Univerzita Karlova v Praze

1. lékařská fakulta

Studijní program: Biomedicínská informatika

Study programme: Biomedical Informatics

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Reunified description of acid-base physiology and chemistry of blood plasma

Znovusjednocení popisu acidobazické fyziologie a chemie krevní plazmy

PhD Thesis

Dizertační práce

Supervisor / Školitel: doc. MUDr. Jiří Kofránek, CSc.

Praha, 2013

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Identification record

MATOUSEK, Stanislav. *Reunified description of acid-base physiology and chemistry of blood plasma.* Prague 2013. 152 p., 0 suppl. PhD. thesis, Charles University in Prague, First Faculty of Medicine, Institute of Pathological Physiology. Supervisor Jiri Kofranek.

Identifikační záznam

MATOUŠEK, Stanislav. *Znovusjednocení popisu acidobazické fyziologie a chemie krevní plazmy [Reunified description of acid-base physiology and chemistry of blood plasma].* Praha 2013. 152 s., 0 příl. Dizertační práce. Univerzita Karlova v Praze, 1. lékařská fakulta, Ústav patologické fyziologie. Školitel Jiří Kofránek.

Content

Acknowledgements

I would like to thank most sincerely to my supervisor doc. MUDr. Jiří Kofránek, CSc. for all his help and support, insightful discussions, kindness, friendship and teaching me that despite all the difficulties, we can sometimes touch the stars.

I would also like to thank to my Welsh, English and Danish co-author, Prof. Stephen E. Rees, PhD, for all the inspiration, friendship, know-how and support he has given me, as well as being an extraordinary host during my research stays in Aalborg, Denmark.

And finally, I would like to express many thanks to all those, who have given me support on this difficult journey. This includes the biggest fan, my father. Thank you. Without all the support that I felt, I would not have managed to come to the end.

I would like to dedicate this work to:

RNDr. Jaromír Neužil

The most amazing chemistry teacher I have ever known. Without the detailed, structured, old-school knowledge he has provided to his students at high-school Gymnázium Zlín, this work would never have been possible.

Abstrakt

Tato dizertace se zabývá důležitým problémem biomedicínské acidobazické teorie, kde existují dva zdánlivě neslučitelné způsoby popisu acidobazického stavu krevní plazmy, zatímco popisovaná fyziologie a chemie evidentně musí být pouze jedna. Tyto dva způsoby popisu se nazývají klasický přístup, který je založený především na práci Ole Siggaard-Andersena, a moderní přístup, který je založen na práci Petra Stewarta a jeho následovníků. Zdroje, ze kterých vycházím, jsou troje. Prvním zdrojem jsou obecně přijímané základní koncepty acidobazické chemie a fyziologie. Druhým zdrojem je elegantní formalizmus popisu acidobazických dějů v roztocích komplexního složení vytvořený Guentherem. Úvodní část dizertace je založena na těchto dvou východiscích, na nich pak staví detailní analýza podpořená použitím jednoduchých počítačových modelů. Výsledkem je formální popis několika složitějších koncepcí, včetně titračních křivek proteinů, chování bikarbonátu a proteinových pufrů ve společném roztoku, vztahu mezi nábojem silných iontů a nábojem proteinových pufrů a neostré hranice mezi silnými ionty a ionty pufrů ve fyziologii. Následuje porovnání různých modelů albuminu jakožto hlavního bílkovinného pufru krevní plazmy. Některé teoretické koncepce této práce, jako například pH - pK_A kriterium jsou validovány chováním těchto modelů. Třetím východiskem této práce je detailní znalost formulace obou přístupů. Následující část dizertace staví jak na této znalosti, tak na výsledcích obecné teorie rozvinuté v první části. Předložené porovnání obou přístupů je nejdetailnějším a nejdůkladnějším, jaké kdy bylo publikováno, alespoň pokud je mi známo. Je osvětleno několik fundamentálních slabin moderního přístupu, některé z nich jsou, zdá se, poprvé podrobeny vážné diskusi. Je odvozena transformace mezi rovnicemi a proměnnými obou přístupů, explicitní forma transformačních vztahů je původním příspěvkem k teorii. Existence transformace ukazuje, že žádný z přístupů neobsahuje informaci, která by v druhém přístupu chyběla. Na základě tohoto porovnání je navrhnut znovusjednocený popis, který by jak kombinoval silné stránky obou přístupů, tak eliminoval slabiny. Větší část je převzata z klasického přístupu, kde se několik klíčových konceptů jeví být lépe zakotvených buď v klinických potřebách, nebo v experimentu. Závěrem je demonstrováno využití získaného vhledu při tvorbě výukových acidobazických simulátorů, což bylo i původním cílem práce.

Klíčová slova: Acidobazická chemie, acidobazická fyziologie, Stewartova teorie, moderní přístup, přístup využívající silné ionty, klasický přístup, počítačové modelování, albumin, titrační křivky, rozdíl silných iontů, SID, přebytek bazí, base exces, BE, hypoproteinemická alkalóza.

