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Samuelsen, Lisa; Holm, René; Lathuile, Audrey; Schönbeck, Jens Christian Sidney

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Buffer Solutions in Drug Formulation and Processing: How $pK_a$ values Depend on Temperature, Pressure and Ionic Strength

Lisa Samuelsen$^a$, René Holm$^{a,b,*}$, Audrey Lathuile$^b$, and Christian Schönbeck$^a$

$^a$Department of Science and Environment, Roskilde University, Universitetsvej 1, DK-4000 Roskilde, Denmark; $^b$Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

*Corresponding author: rholm@ITS.JNJ.com
Abstract

Solution pH is an important factor during drug formulation and processing. Changes in pH present challenges. Regulation of pH is typically managed by using a buffer system, which must have a suitable pKa. The pKa value of buffers depends on temperature, pressure and ionic strength. In addition, the pKa value can also be affected by the polarity of the solvent, e.g., by the addition of a co-solvent. Theoretical considerations and accessible experimental data were used to understand how the pKa values of pharmaceutically relevant buffers depend on these factors. Changes in temperature also affect the buffer pKa. Carboxylic acid moieties were least affected by changes in temperature. Buffers containing amino groups were most affected by changes in temperature, and the pKa decreased as temperature was increased. It was possible to predict accurately how buffer pKa varies with temperature, based on changes in enthalpy and heat capacity for the ionization reactions. Changes in pressure had a limited effect on buffer pKa for pressures <100 MPa. At higher pressures, buffer pKa varied by up to 0.5 pH units. Altering the ionic strength or polarity of the solvent influenced buffer pKa slightly. However, it is possible to keep both the ionic strength and the polarity of the solvent constant during drug formulation and processing.

Keywords: pH; pharmaceutical processing; temperature-dependency; chemical thermodynamics; liquid formulations; autoclaving;
1. Introduction

Regulation of pH is often essential, e.g., biological and biochemical processes are controlled by proton transfer (Fukada and Takahashi, 1998). Energy production in mitochondria by reduction of NAD$^+$ to NADH is one example of a biological process governed by proton transfer, and many biochemical studies are performed under pH-buffered conditions (Fukada and Takahashi, 1998; Karow et al., 2013; Mauger, 2017). pH is also an important factor in drug formulation and processing, as the pH facilitates folding and unfolding of proteins, chemical degradation and solubility of drug compounds (Babic et al., 2007; O’Brien et al., 2012; Stoll and Blanchard, 1990). It is estimated that two-thirds of marketed and potential drugs are ionizable compounds (Williams et al., 2013). Most ionizable drugs are weak electrolytes, thus their dissociation is partially pH-dependent (Williams et al., 2013). Regulation of pH is used to ensure the quality and performance of drug formulations, since pH can improve the overall thermodynamic parameters of reactions, chemical stability and solubility. During pharmaceutical processing such as autoclaving, homogenization, stability testing and storage, controlling pH can be considered as a critical quality attribute in many systems and formulations.

Buffer systems are central in the control of pH, making it important to understand buffer properties in order to obtain a robust formulation. All weak acids or bases and their corresponding base or acid in aqueous solution can be a buffer solution. It is easy to imagine the large numbers of potential buffers that exist (Stoll and Blanchard, 2009), though within drug formulation a limited number of buffers are usually used. In general, buffers can be characterized based on their chemical structure as, i) carboxylic acid buffers, ii) phosphate buffers, iii) zwitterionic buffers and iv) Tris-based buffers (Mauger, 2017). Buffers should: (1) have a relevant p$K_a$, (2) have a high aqueous solubility to achieve a sufficient buffer capacity, (3) be chemically stable, (4) exhibit minimal toxicity, and (5)
be inexpensive, easily purified and accessible (Good et al., 1966; Mauger, 2017; Stoll and Blanchard, 1990; Yalkowsky, 1999). Selecting a buffer with a relevant pKₐ is important and it should reflect the experimental conditions, i.e., as a general rule buffers are most effective in a range of ± 1 pH units of their pKₐ (Mauger, 2017).

Buffer solutions and their chemistry have been investigated since the beginning of the 1900s. The pKₐ of various buffer species vary as a function of temperature, pressure and ionic strength (Fukada and Takahashi, 1998; Good et al., 1966; Mauger, 2017; Stoll and Blanchard, 1990). These three parameters can all be varied in a drug formulation and during processing of the formulation. It is, therefore, important for the pharmaceutical scientist to understand how the pKₐ of buffers varies with these parameters to ensure both a robust formulation during processing, e.g. during sterilization, and during storage (Karow et al., 2013). To understand the variation of pKₐ, knowledge of thermodynamic data for the ionization of acids and bases is necessary.

