al. 2001b). These models were validated and calibrated against independently collected data sets. Results are discussed in more detail in Tijskens et al (2001c). These results strongly indicate that the approach followed is valid and generally applicable.

## pH AND HYDROGEN IONS

For a general acid-base system, the mechanisms of ionic dissociation of acids, bases and salts can briefly be summarised as:

$$HA \xleftrightarrow{K_A} H^+ + A^- \tag{1}$$

Since we can assume all reaction involving hydrogen ions to be very fast compared to ordinary reactions, this system is in continuous equilibrium and obeys the following relation:

$$K_A = \frac{A^- \cdot H^+}{HA} \tag{2}$$

Since pH is defined as the negative logarithm of the hydrogen ion concentration, we can convert this equation to express the pH at any situation of the equilibrium in the more usual p-notation:

$$pH = pK_A + \log_{10}\left(\frac{HA}{A^-}\right) \tag{3}$$

### Purely buffered systems

pH buffering is frequently applied to control pH at a fixed value. As a consequence, effects of pH are minimised and in most cases not taken into account in model formulations and data analyses. However, when the effects of pH is studied at different buffered pH values, these effects have to be included in the model. Based on the vast theoretical knowledge available the easiest way is to consider hydrogen ions simply as reagents in the system under study.

A model for a (pseudo) first order reaction of some component by action of hydrogen ions can be set up. An example for this type of behaviour is the degradation of green colour over time at various pH values. Basically, the green colour of blanched vegetables can be attributed to a simple (first order) extraction of the $Mg^{++}$ ion from the porphyrin ring that constitutes the chlorophyll pigment (Tijskens et al. 2001b). Since the degradation is enhanced by hydrogen ions, the mechanism can be represented as:

$$C + H^+ \xrightarrow{k_e} P \tag{4}$$

where C is the concentration of the substrate (chlorophyll), P is the decay product (pheophitines), and $k_e$ is the reaction rate constant. Applying the fundamental rules of chemical kinetics, this mechanism can be converted into a differential equation:

$$\frac{dC}{dt} = -k_e \cdot H^+ \cdot C \tag{5}$$

Solving equation 5 for constant external conditions of temperature and pH, one obtains an analytical solution that can be used to analyse the experimental data:

$$C = C_0 \cdot e^{-k_e \cdot H^+ \cdot t} \tag{6}$$

Expressing Eq. 6 in the usual p-notation one obtains:

$$C = C_0 \cdot e^{-k_e \cdot 10^{-pH} \cdot t} \tag{7}$$

Where the reaction is affected by the presence of hydroxyl ions, a similar equation can be derived:

$$C = C_0 \cdot e^{-k_e \cdot \frac{10^{-pH}}{K_w} \cdot t} \tag{8}$$

where $K_w$ is the dissociation constant of water ($10^{-14}$).

As in all chemical reactions, the rate constants depend on temperature according to Arrhenius' law:

$$k_i = k_{i,ref} \cdot e^{\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right)} \tag{9}$$

The combination of Eq. 7 (or 8) and 9 provide a model that describes the discoloration of green vegetables over the complete range of temperatures and pH values normally encountered. In **Fig. 1** an example is shown for the degradation of some compound affected by $H^+$ ions at different but constant pH values. Clearly visible is the tremendous impact the pH has on the rate of reaction. At pH 9 and higher, the rate is negligible. At pH of about 6, all substrate has disappeared in about 10 time units, while at pH 5 and lower, the conversion is almost instantaneous.