Abstract

This thesis addresses an important problem of biomedical acid-base theory, where there are two apparently contradictory ways of describing the acid-base status of blood plasma, while the underlying physiology and chemistry obviously has to be only one. The two descriptions are called the traditional approach, based mainly on the work of Ole Siggaard-Andersen and the modern approach, based on the work of Peter Stewart and his followers. This work has three starting points. First are generally accepted basic concepts of acid-base chemistry and physiology. Second is an elegant formalism to the description of acid-base phenomena in complex solutions developed by Guenther. First part of this thesis builds on these two starting points, which serve as a basis for a detailed analysis augmented by the use of simple computer modelling. This results in formal description of several more advanced concepts, including the titration curves of proteins, behaviour of bicarbonate and protein buffers in single solution, relationship between strong ion charge and protein buffer charge and fuzzy division between strong ions and buffer ions in physiology. The modeling work then proceeds to comparing various models of albumin, principal protein buffer of blood plasma. Theoretical concepts of this work, such as $pH-pK_A$ criterion are validated by the behaviour of these models. Third starting point is the detailed knowledge of the formulations of both approaches. Second part of this thesis builds on the results of the general theory developed in the first part; the presented comparison of the two approaches is the most detailed and comprehensive so far, at least to my knowledge. Several fundamental weaknesses of the modern approach are uncovered; some of them seem to be seriously discussed for the first time. The transformation between the two approaches is derived; the explicit form of the transformation relationships is an original contribution. The existence of the transformation shows that neither approach contains extra information to the other one. Out of this comparison, reunited description is suggested, combining the strengths of both approaches and eliminating the weak points. More is taken from the traditional approach, where several key concepts appear more rooted either in clinical needs or in experiment. The thesis concludes by showing the use of the gained insight in building educational acid-base simulators, which was the original purpose of this work.

Keywords: acid-base chemistry, acid-base physiology, Stewart theory, modern approach, strong ion approach, traditional approach, computer modelling, albumin, titration curve, strong ion difference, SID, base excess, BE, hypoproteinemic alkalosis

Preface

Acid-base chemistry and the regulation of the acid-base ballance is a complex part of human biochemistry and medical physiology. It is connected to the blood gas transport, metabolism, regulation of volume and composition of the internal environment and proper renal and pulmonary function. Pathological disturbances of acid-base ballance are encountered in various fields of clinical medicine, including intensive care, nephrology and pneumology. Quantification of these disturbances involves mathematical description that can seem nontrivial.

As such, acid-base has often been difficult to understand for both students and medical professionals. The situation is further complicated by the fact that there is a disagreement about some relevant concepts even among experts. All this makes it an area, where a systematic analysis based on formalized mathematical description and generally accepted chemical principles can lead to a better insight, the use of educational computer simulators can help to transfer this insight into brighter and more effective teaching and both can hopefully bring about a more focused clinical approach aware of what is a relevant description of patients' pathological condition and what is less so.

In the past two decades, acid-base chemistry and its description in the context of human medicine have been divided into two opposing approaches: So called modern approach, conceived by Peter Stewart mainly in early 1980's, and the so called traditional approach, based on the work that Ole Siggaard-Andersen, Peter Astrup and others advanced mainly in the 1960's. Followers of Stewart's approach have seen their view as a "revolution in our understanding" of acid-base (1), providing "a unique insight into the pathophysiology of acid-base derangements'' (2) and describing true causal mechanism by which acid-base disorders develop (2). These researchers also claimed that the traditional approach has missed one important determinant of acid-base status, called total concentration of weak acid (A_{tot}), (3). This parameter has been shown to depend mainly on the concentration of albumin and associated with significant acid base disturbances (4), (5). However, Ole Siggaard-Andersen and others have maintained very skeptical look at the modern approach, countering with the statement that Stewart's approach "is anachronistic and the terminology misleading, confusing anions and cations with acids and bases." (6)

In this situation, I have become part of a research team, lead by my supervisor dr. Kofranek. Our group has been interested in building large scale computer models of internal environment and of acid-base physiology and later also smaller scale educational simulators. Given my deep interest in

acid-base chemistry, I was naturally drawn into trying to figure out how the two approaches fit together and how well do elements of each theory describe the underlying acid-base physiology, which obviously has to be only one. Meanwhile, common links between the approaches gradually started to be published (7), (8), a process to which I have contributed as well (9). My work was needed in our computer modelling, but in the end, it also gave results that can be used directly in the clinical practice.

The work that has been done is mostly analytical or deductive, amender with the use of simple computer models needed to plot the accompanying graphs (an example of such a simple model would be for example the Figge-Fencl model of albumin buffering properties). These models were implemented in Microsoft Excel 2007, Wolfram Mathematica 8.0 and Modelica / Dymola, resulting graphs were plotted mostly in Microsoft Excel, 3D graphs in Wolfram Mathematica. No underlying data were needed, as both theories have already been shown to fit experimental data; the disagreement is in the level of interpretation of known facts rather than data precision.

The thesis is structured into four chapters, starting with general concepts and gradually moving to the deductions drawn from these concepts.

Chapter one covers the general description of acid-base chemistry, applicable to any field. Topics covered include the dissociation of water and definition of pH; description of acids, bases and buffers and general description of mixture solutions containing more than one substance with possibly more than 1 dissociation step. Two master equations that summarize the acid-base behavior of complex mixture solutions in a nicely concise form are given, one for total titratable acid (C_H) and the other for total titratable base (C_B).