The present work aims to provide formulation scientists with an overview of how the pH of the buffers used in pharmaceutical drug formulation is influenced by temperature, pressure and ionic strength based on accessible experimental data and theoretical considerations.

2 Theoretical background

A buffer solution or buffering agent is an aqueous solution of a weak acid (HA) or base (A⁻) in equilibrium with its corresponding base or acid.

\[
HA \rightleftharpoons H^+ + A^- \quad \text{(Eq. 1)}
\]
The equilibrium constant is referred to as the acid dissociation constant $K_a$ or ionization constant. It relates to the degree of dissociation of the acid, and generally it is calculated based on the concentrations of the reacting species:

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad \text{(Eq. 2)}$$

So the $K_a$ is a measure of the strength of the acid. Strong acids have a high $K_a$, which means a high degree of dissociation. Most often, acid strengths are reported as $pK_a$ values, the negative logarithm of $K_a$. This value is strongly related to the pH of the solution, and the Henderson-Hasselbalch equation describes how the pH depends on the concentration of the buffer components and $pK_a$:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad \text{(Eq. 3)}$$

The buffer $pK_a$ depends on temperature, pressure and ionic strength, and the pH of the solution is affected accordingly. To understand how such factors influence the $pK_a$, it is important to understand the thermodynamics behind the equilibrium constant. $\Delta G^\circ$ (Gibb’s free energy) is the thermodynamic parameter quantifying the energy that drives reactions. At constant temperature (T in K) and pressure, $\Delta G^\circ$ is defined by the change in enthalpy ($H$) and entropy ($S$):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad \text{(Eq. 4)}$$

For a system in chemical equilibrium, $\Delta G^\circ$ is related to the equilibrium constant:

$$\Delta G^\circ = -RT\ln K_a \quad \text{(Eq. 5)}$$

where $R$ is the gas constant, and $T$ is the temperature.

2.1 Theoretical influence of temperature

Through standard Gibb’s free energy in eq. 4 and 5, the equilibrium constant can be linked to enthalpy and entropy:
\[ \ln K_a = -\frac{\Delta H^*}{R} + \frac{\Delta S^*}{R} \]  
(Eq. 6)

It is possible to differentiate the expression in equation 7 with respect to T and get the van’t Hoff equation, which describes how \( K_a \) depends on temperature.

\[ \frac{\partial \ln K_a}{\partial T} = \frac{\Delta H^*}{RT^2} \]  
(Eq. 7)

Eq. 7 shows that the temperature dependence of \( K_a \) is governed by the protonation enthalpy. A large absolute value of \( \Delta H \) means that \( K_a \) is strongly temperature dependent. The van’t Hoff equation is typically written for a specific integral between temperatures \( T_1 \) and \( T_2 \):

\[ \ln \frac{K_2}{K_1} = -\frac{\Delta H^*}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right) \]  
(Eq. 8)

This equation shows the link between \( K_a \) and temperature with the assumption that \( \Delta H \) is a constant in the given temperature range. However, \( \Delta H \) does change with temperature. The change in enthalpy as a function of temperature is described by a quantity termed the heat capacity, \( C_p \):

\[ \Delta C_p = \frac{\partial \Delta H}{\partial T} \]  
(Eq. 9)

By combining eq. 8 and 9, it is possible to derive an extended van’t Hoff equation:

\[ \ln K_2 = \frac{\Delta H^* - T_1 \Delta C_p}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) + \frac{\Delta C_p}{R} \ln \left( \frac{T_2}{T_1} \right) + \ln K_1 \]  
(Eq. 10)

where the subscripts refer to the values at temperatures 1 and 2. Thus, the correlation between \( K_a \) and temperature depends on the thermodynamic quantities \( \Delta H \) and \( \Delta C_p \), and it possible to calculate \( K_2 \) at any temperature \( T_2 \) as long as \( K_1 \), \( \Delta H \) and \( \Delta C_p \) are known at temperature \( T_1 \).

2.2 Effect of pressure

For buffers in solution, the effect of pressure on the equilibrium constant is closely related to the change in volume (Hayert et al., 1999; Quinlan and Reinhart, 2005; Van Eldik et al., 1989). From a chemical thermodynamic perspective, the relationship between the pressure and volume of liquids
is described by compressibility, which is the decrease in volume upon pressure increase. In chemical thermodynamics, the chemical potential is defined as the partial molar Gibb's free energy:

\[ \mu = \frac{\partial G}{\partial N} \]  

(Eq. 11)

The chemical potential is affected by composition, temperature and pressure. The variation of chemical potential of a solute species at constant temperature is a function of the pressure P and the volume V of the solute species:

\[ \frac{\partial \mu}{\partial P} = V \]  

(Eq. 12)

By combining eq. 5, 11 and 12, the relation between the volume change and the equilibrium constant at constant temperature with change in pressure becomes evident:

\[ -RT \frac{\partial \ln K_a}{\partial P} = \Delta V \]  

(Eq. 13)

Liquids are often practically incompressible due to tight packing of the species in solution. This is due to intermolecular repulsive forces in solution. In reality, liquids are slightly compressible, and pressure increase leads to small volume changes. At pressures <300-400 MPa, the volume changes with pressure are minimal (Quinlan and Reinhart, 2005). This means that small changes in pressure have a limited effect on the composition and thus the equilibrium in solution.