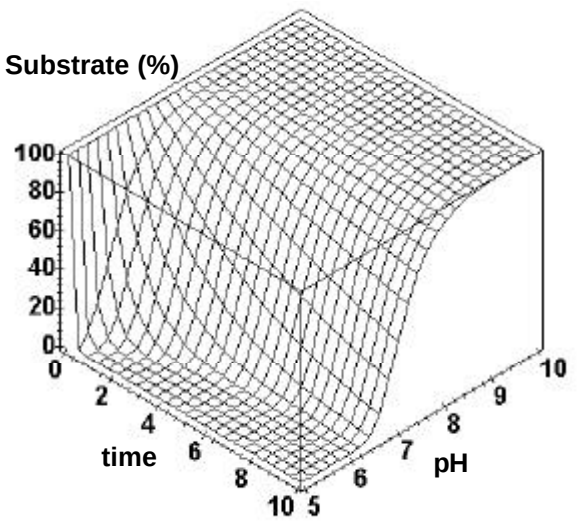


**Fig. 1** Substrate changes as function of time and pH at a constant temperature

**Purely unbuffered systems**

When a system is unbuffered, which is very unlikely in food matrices, the hydrogen ions may be consumed by the ongoing reaction. Using the law of preservation of mass, the analytical solution of the differential equation (Eq. 5) when $H^+$ can not be considered constant, is:

$$C = \frac{C_0 \cdot (C_0 - H_0)}{C_0 - H_0 \cdot e^{(k \cdot (C_0 - H_0) \cdot t)}} \qquad (10)$$

When the initial concentrations $C_0$ and $H_0$ are the same, eq. 10 reduces to the standard formulation for a second order reaction:

$$C = \frac{C_0}{1 + C_0 \cdot k \cdot t} \qquad (10b)$$

So, by the simple external condition of a complete unbuffered system, the simple exponential equation turns itself into a kind of logistic equation (note the negative sign in the denominator) of colour change. Of course, the lesser amount of one of the two reagents (C and $H^+$) will determine the extent of conversion at infinite time. (see **Fig. 2**).

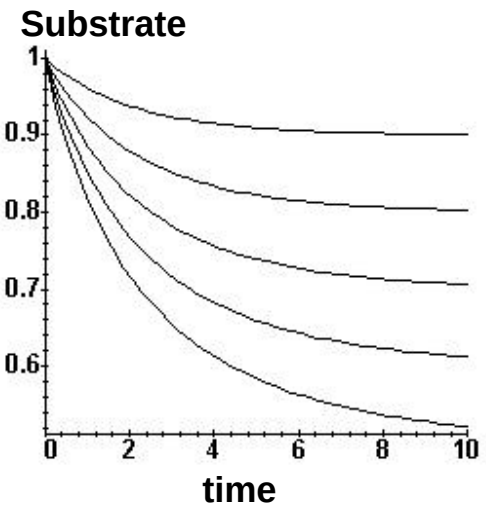


**Fig. 2** Decreasing amount of substrate as a function of time at constant temperature as affected by a decreasing H+ concentration.

**Real buffering systems and buffer capacity**

When working with real buffering systems, one has to be deal with many more aspects of pH effects. The buffering capacity of a buffering system has to be taken into account. This is defined (based on a rearrangement of eq. 2) as the change in pH value when the base (or acid) concentrations change, e.g. by adding or removing hydrogen ions. Especially when setting up a specific pH value by adding acids or bases to an equi-molar buffering combination, one has to be careful not to overstep the buffering capacity. In **Fig. 3** an example is given for the frequently used McIlvaine (citrate) buffer at low pH values (model Chang 1981). In cases where real food matrices are involved, one has to be careful with the mostly unknown buffering capacity of the food material itself. Most often, a pH gradient is built up between the applied buffering solution and the contents of the food material. In the Tijskens et al. (2001c), an example of this pH gradient is worked out in more detail.

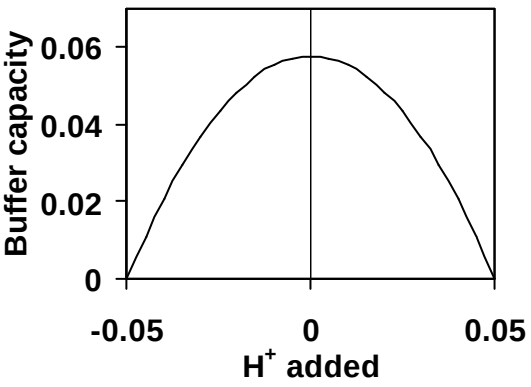


**Fig. 3** Buffering capacity of McIlvaine buffer at equi-molar concentrations of 0.1 M.

**ACTIVITY OF ENZYMES**

The activity of most enzymes greatly depends on pH. The so-called optimal pH range is provided in many textbooks on enzyme behaviour and properties (Godfrey and West 1996). Sparingly, some fundamental explanation is provided for this pH dependent behaviour (Fersht 1984, Whitaker 1994).