Chapter two is concerned with those notions of acid-base chemistry pertinent to human (and animal) physiology. These include special role of carbon dioxide – bicarbonate buffer, concept of linearization in describing buffering properties of proteins, description of a mixture of bicarbonate and protein buffers, relationship between charge of protein buffers and their buffering properties (i.e. relationship between BB and SID), recent advances in modelling of albumin and their implications and electroneutrality equation in plasma.

Chapter three compares the two most popular approaches to the mathematical modeling of acid-base chemistry of blood; that of Ole Siggaard-Andersen and colleagues and that of Peter Stewart. First, both approaches are introduced in their historical perspective. The theory developed in the first two chapters is then used to get further insight into each one. Severe weaknesses of the modern approach are uncovered during this process. The general theory is also used for a detailed

comparison of the two approaches, showing that there is a transformation or a mapping between the description of the traditional approach and that of the modern approach. The description of this transformation develops the theory, published in one of the papers of this thesis (9), and similar to the conclusions of other authors (10), (7). Still, the version published in this thesis seems to be the most detailed and comprehensive coverage of the topic so far, containing parts that have not been published so far. The existence of the transformation means that in principle both approaches could be used to draw same conclusions in clinical practice. However, it does not necessarily mean that the information is structured equally well in terms of its usefulness for direct interpretation of patient data.

Chapter four shows how the insight gained when developing the general theory and comparing the approaches can be implemented in educational simulators. Various aspects of acid-base physiology can be efficiently demonstrated with the help of current technology, including detailed behaviour of buffers, bicarbonate buffer and its role in physiology, relationship between strong ions and buffers and a relationship between bicarbonate and non-bicarbonate buffers. Reunited description of acid-base disorders is suggested out of the comparison of the previous chapter, combining the strengths of both approaches and eliminating the weak points. More is taken from the traditional approach, where several key concepts appear more rooted either in clinical needs or in experiment. Final summary discusses the content of this work and its contribution from various points of view. Current and past development of acid-base theory is explored and the ideas of this thesis are put into context. Limitations of this work are also discussed, as well as possible future development of the field.

Chapter 1

General theory of acid-base chemistry

Chapter one covers the general description of acid-base chemistry, including the necessary equations. Topics covered include the dissociation of water and definition of pH; description of acids, bases and buffers; general description of mixture solutions containing more than one substance and mathematical treatment of substances with more than 1 dissociation step, first alone and then in mixture. Complex mixture solutions are described using two master equations, one for total titratable acid (C_H) and other for total titratable base (C_B). These are, in fact, mirror descriptions of the same chemical phenomenon.

Elementary concepts of acid-base chemistry

Acid-base chemistry is concerned with reversible reactions of oxonium (hydronium) and hydroxide ion with other substances, typically dissolved in water solution. Oxonium and hydroxide ions are mostly in dynamic balance and if not, the balance is attained almost instantaneously (i.e. in microseconds and faster (11)) through the following chemical reaction

$$H_2O + H_2O \leftrightarrows H_3O^+ + OH^-$$ (1.1)
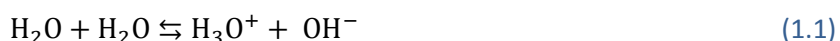
The reaction is known as hydrolysis of water (to the right) and neutralization (to the left). The reaction can be simplified by ignoring one of the water molecules on both sides. On the right side, this means substituting hydrogen ion in place of the oxonium ion. Reaction (1) then becomes

$$H_2O \leftrightarrows H^+ + OH^-$$ (1.2)

Reaction equilibrium can be expressed as a (mathematical) product of reaction product concentrations over the reactant concentration, where K_w' is the equilibrium constant

$$K'_w = \frac{[H_3O^+] * [OH^-]}{[H_2O]^2}$$ (1.3)

Because the concentration of water is typically large and practically does not change, the reaction equilibrium can be written in an equation that excludes water, where K_w equals $K_w'/[H_2O]^2$., K_w is called ionic product of water

$$K_w = [H^+] * [OH^-]$$ (1.4)

The value of K_w is $1*10^{-14}$ at temperature of 25°C. Whenever disturbance to the reaction equilibrium occurs, as in adding an acid (that contains high concentration of oxonium ions) into a solution, some oxonium ions react with hydroxide ions until a new balance is established and ionic product of water is satisfied again. There are situations, where majority of added ions react this way and "disappear" by forming water, an example of this being a situation where the concentration of hydroxide ions was high in the beginning and acid was added (neutralization reaction). However, there are also situations, where the amount of ions that have reacted and formed water is practically unmeasurable, as in adding acid to already acidic solution. This variability in behavior is caused by a great variability in concentration of oxonium (hydrogen) and hydroxide ions, which are present in concentrations differing by 14 orders of magnitude.