### 2.3 Ionic strength

Ions exhibit strong interactions in solution, resulting in deviation from ideal behavior. In eq. 2, \( K_a \) is expressed in terms of the concentrations of the reacting species. However, this is too simplistic and it is inadequate to describe the true nature of \( K_a \) this way, due to solutions deviating from ideal behavior. Therefore, the activity of the reacting species must be used instead:

\[ K_a = \frac{a_{H^+} a_{A^-}}{a_{HA}} = \frac{\gamma[H^+] \gamma[A^-]}{\gamma[HA]} \]  

(Eq. 14)
where $a$ is the activity, and $\gamma$ is the activity coefficient. By using the activity term, a more precise equilibrium constant can be determined. At low ionic strength, the activity coefficient of an ionic solution ($\gamma$) can be calculated from the Debye-Hückel equation:

$$\log \gamma = -\frac{z^2 A}{2^{1/2}} I^{1/2}$$

(Eq. 15)

where $A$ is a constant, $z$ is the charge and $I$ is the ionic strength. $A$ and $I$ can be calculated (Kennedy, 1990):

$$A = 1.824 \cdot 10^6 \cdot (\epsilon T)^{-3/2}$$

(Eq. 16)

A depends on temperature and the dielectric constant $\epsilon$ of the solvent. The ionic strength ($I$) describes the intensity of the ion atmosphere of the entire solution (Kennedy, 1990):

$$I = \frac{1}{2} \sum z^2 C$$

(Eq. 17)

The sum of charge ($z$) and concentration ($C$) refers to all of the various ions in the solution. The Debye-Hückel equation is valid at low ionic strength. Better estimates of the activity coefficient at higher ionic strength is possible by including terms for the effective radius of the ions or with empirical constants (Goldberg et al., 2002; Kennedy, 1990; Roy et al., 2006):

$$\log \gamma = -\frac{A z^2 \sqrt{I}}{1 + B a^* \sqrt{I}} + C I$$

(Eq. 18)

where $B$ is constant, $a^*$ is the ion size parameter, and $C$ is an adjustable parameter based on curve-fitting. As shown by these equations, the activity coefficient depends on the ionic strength, the charge and the temperature, thus making $pK_a$ dependent on ionic strength.

3. Experimental determination of thermodynamic quantities $pK_a$, $\Delta H$, and $\Delta C_p$

Some methods used to determine $pK_a$ include potentiometry, spectrometry, conductometry, solubility, NMR, electrochemical cells with and without liquid junctions and calorimetry (Babic et al., 2007; Goldberg et al., 2002; Nowak et al., 2015; Reijenga et al., 2013). By combining
experiments and thermodynamic equations, it is possible to determine how pKa depends on
temperature, and thus also how to use this information during drug formulation and processing.

The methods mentioned above are all widely used and accepted. However, a discussion of which
techniques are superior is complex and beyond the scope of this work. Instead, interested readers
are referred to papers such as Reijenga et al. (2013) and Babic et al. (2007) for descriptions of
techniques and comparison between methods. It is worth noting though, that differences in results
are seen between various experimental methods used to determine thermodynamic quantities. Also,
there are differences in how well results and experimental conditions are reported. Potentiometric
titration is an inexpensive and simple technique (Reijenga et al., 2013), but results are often
summarized and the experimental data and conditions are not always reported in full detail
(Goldberg et al., 2002). For conductivity measurements, electrochemical cell setups, and
calorimetric studies, the experimental conditions, the composition of solutions as well as the
primary data are often reported, making it possible to recalculate the thermodynamic results based
on these methods (Goldberg et al., 2002). By reporting experimental conditions and results more
clearly, it enables recalculation of data (Goldberg et al., 2002). This provides an important tool in
validating existing and potential thermodynamic models.