Enzymes consist of one or more special active sites attached to a protein backbone. These active sites can bind to a specific substrate and perform some conversion on that substrate. Being proteins, the amino and acidic groups are omnipresent, both in the backbone as well as in or near the active site. By taking up or releasing hydrogen ions, the active site does not change much in stereo configuration, since the $H^+$ ion is very small. The enzymatic activity, however, may change quite dramatically by the change in electric charge at the active site. Again, by considering the $H^+$ concentration as a reactant, the same line of reasoning can be applied to postulate a plausible mechanism. In eq. 11, a possible mechanism is shown that describes the effects on pH through the concentration of $H^+$ on the activity of an enzyme (Tijskens et al. 2001a):

$$\begin{aligned} En + H^+ &\xleftrightarrow{K_{EH}} EnH^+ \\ En + OH^- &\xleftrightarrow{K_{EOH}} EnOH^- \end{aligned} \tag{11}$$

where En is the active enzyme configuration and EnH and EnOH are the inactive configurations. $K_{EH}$ and $K_{EOH}$ are the equilibrium constants of the reactions.

The water dissociation is defined as usual as:

$$K_w = H^+ \; OH^- \cong 10^{-14} \qquad \text{and} \qquad OH^- = \frac{K_w}{H^+} \tag{12}$$

The amount of EnH and EnOH can now be expressed in terms of actual amount of active enzyme and pH by:

$$EnOH^- = \frac{K_w \cdot En}{K_{EOH} \cdot H^+} \;\&\; EnH^+ = \frac{En \cdot H^+}{K_{EH}} \tag{13}$$

The total amount of enzyme in any configuration has to remain constant:

$$En_{tot} = EnH^+ + EnOH^- + En \tag{14}$$

Combining the equations 13 and 14, and solving for En, an expression is obtained for the active enzyme at any $H^+$ concentration (or pH):

$$En = \frac{En_{tot}}{1 + \frac{H^+}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{H^+}} \tag{15}$$

Eq. 15 describes the concentration of an enzyme as function of pH. To convert into activity, the concentration has to be multiplied by the specific activity rate constant $k_s$. The effects of temperature are hidden in the rate constants and equilibrium constants. They all depend on temperature according to Arrhenius' law. This equation has

successfully been applied to the activity of phytases of seven different origins (Tijskens et al. 2001a, 2001c).

In most food-related cases, a system of iso-enzymes is present. For each of the active iso-enzyme configuration, the model has to be applied separately, with a specific value for $K_{EH}$ and $K_{EOH}$ for each of the configurations. This model has been applied to an iso-enzyme system in lipase in hazelnuts (Seyhan et al. 2001, Tijskens et al 2001c). An example of the behaviour of enzyme activity in a system of two active iso-enzymes is shown in **Fig. 4**. An unlimited range of apparent observed activity behaviour can be constructed / described by putting together two to three iso-enzyme configurations.

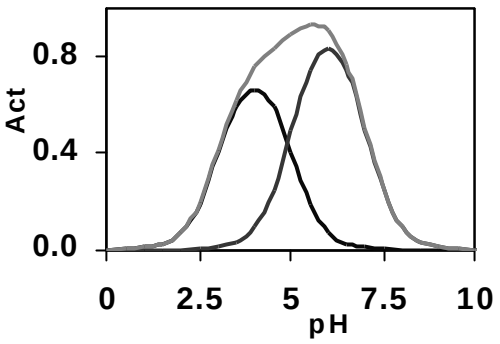


**Fig. 4** Example of behaviour of enzyme activity as a function of pH for two simultaneously active configurations

## CONCLUSIONS

By considering hydrogen ions as a reagent in basic mechanisms and applying the well-known rules of chemical kinetics and equilibria, useful and fundamental expressions can be developed that describe the effect of pH in a variety of processes and conditions. Empirical expressions and polynomials can be avoided altogether. All parameters in the models deduced have a chemical or physical meaning, well within the views of current theories. By consistently applying all available knowledge, theoretical as well as practical, practical models can be developed and some structure and understanding can be brought into the modelling of pH in action.

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