That is why it is desirable to set up a logarithmic measure of oxonium (hydrogen) ion concentration defined[1] as:

$$pH \equiv -\log_{10}[H_3O^+] \equiv -\log_{10}[H^+] \tag{1.5}$$

Neutral pH is such at which $[H_3O^+]$ equals $[OH^-]$. This happens when they both equal $\sqrt{K_w}$, which numerically is $1*10^{-7}$ at 25°C. According to the eq. 5, pH equals 7 at this hydrogen ion concentration. Acidic solutions have pH < 7, i.e. $[H^+]$ is greater than 10^{-7}, alkalotic (basic) solutions have pH > 7, i.e. $[H^+]$ is smaller than 10^{-7}. However, K_w varies with temperature (12), increasing both with higher temperature. At 37 °C, K_w equals to $2.5*10^{-14}$, which corresponds to neutral pH of 6.8. K_w also varies with ionic strength. Note that the definition equation (1.5) is mathematically equivalent to

$$[H^+] = 10^{-pH} \tag{1.6}$$

There are still two major definitions of what is acid and what is base in use in various texts concerning acid base physiology. In the older definition, proposed by Svante Arrhenius in 1884, an acid is a substance that increases concentration of hydrogen ion in the solution, while base is a substance that increases the concentration of hydroxide ions in the solution. Examples would include lactic acid and sodium hydroxide that react as follows

$$HLac \rightarrow H^+ + Lac^- \tag{1.7}$$

$$NaOH \rightarrow Na^+ + OH^- \tag{1.8}$$

By this definition, HLac is an acid (weak acid in this case), while NaOH is a base. A substance is considered an independent entity in its undissociated, electroneutral state (on the left). The

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[1] More correctly, pH is defined with activities and not relative concentrations, where activity is relative concentration times activity coefficient γ. In ideal case, activity coefficient should approach 1, the two measures being equal. Real world situation differs from this ideal world situation most commonly in order of %. However, certain situations bring about more marked differences.

dissociated state (on the right) is considered to be derived and both ions are taken together as something that has originated from single substance by the dissociation reaction. Although older and less precise, Arrhenius definition is still in use, because the concept is easier to grasp during the chemistry curriculum. Modern approach of Peter Stewart uses this definition (13), which has been criticized by Siggaard-Andersen and Fogh-Andersen (6).

More recent definition of Brønsted and Lowry (1923) is based on the following general reaction

$$HB_i \leftrightarrows H^+ + B_i^-$$ (1.9)

where HB_i is the acid form of the substance and B_i^- is the base form of it, also known as a conjugate base. The two (and sometimes more) forms are also called species. In this definition, an acid is a species prone to donate a hydrogen ion, and base is a species prone to receive it. (OH remark) Thus lactic acid *HLac* is still an acid, but lactate anion *Lac^-* is now defined as a base. The general equation (1.9) can be applied in the case of lactic acid and sodium hydroxide as can be seen in figure 1

Conjugate acid	*Hydrogen ion*	*Conjugate base*

$$HB_i \leftrightarrows H^+ + B_i^-$$

$$HLac \leftrightarrows H^+ + Lac^-$$

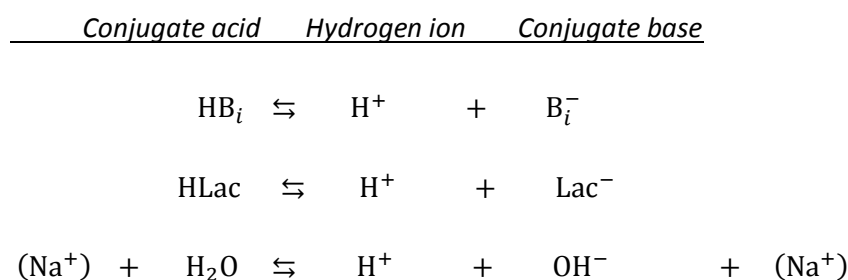$$(Na^+) + H_2O \leftrightarrows H^+ + OH^- + (Na^+)$$

Figure 1: Conjugate acid dissociates to form hydrogen ion and conjugate base. The top row shows general case. The middle row show dissociation of lactic acid into hydrogen ion and lactate. The bottom row shows that in Brønsted and Lowry theory, conjugate acid to the hydroxide ion in fact water, Na^+ being only a bystander ion. Note that typically, only very small amounts of hydroxide ions are going to react this way (to the left). For instance, mixing NaOH into pure water so that the resulting concentration is 0.1 mmol/l reduces H^+ concentrations (by reaction to the left) from 10^{-7} mol/l to 10^{-10} mol/l. Hence, as a net effect, no more then 10^{-7} mol/l of H^+ and OH^- react to the left.

In the Brønsted and Lowry definition, sodium lactate (NaLac) is a base (its pH is higher than 7) because it contains lactate Lac^- (to a much higher extent than lactic acid HLac). It is not a base because of its sodium content; sodium ion is theoretically considered an aprote (species that does not react with hydrogen ion) (6). On the contrary, in the Arrhenius definition, sodium lactate would be a base, because it (possibly) originated as salt solution by mixing strong base (NaOH) and a weak acid (HLac). If in equimolar concentration, the strong base prevails over the weak acid and the result is an alkaline solution (its pH is higher than 7).