4. Data selection

During drug formulation and processing, it is important to choose a suitable buffer. For the present
work some pharmaceutically relevant buffers have been selected, where the criteria for selecting
buffers were 1) relevance for drug formulation, 2) availability of literature regarding
thermodynamic quantities at various temperatures, pressure and ionic strength, and lastly 3) buffers
with different chemical structure should be included to highlight any potential differences based on
the chemical structure. Values of $pK_a$, $\Delta H^*$, and $\Delta C_p$ at 25°C of selected buffers are presented in Table 1.
Table 1. The table shows pKₐ, ΔH and ΔCₚ at 25°C for selected buffers used regularly in the pharmaceutical industry. a(Goldberg et al., 2002). b(Nagai et al., 2008). c(pK₁ including aqueous CO₂). na = data not available.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pKₐ</th>
<th>ΔH* (kJ mol⁻¹)</th>
<th>ΔCₚ⁺ (J K⁻¹ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>4.76</td>
<td>-0.41</td>
<td>-142</td>
</tr>
<tr>
<td>Carbonate</td>
<td>pK₁</td>
<td>3.60</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>10.33</td>
<td>14.70</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>6.35</td>
<td>9.15</td>
</tr>
<tr>
<td>Citrate</td>
<td>pK₁</td>
<td>3.13</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>4.76</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>6.40</td>
<td>-3.38</td>
</tr>
<tr>
<td>Succinate</td>
<td>pK₁</td>
<td>4.21</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>5.64</td>
<td>-0.5</td>
</tr>
<tr>
<td>Phosphate</td>
<td>pK₁</td>
<td>2.15</td>
<td>-8.0</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>7.20</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>12.33</td>
<td>16.0</td>
</tr>
<tr>
<td>Tris</td>
<td>8.06</td>
<td>47.45</td>
<td>-59</td>
</tr>
<tr>
<td>HEPES</td>
<td>pK₁</td>
<td>3.00</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>7.50</td>
<td>20.4</td>
</tr>
<tr>
<td>MES</td>
<td>6.27</td>
<td>14.8</td>
<td>5</td>
</tr>
<tr>
<td>TAPS</td>
<td>8.44</td>
<td>40.4</td>
<td>15</td>
</tr>
<tr>
<td>Histidine</td>
<td>pK₁</td>
<td>1.56</td>
<td>-2.3</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>6.07</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>9.34</td>
<td>43.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>pK₁</td>
<td>1.85</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>9.09</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>10.90</td>
<td>43.5</td>
</tr>
<tr>
<td>Glutamate</td>
<td>pK₁</td>
<td>2.19</td>
<td>-0.366</td>
</tr>
<tr>
<td></td>
<td>pK₂</td>
<td>4.45</td>
<td>-0.798</td>
</tr>
<tr>
<td></td>
<td>pK₃</td>
<td>10.10</td>
<td>4.5</td>
</tr>
</tbody>
</table>
ΔH describes how the equilibrium constant depends on temperature. For ΔH close to 0, the equilibrium constant shows little variation with temperature as described by Eq. 8. ΔC_p describes how ΔH depends on temperature. A ΔC_p value close to 0 means that ΔH varies little with temperature.

5. Pressure has a negligible effect on buffer pK_a at pressures <100 MPa

Theoretically, a small change in pressure will lead to minimal changes in K_a. For carboxylic acids such as acetate, succinate and citrate, the pK_a decreases as pressure is increased (Hayert et al., 1999; Kumar, 2005; Quinlan and Reinhart, 2005). The decrease in pK_a is relatively small. For acetic acid, a decrease of 0.1 pH units is observed when pressure is increased from 0.1 MPa to 25.3 MPa (Kumar, 2005), and a decrease of 0.2 pH units when pressure is increased to 100 MPa (Hayert et al., 1999). Evidently, the reduction in pK_a is minimal for these buffers, even when pressure changes significantly.

Increase in pressure decreases the pK_a of phosphate buffer (Hayert et al., 1999; Quinlan and Reinhart, 2005). Phosphate is slightly more sensitive to pressure changes than carboxylic acid buffers, and for the range 0.1-100 MPa pK_a decreases by 0.3 pH units (Hayert et al., 1999). Phosphate buffer is increasingly more sensitive at higher pressures (Hayert et al., 1999).

Tris buffers and biological buffers such as HEPES and MES also show sensitivity towards pressure. However, in contrast with carboxylic acid buffers, pK_a increases with pressure (Hayert et al., 1999; Quinlan and Reinhart, 2005). The magnitude is similar to that of carboxylic acid buffers (Quinlan and Reinhart, 2005). Tris is thought to be almost pressure insensitive (Neuman et al., 1973). The
difference among the various groups of buffers is thought to be related to charges in the solution, as volume contractions occur due to packing of water molecules around the charged species (Hayert et al., 1999).

It has been shown in several studies that the relationship between pK$_a$ and pressure is approximately linear for the pressure range of 0-200 MPa (Hayert et al., 1999; Li and Duan, 2007; Quinlan and Reinhart, 2005), thus making it possible to interpolate the change in pK$_a$ value for pressures relevant during pharmaceutical processing. During autoclaving, pressure changes occur for liquid systems as the pressure increases to around 0.304 MPa (3 atm) to obtain the sterilization temperature. The change in pressure during autoclaving corresponds to a decrease of 0.0005 pH units for acetic acid, so the influence of pressure on the pK$_a$ value during autoclaving can be regarded as negligible. Other pharmaceutical processes where pressure changes may be relevant include homogenization, lyophilization, liposome extrusion, and transfer of liquid between compounding vessels. During production of e.g., suspensions or emulsions, homogenization is often used. Thus, a pressure of 10 MPa (100 atm) may be applied. Under these conditions the pK$_a$ of acetic acid decreases less than 0.04 pH units, which is still considered a small change. For high pressure homogenization, a pressure around 150-200 MPa can be applied, which will affect the pK$_a$ of most buffers by about 0.5 pH units. This can potentially influence the stability of drug formulations and should hence be considered.

6. Temperature effect on pK$_a$ depends on chemical structure

Carboxylic acid buffers are least affected by temperature changes (Goldberg et al., 2002; Karow et al., 2013; Mauger, 2017). The pK$_a$ values as a function of temperature for some carboxylic acid buffers are shown in figure 1, which is based on data reported by Goldberg et al. (2002).
An increase in temperature leads to a slight decrease in pKa values at low temperatures (0-50°C), and pKa increases as the temperature exceeds 50°C (Goldberg et al., 2002; Li and Duan, 2007). The changes are, however, small. The pKa of acetate decreases by 0.025 pH units from 0-25°C, and increases 0.290 by pH units in the interval 25-125°C (Goldberg et al., 2002). Similar small changes in pKa values are reported for many carboxylic acids - among others citrate and succinate (Goldberg et al., 2002; Quinlan and Reinhart, 2005). By curve fitting the data from figure 1, it is evident that there is a quadratic relationship between pKa and temperature for all three buffers. They fit a second-order polynomial function with similar quadratic coefficients, which means that the curvature is similar. Based on these data, the relationship between pKa and temperature is generally similar for the carboxylic acid buffers. Common for these buffers are also dissociation enthalpy close to 0 (Table 1) (Fukada and Takahashi, 1998; Goldberg et al., 2002). This means that pKa hardly changes with temperature.

The dissociation of the first two protons of phosphate also have enthalpy values close to 0, and pKa changes only slightly with temperature (Karow et al., 2013; Mauger, 2017). In the temperature interval 25-125°C, the pK1 of phosphate increases by 0.552 pH units (Goldberg et al., 2002). The enthalpy of the third proton dissociation of phosphate is larger and theoretically the temperature-dependency would thus be greater. However, experimental data is only available up to 37°C. The dissociation of carbonate has higher enthalpy changes, and thus also a greater temperature dependency.

The buffer most sensitive to temperature changes is Tris (Goldberg et al., 2002; Mauger, 2017). The dissociation of Tris also shows the highest enthalpy change. Dissociation of biological buffers such as MES, HEPES and TAPS also exhibits high enthalpy changes (Table 1), and in general these
buffers are more sensitive to temperature changes compared to carboxylic acid buffers. Figure 2 shows how the pK$_a$ values of some biological buffers vary with temperature. In contrast to carboxylic acid buffers, biological buffers and Tris buffer have decreasing pK$_a$ values as temperatures are increased (Roig et al., 1993; Roy et al., 2009; Roy et al., 2011). This indicates that increasing temperature does not favor ionization of the buffer. The pK$_a$ of Tris decreases 1.987 pH units in the interval 25-125˚C (Goldberg et al., 2002). A similar decrease in pK$_a$ is seen for TAPS, whereas MES and HEPES show slightly smaller decreases in pK$_a$ values (Fukada and Takahashi, 1998).