Both acids and bases can be divided into strong and weak. Standard definition states (14) that strong acid is such that dissociates (deprotonates) fully while weak acid dissociates only partially. General chemistry is often concerned with acids as the only substance in the solution. For strong acids pH of such solution is easy to calculate based on the definition equation of pH (eq. 1.5). For instance, solution hydrochloric acid in concentration 1 mmol/l (10^{-3} mol/l) is going to have pH of 3. This concept is apparent in table 1. Similarly, strong bases are going to get protonated fully, while weak bases get protonated only partially. Common strong bases are those containing hydroxide ion (OH^-), for instance soluble hydroxides (OH^-) of alkali metal and alkaline earth metal elements. Common weak base is ammonia. Hydroxides are bases according to the Brønsted and Lowry theory, because they eagerly accept hydrogen ion, for instance from surrounding water molecules. In such a case, the OH^- itself turns into H_2O, creating OH^- from the donor molecule instead.

Concentration	0.01 mmol/l	0.1 mmol/l	1 mmol/l	10 mmol/l	0.1 mol/l	1 mol/l
pH of strong acid (e.g. HCl)	5.00	4.00	3.00	2.00	1.00	0.00
pH of weak acid (example HLac)	5.03	4.17	3.50	2.95	2.43	1.92

Table 1: pH of strong acid (HCl) as a function of acid concentration. pH of example weak acid (Lactic acid, HLac) is presented for comparison. Any strong acid is going to behave as hydrochloric acid, pH can be calculated easily as $-log_{10}(Ci)$, where Ci is the acid concentration in mol/l. For any given concentration, pH of weak acid is going to be higher than that of strong acid, which is a theoretical limit. pH of weak acid depends both on its concentration and its acid dissociation ability, characterized by a constant pK_A. Value of pK_A of lactic acid is 3.87. For calculation of pH of a weak acid as a function of concentration, see for example (14).

For each substance, there exists equilibrium of the reversible dissociation reaction (1.8). It is given by mass action equation (i.e. equation of the reaction equilibrium), which states that[2]:

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[2] Again, more correct treatment would require use of activities instead of relative concentrations. However, according to Wooten's analysis (7), medical texts concerned with acid-base chemistry use relative concentrations in the mass action equations. Exception is the relative concentration of hydrogen ion [H^+], where activity is used instead, generally without explicitly mentioning it. This is probably due to the calibration of the pH measuring equipment, where the manufacturers commonly use activities.

$$K_A = \frac{[\text{H}^+] * [\text{B}_i^-]}{[\text{HB}_i]} \qquad (1.10)$$

K_A is the equilibrium constant of the substance *i*, so it should be written as $K_{A(i)}$, but I am going to omit the subscript for now. The equilibrium reaction can also be expressed in its logarithmic form

$$pH = pK_A + \log_{10} \frac{[\text{B}_i^-]}{[\text{HB}_i]} \qquad (1.11)$$

Here, pK_A is equal to $-\log_{10}(K_A)$. Equation (1.11) is known as Henderson-Hasselbalch equation. Generally, the total concentration of the species *i*, i.e. both forms of the substance taken together, stays constant during addition of hydrogen or hydroxide ions (process known as titration or buffering).

$$C_i = [B_i^-] + [HB_i] \qquad (1.12)$$

This addition of hydrogen ion (acid) or hydroxide (base) is called titration, or buffering, depending on the context. The charge of the acidic and alkalotic form of the buffer does not necessarily have to be 0 and -1. Basically, any charge is possible, with the charge of base form being always one smaller than the charge of the acidic form, as is illustrated in figure 2.
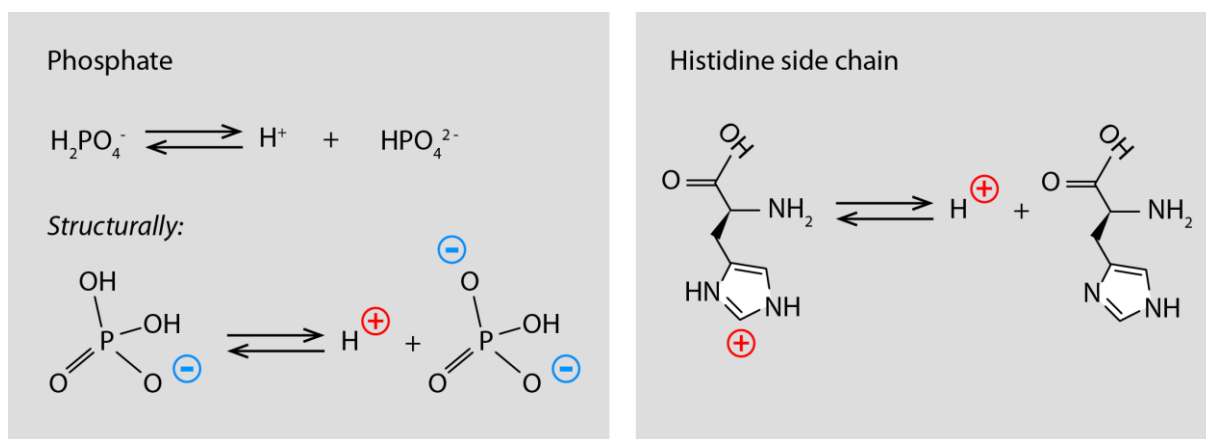

Figure 2: *The charge of acid and base form (conjugate acid and conjugate base) does not have to be 0 and -1. This is a common case, but there are many possibilities. In case of the second dissociation step of phosphate (pK$_A$ = 7.2), the charge of the acid form is -1 and that of the base form -2. In case of the histidine side chain (pK$_A$ = 6.0), the charge of the acid form is +1, the charge of the base form is 0.*