Other buffers with high dissociation enthalpy changes include the amino acids histidine and lysine. These buffers have high enthalpy values for the second and third dissociation represented by pK$_2$ and pK$_3$. Histidine has a pK$_2$ change of 0.46 pH units in the range 25-60˚C, and similarly, lysine has a pK$_2$ change of 0.75 pH units (Nagai et al., 2008). For the amino acid glutamate, only pK$_3$ is temperature sensitive (Gupta et al., 2013; Nagai et al., 2008). No experimental values for the pK$_a$ of amino acids at temperatures >60˚C could be found. However, it is possible to extrapolate these values to higher temperatures based on $\Delta H$ and $\Delta C_p$ (Table 1) using Eq. 8 and 10. The extrapolation of pK$_a$ values for histidine, lysine, and glutamate are shown in figure 3. Figure 3 shows both experimental data from Nagai et al. (2008) and extrapolated pK$_a$ values for temperatures above 60˚C. The extrapolation shows that pK$_2$ and pK$_3$ for lysine and pK$_3$ for histidine are sensitive to temperature. The pK$_1$ and pK$_2$ values for glutamate are insensitive to temperature, and so is pK$_1$ for lysine and pK$_2$ for histidine. The extrapolation of pK$_3$ value for glutamate does not fit well with the experimental data. The values are calculated based on $\Delta H$ and Eq. 8, and the fit would likely be improved if $\Delta C_p$ was known.
There is a clear correlation between dissociation enthalpies close to 0 and lower sensitivity towards temperature increase. This has been described previously (Fukada and Takahashi, 1998; Karow et al., 2013). The selected biological buffers and Tris have high dissociation enthalpy values at 25°C, and $\Delta C_p$ is close to 0. This means the $\Delta H$ values will not change significantly when temperatures are increased, which means that these buffers are sensitive to temperature changes as seen in figure 2. For carboxylic acid buffers, small enthalpy changes at 25°C are most commonly seen, and there is thus a small temperature dependency. The selected carboxylic acid buffers have high $\Delta C_p$ values, which results in larger changes in dissociation enthalpy at higher temperatures, and this explains why $pK_a$ values of carboxylic acids become increasingly more sensitive as temperatures are increased (see figure 1). The carboxylic acid moiety of amino acids exhibits dissociation enthalpies close to 0, and these $pK_a$ values are insensitive to temperature changes (see figure 3). By comparing $pK_a$ values of amino acids with the various functional groups in each amino acid, it becomes evident that the functional groups reflect the sensitivity towards temperature. Histidine and lysine have one carboxylic acid moiety each represented by $pK_1$, and both moieties have low dissociation enthalpies. On the other hand, glutamic acid has two functional carboxylic acid moieties i.e., $pK_1$ and $pK_2$, and both of these moieties have low dissociation enthalpies. The two amino moieties of histidine and lysine have higher dissociation enthalpies, and are thus more sensitive to temperature changes (figure 3). The functional groups reflect the sensitivity towards temperature, as histidine and lysine, with only one carboxylic acid moiety, are more sensitive to changes in temperature compared to glutamic acid. Further, it is observed that buffers containing amino groups are very temperature dependent. The effect of temperature on the $pK_a$ values of buffers is linked to the chemical structure. This knowledge can be used to qualitatively predict the temperature sensitivity of a buffer system.
During drug formulation, processing and storage temperatures can vary. Storage temperatures are often as low as 5°C, but may vary from country to country. In warmer climates, storage temperatures may be up to 30°C. During processing such as autoclaving temperatures are up to 130°C. The pKₐ of Tris is 8.06 at 25°C, and thus it has a buffering capacity in the range 7-9 at this temperature. As temperature is increased to 130°C, pKₐ decreases to 6.07, and correspondingly the pH of the solution decreases by 2 pH units. Tris is considered to be the most temperature-sensitive buffer, and the above mentioned example was therefore based on this buffer. Also during homogenization of formulations, temperature can vary, which can affect the pKₐ. As the liquid is compressed, the temperature will rise 2-2.5°C per 10 MPa. The pressure increase during homogenization is approximately 50 MPa, meaning the temperature will increase by 10-12.5°C.

During homogenization, this will correspond to a decrease in pKₐ of approximately 0.3 pH units for Tris buffer. However, during high pressure homogenization, the pressure increase is 100-200 MPa, and thus the temperature increase will be 20-50°C. In addition to temperature rising with increasing pressure, the formulation is sometimes preheated prior to homogenization. The maximum temperature during high pressure homogenization can reach 140°C, which would affect the pKₐ of buffers. As drug formulations highly depend on the regulation of pH, this is potentially a problem that should be incorporated into the formulation and process design. One way of doing this could be to use a flowchart (figure 4) that visualizes how temperature and pressure should be considered when selecting a suitable buffer. Altering the pKₐ of a system changes the pH of the solution, which influences the solubility and chemical stability of the product. Amino-containing buffers appear most sensitive to temperature changes, according to the data presented in this work. However, to evaluate other buffer species more closely, it is suggested to consider ΔH and ΔCₚ of the reaction. If ΔH is known, it is possible to predict the variation of pKₐ with temperature over small temperature intervals by using Eq. 8. By knowing ΔH and ΔCₚ, it is possible to make more accurate predictions.
for $pK_a$ across a wider range of temperatures using Eq. 10. These two parameters should be considered and used as a guide during drug formulation or processing, as illustrated in figure 4.

The temperature-dependency is sometimes crucial for selecting a suitable buffer, and in such situations a carboxylic acid buffer would be preferred. However, there might be situations where carboxylic acid buffers are not suitable due to unwanted interactions. Alternatively, it should be possible to use a mixture of buffers to obtain a suitable system that is less affected by temperature. In a study by Quinland and Reinhart (2005), it was proposed that insensitivity of $pK_a$ values to pressure might be achieved by mixing two buffers; one with increasing $pK_a$ as pressure is increased, and one with decreasing $pK_a$ as pressure is increased. Glutamate is a buffer that contains two carboxylic acid groups and an amino group, and the amino acid moiety is less affected by changes in temperature compared to other amino-containing buffers. Thus, a mixture of a biological or Tris based buffer with carboxylic acid buffer could lead to a buffer less sensitive to changes in temperature than biological buffers by themselves. However, it would be necessary to verify this experimentally.