Equation (1.11) can be used to calculate the ratio of acid form to the base form at any given pH. A special case arises when pH equals pK$_A$, leading to the ratio of [HB] to [B⁻] being exactly 1:1. This is the ratio at which the substance buffering properties are most pronounced, as is going to be yet presented. Table 2 summarizes ratios of acid and base forms as a function of pH for two substances, HLac – Lac⁻ pair with pK$_A$ of 3.84 and NH$_4^+$ - NH$_3$ pair with pK$_A$ = 9.23.

pH	2.84	3.84	4.84	5	6	7	8	8.23	9.23	10.23
HLac : Lac-	10:1	1:1	0.1:1	0.07:1	0.007:1	0.0007:1				
NH_4^+ : NH_3				17000:1	1700:1	170:1	17:1	10:1	1:1	0.1:1

Table 2 *Ratio of the acid form and the base form as a function of pH. Two different substances, lactic acid/lactate (pK_A = 3.84) and ammonium (pK_A =9.23) are considered, being example species of weak acid and weak base, respectively. Note that the more acidic the pH, the higher the tendency of a species to be in its acid form. Note also a fact that might seem bit paradoxical at first: At pH close to neutral, weak acids tend to be in its base form (Lac$^-$), while weak bases are more in their acid form (NH_4^+).*

When looking at table 2, one can see that substances recognized as acids in the Arrhenius theory (as is lactic acid) have pK_A lower than 7; at pH around 7, they are mostly in their base form (lactate). This is because their acid form is really "eager" to donate a hydrogen ion and become the conjugate base (lactate). Conversely, substances recognized as bases (ammonium) have pK_A higher than 7 and are mostly in their conjugate acid form at neutral pH. This is because their base form is eager to receive hydrogen ion and become conjugate acid (ammonium ion).

Table 2 has touched on the fact that pK_A of each substance can be used to divide substances into acids and bases. Both weak acids and weak bases can function as buffers. According to Wikipedia (15), buffer is "an aqueous solution consisting of a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. Its pH changes very little when a small amount of strong acid or base is added to it and thus it is used to prevent changes in the pH of a solution.... Many life forms thrive only in a relatively small pH range so they utilize a buffer solution to maintain a constant pH. One example of a buffer solution found in nature is blood."

The ability of the buffer solution to resist pH changes can be measured using buffer capacity β, also known as buffer value. Buffer value of a solution is basically the slope of the titration curve, i.e. how much base (in experiment most commonly OH$^-$) needs to be added to cause a change in pH. Mathematically, this is same as the derivative of total titratable base C_B (i.e. OH$^-$ ions added) with respect to pH (in determining the slope of the titration curve, we can also add acid, which causes negative change in C_B and negative change in pH, resulting in same buffer value).

$$\beta \stackrel{\text{def}}{=} \frac{dC_B}{d\text{pH}} \tag{1.13}$$

C_B is going to be defined more rigorously in the next section, now it only suffices to say that for one substance only (the buffer substance), it equals

$$C_B = [\text{OH}^-] - [\text{H}^+] + [\text{B}_i^-] \tag{1.14}$$

Substituting equation (1.12) into equation (1.10) $[\text{B}_i^-]$ term can be expressed as

$$[\text{B}_i^-] = C_i * \frac{K_A}{K_A + [\text{H}^+]} \tag{1.15}$$

This formula can be used as a substitute for the $[\text{B}_i^-]$ term in the equation (1.14). We can also substitute pH for the $[\text{H}^+]$ terms, using equation (1.6) and for the $[\text{OH}^-]$ using ionic product of water (1.4). All together, this yields

$$C_B = \frac{K_w}{10^{-pH}} - 10^{-pH} + C_i * \frac{K_A}{K_A + 10^{-pH}} \tag{1.16}$$

Formula (1.16) can be differentiated with respect to pH under the assumption of constant C_i, using the rules of calculus

$$\beta = \frac{dC_B}{d\text{pH}} = 2.303 \left(\frac{K_w}{10^{-pH}} + 10^{-pH} + C_i * \frac{K_A * 10^{-pH}}{(K_A + 10^{-pH})^2} \right) \tag{1.17}$$

The coefficient 2.303 is an approximation of natural logarithm of 10, *ln*(10). It comes from differentiating powers of 10 and not of *e* (Euler's number). When we substitute back in the original variables, we get

$$\beta = 2.303 \left([\text{OH}^-] + [\text{H}^+] + C_i * \frac{K_A * [\text{H}^+]}{(K_A + [\text{H}^+])^2} \right) \tag{1.18}$$

The first two terms represent free hydroxide and hydrogen ion. They are related to the fact that at very low and very high pH, we have to add or subtract a lot of acid or base in order to change pH, which is due to the logarithmic nature of pH. These extremes are not interesting in acid-base physiology which is mostly concerned with fluids of pH between 5 and 9, where neither term is larger than 10 µmol/l. The third term is the most interesting, representing the internal buffer capacity of the substance *i*, called β_i. Written separately

$$\beta_i = 2.303 * C_i * \frac{K_A * [\text{H}^+]}{(K_A + [\text{H}^+])^2} \tag{1.19}$$