7. Influence of ionic strength on $pK_a$ of selected buffers

The ionic strength of a solution describes the interactive forces and distances between the ions in the solution. Ionic strength can be increased by increasing the concentration of existing components or by adding ionic compounds. It is possible to keep the ionic strength practically constant by having a large concentration of background electrolytes (e.g. buffer) compared to the ionic contribution of added species (Ferra et al., 2011). As described previously, changes in ionic strength affect the activity coefficient, and will thus lead to changes in $pK_a$ values. For carboxylic acid buffers, the $pK_a$ decreases with increasing ionic strength until a minimum is reached; further
increase of the ionic strength leads to an increase in pK_a. The minimum for carboxylic acids is reached for ionic strength around 0.42-1.5 mol/kg. The pK_a of acetate decreases around 0.3 pH units as ionic strength is increased in the interval 0-0.5 mol/kg at 25°C (Ferra et al., 2011; Harned et al., 1937). Similar decreases in pK_a are seen for citrate and succinate in the interval 0-1 mol/kg at 25°C (Bénézeth et al., 1997; Kettler et al., 1995). The pK_a of carbonate also decreases by a similar magnitude in the interval 0-1.5 mol/kg (He and Morse, 1993; Millero et al., 2007). Phosphate shows a relationship between pK_a and ionic strength that is similar to that of carboxylic acid buffers. The pK_a decreases for ionic strength 0-3 mol/kg, and increases for higher ionic strength (Hershey et al., 1989).

Tris behaves differently from carboxylic acid buffers when ionic strength is increased, as was also seen for the pK_a-temperature relationship. The pK_a of Tris increases by about 0.2 pH units in the interval 0-1 mol/kg at 25°C (Izaguirre and Millero, 1987; Palmer and Wesolowski, 1987). An increase in pK_a with increasing ionic strength is also observed for biological buffers (Roig et al., 1993; Roy et al., 2002; Wesolowski and Palmer, 1989).

It is evident that the structure of the buffers dictates how they are affected by ionic strength. This is similar to what is observed for the temperature effect on buffer pK_a. However, whereas the pK_a of Tris and biological buffers decreases with temperature, pK_a values increase with ionic strength. For carboxylic acid buffers, the effect of ionic strength on pK_a is interval specific. Manipulating ionic strength can be exploited during drug formulation to increase chemical stability or improve solubility without having to change the buffer system. Increasing the ionic strength of Tris buffer will increase the pK_a, and thus alter the appropriate range of the buffer. At 0 mol/kg ionic strength the pK_a of Tris is 8.06, but at 2 mol/kg, the pK_a increases to 8.41, thus slightly changing the
appropriate buffer range to 7.5-9.5. Exploiting the variation of pKₐ has the potential to make buffers more versatile, as a relevant pKₐ value is essential in choosing the appropriate buffer system.

As described previously, the thermodynamic equilibrium constant is defined by the concentration and the activity coefficient, thus this should be accounted for when reporting pKₐ values. By adjusting the pKₐ values to 0 mol/kg ionic strength, it would become easier to compare pKₐ values reported in different studies. In many cases, the activity coefficients are unknown, but these can be estimated by the Debye-Hückel model or similar models. The biggest challenge in doing so is choosing the correct model, as the Debye-Hückel equation is valid at low ionic strength, and the inclusion of empirical constants is needed to account for a more complex ion environment and specific interactions. Extending and understanding the ion activity models are a field of study of its own, but accurate reporting of thermodynamic pKₐ values will make it easier to compare values obtained in different studies.

8. Role of organic co-solvents in relation to buffer pKₐ

Organic co-solvents have a lower dielectric constant than water. The dielectric constant is a measure of the polarity of a solvent, and thus the addition of organic co-solvents will reduce the overall polarity of a solution. The concentration of organic co-solvent is often varied during pre-formulation to find a suitable concentration, but during later pharmaceutical processing, the concentration will be constant. From Eq. 15 and 16, it can be seen how the activity coefficient depends on the dielectric constant. Reduction in the dielectric constant results in a decreased polarity. Changes in the polarity of the solution will lead to changes in pKₐ values (Avdeef et al., 1999; Yalkowsky, 1999; Yasuda, 1959). The variation in pKₐ depends on the fraction of the co-solvent, the pH of the solution and the buffer species (Subirats et al., 2007). For carboxylic acids,
addition of co-solvents will increase the $pK_a$ values (Williams et al., 2013). The magnitude of the increase depends on the polarity and amount of the co-solvent. The $pK_a$ of acetic acid increases from 4.76 to 5.89 in a 50:50 mixture of water-DMSO (Yalkowsky, 1999). Also in a 50:50 mixture of water-ethanol is a changed in the $pK_a$ value of acetic acid observed, where it has been reported to be 5.62 (Yalkowsky, 1999). The $pK_a$ of phosphate buffer also increases as the polarity of the solvent decreases (Yalkowsky, 1999).