This is an established formula for the relationship between buffer capacity and the law of mass action of the substance *i*.
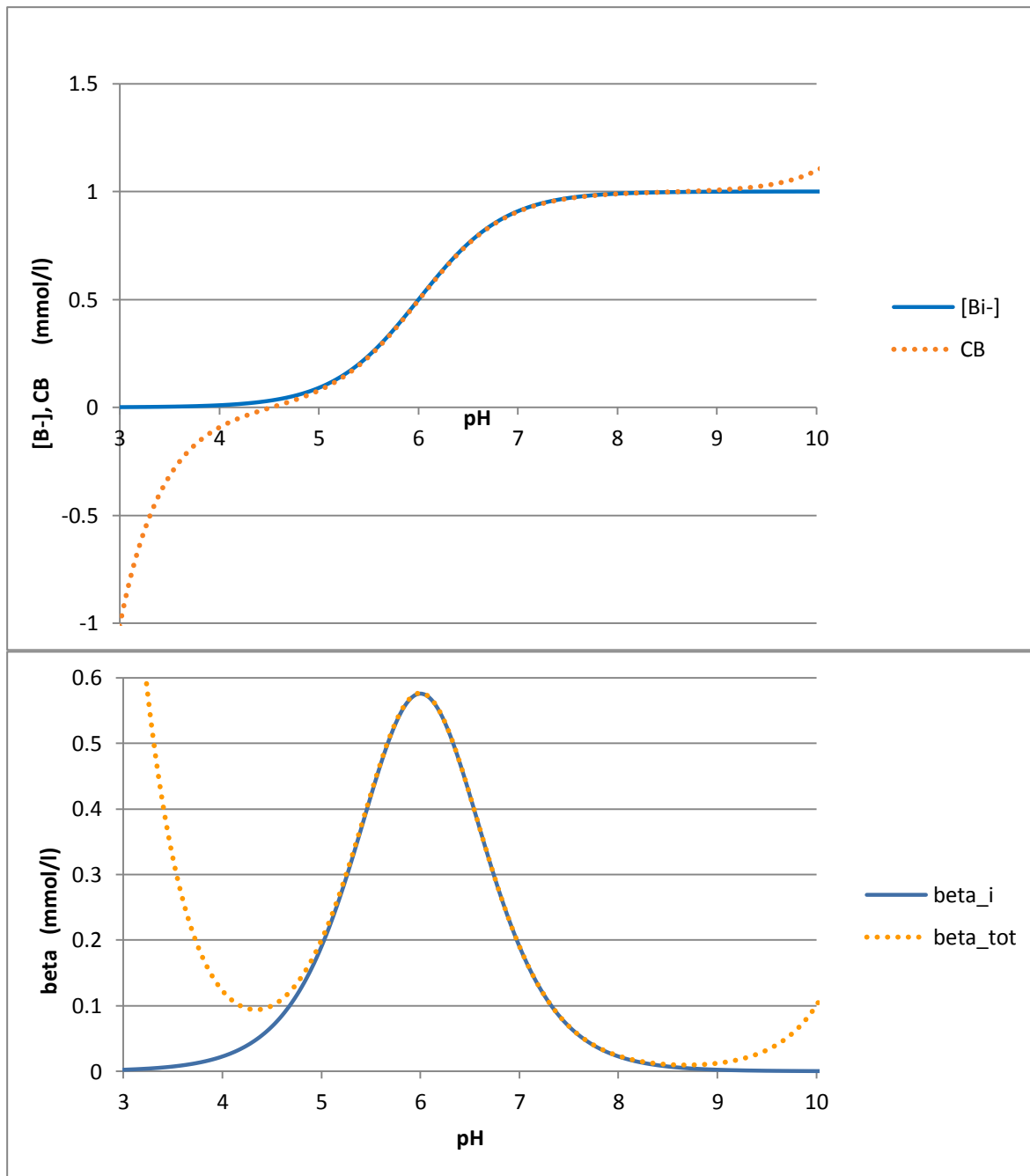
Figure 3: *The upper graph shows the titration curve of 1 mmol/l concentration of buffer substance (which could be histidine side chain) with pK_A =6.0 (orange dotted line). The y axis shows the amount of OH^- (strong base) added during the titration. The blue line represents the buffer behaviour, i.e. its transition from the acid form to the base form as pH increases. The orange dotted line is, in fact, a superposition of the substance internal buffer curve with the titration curve of pure water. The lower graph shows buffer capacity β of the same system (orange dotted line), which is again composed of the internal buffer capacity $β_i$ of the histidine buffer (blue line) and the effect of the water environment. The lower graph is a derivative (slope) of the upper graph. Note that the substance internal buffer capacity is symmetrical, with the highest point at $pH = pK_A$.*

Formula (1.19) will be used when comparing the traditional approach of Siggaard-Andersen to the modern approach of Stewart. It can also be written in slightly different form, using equations (1.15) and (1.46) in the process

$$\beta_i \;=\; 2.303 * C_i * \frac{K_A}{K_A + [H^+]} * \frac{[H^+]}{K_A + [H^+]} \;=\; 2.303 * \frac{[B^-] * [HB]}{C_i} \tag{1.20}$$