The $pK_a$ of Tris decreases from 8.06 to 7.66 in a 50:50 mixture of water-DMSO (Yalkowsky, 1999). Biological buffers, i.e. HEPES, MES TAPS etc., show a similar decrease in $pK_a$ values by addition of organic co-solvents. In general, the effect on $pK_a$ values by co-solvents is smaller with Tris and biological buffers compared to carboxylic acid buffers. In general, when adding co-solvents, the $pK_a$ value of a base is decreased by half as much as the $pK_a$ value of an acid is increased (Yalkowsky, 1999).

The acidity of the buffer dictates the effect of added organic co-solvents. In drug formulation and processing, addition of organic co-solvents can be a necessity. Organic co-solvents are used to overcome issues with low aqueous solubility (Avdeef, 1998). This strategy is often applied to non-polar compounds which are practically insoluble in water (Williams et al., 2013). The amount of organic co-solvent needed depends on the insoluble compound and the desired concentration. The effects of an organic co-solvent on the $pK_a$ of the buffer can be minimized by using a small amount of organic co-solvent and choosing a co-solvent with a dielectric constant close to that of water.

It is possible to extrapolate the obtained $pK_a$ values to zero organic solvent content (Avdeef et al., 1999; Rived et al., 2001). Several extrapolation equations exist, but the most popular relates the
dielectric constant to the pKₐ value through a linear relationship (Ruiz et al., 2005). The pKₐ value must be measured at various water-methanol concentrations to obtain empirical constants that can be used for the extrapolation. A linear relationship is seen for 0-60 % methanol content. More advanced models also exist.

9. Summary

The pH of a buffer solution is an important characteristic during drug formulation and processing, and it depends on the buffer pKₐ. Altering the temperature affects the buffer pKₐ as described by the van’t Hoff equations, and the effect on pKₐ can be predicted based on ΔH and ΔCₚ. The effect of temperature depends on the functional groups of the buffer. Carboxylic acid buffers and carboxylic acid moieties in amino acids have a low temperature-dependency, while amine containing buffers are very temperature-dependent. Pressure changes within an interval of 0-100 MPa result in practically no changes in pKₐ values for any of the buffer species. At higher pressures, changes in pKₐ up to 0.5 pH units should be expected.

Altering ionic strength or the polarity of the solvent can be used to increase solubility of poorly soluble compounds, but these changes will affect the pKₐ value due to the effect on the activity coefficient. The ionic strength affects the pKₐ value in an interval-dependent manner depending on the structure of the buffer. Co-solvents reduce the polarity of the solvent resulting in decreased pKₐ values for bases and increased pKₐ values for acids.

The effect on pKₐ can be exploited to increase chemical stability. However, changes in pKₐ values can also present challenges during drug formulation and processing, i.e., during autoclaving, storage or homogenization where temperature or pressure increase. A carboxylic acid buffer or phosphate
buffer would be most suited to avoid changes in pKₐ value of the buffer due to temperature changes. To avoid changes in pKₐ of buffer species, the ionic strength and polarity of the solvent should be kept constant.

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References


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Figure legends

Figure 1: pK<sub>a</sub> values as function of temperature (°C) for selected carboxylic acid buffers; acetate, citrate, and succinate. The pK<sub>a</sub> values are almost invariant over the temperature range of 0-125°C. The plot is generated based on experimental data available from Goldberg et al. (2002).

Figure 2: pK<sub>a</sub> values as a function of temperature (°C) for Tris buffer and some biological buffers; MES, TAPS, and HEPES. The pK<sub>a</sub> values for all buffers decrease as temperature increases. This plot is based on experimental data available from Goldberg et al. (2002) and Fukada and Takahashi (1998).

Figure 3: pK<sub>a</sub> values as a function of temperature (°C) for the amino acids; lysine, histidine, and glutamate. (●–): pK<sub>1</sub>. (■–): pK<sub>2</sub>. (▲–): pK<sub>3</sub>. The open data points from 0-60°C are experimental data from Nagai et al. (2008). The filled data points from 75-125°C are extrapolated based on ΔH and ΔC<sub>p</sub> in Table 1 and Eq. 8 or 10.

Figure 4: Flowchart illustrating how temperature and pressure should be considered during the selection of a suitable buffer for pharmaceutical processing.