Note that the sum of [B⁻] and [HB] is bound by the equation (1.12) and has to be equal to C_i. It might be interesting to find the maximum of βi. In order to find it, we could, for instance substitute in eq. (1.12) to get rid of one of the variables (for instance [HB]), take a derivative of the resulting β_i as a function of [B⁻] with respect to [B⁻], set it equal to zero and solve. Alternatively, one can notice that the problem is analogical to the task of finding a rectangle with biggest possible area, given a fixed perimeter. Such a rectangle is known to be square, i.e. the situation when [B⁻] equals [HB]. Since (1.12) has to hold as well, they both have to equal (C_i /2). This gives us maximum value of buffer capacity β$_{Max(i)}$

$$\beta_{Max(i)} \;=\; 2.303 * \frac{\frac{C_i}{2} * \frac{C_i}{2}}{C_i} \;=\; \frac{2.303}{4} * C_i \;=\; 0.576 * C_i \tag{1.21}$$

When [B⁻] equals [HB], they are in 1:1 ratio, which is typical for the situation when pH of the solution equals pK$_A$ of the buffer (table 2). Thus, each buffer has highest buffer capacity when pH is around its pK$_A$. This can be appreciated at figure 3, where the buffer capacity of a single buffer with pK$_A$ = 6.0 is shown. Note than the internal buffer capacity is symmetrical around pK$_A$, regardless where that pK$_A$ is.

$\lvert$pH-pK$_A\rvert$	0	0.5	1	1.5	2	2.5	3.0	4.0	5.0
β$_{i\ Relative}$	100%	73%	33%	12%	3.92%	1.26%	0.40%	$4.00*10^{-4}$	$4.00*10^{-5}$
β$_{i\ Absolute}$	0.576	0.420	0.190	0.068	$2.26*10^{-2}$	$7.24*10^{-3}$	$2.30*10^{-3}$	$2.30*10^{-4}$	$2.30*10^{-5}$

Table 3: *Distribution of internal buffer capacity around the maximum at pH = pK$_A$. The buffer is in unit concentration. $\lvert$pH-pK$_A\rvert$ is the difference of absolute values, representing symmetrical variation to both sides. At the pH range of (pK$_A$ - 0.5; pK$_A$ +0.5), βi is relatively constant losing only ¼ of its value (see also figure 3).Then it decreases almost linearly until pH of about pK$_A$ ± 1.5. At each tail, βi decreases exponentially to zero.*

Equation (1.11) can be written in its exponential form, giving the information already summarized in table 2

$$\frac{[B_i^-]}{[HB_i]} = 10^{pH-pK_A} \tag{1.22}$$

This essentially means that if pH is higher than pK_A by 2, only 1% of the substance is in its $[HB_i]$ form and 99% are in the base form $[B_i^-]$. When pH is higher by 3, 99.9% of the substance is in the base form $[B_i^-]$. Thus, when pH is sufficiently high (higher by about 2.5 -3 then pK_A at normal 2-3 digit precision), we can approximate $[B_i^-]$ by C_i. By similar argument, $[HB_i]$ can be approximated by C_i when pH is sufficiently low. Thus, we can write

for $pH \ll pK_A$: $$\frac{[B_i^-]}{C_i} = 10^{pH-pK_A} \tag{1.23}$$

for $pK_A \ll pH$: $$\frac{[HB_i]}{C_i} = 10^{pK_A-pH} \tag{1.24}$$

Substituting these relationships into the equation (1.20), we get a useful approximation of βi

for $pH \ll pK_A$: $$\beta_i = 2.303 * \frac{[B_i^-]}{C_i} * [HB_i] \doteq 2.303 * 10^{pH-pK_A} * C_i \tag{1.25}$$

Analogical expression can be derived for the other tail. This behaviour explains the exponential decrease of β$_i$ as pH gets further from pK_A, as well as the coefficient 2.30 seen in the last columns of table 3. For the relative buffer capacity, we get a coefficient 4.00, as can be seen from eq. (1.25) and (1.21)

for $pH \ll pK_A$: $$\beta_{i\,Relative} \overset{def}{=} \frac{\beta_i}{\beta_{Max(i)}} \doteq \frac{2.303 * 10^{pH-pK_A} * C_i}{\frac{2.303 * C_i}{4}} = 4 * 10^{pH-pK_A} \tag{1.26}$$

and analogically for the other tail. This behaviour can be also seen in table 3, where the values have been computed using eq. (1.19).

As has become apparent from this analysis, buffer behaviour of a substance can be assessed taking into consideration pK_A and the pH of interest. Furthermore, pK_A can be used to make a division between strong acids and weak acids and strong bases and weak bases. In the general chemistry, the dividing pK_A of strong acids is lower than zero and negative (14), while most common strong bases are hydroxides of alkali metals and alkaline earth metals, having pK_A above 12 (in theory, the H_2O - OH^- transition should have pK_A of 14.0).

When strong acids and bases dissociate, the remaining part (as is chloride and sodium) behave as a so called strong ion, not interfering further with acid-base reactions. After dissociation, all the acid-base effects are dependent on free (or bound to other substances) H^+ and OH^- ions.
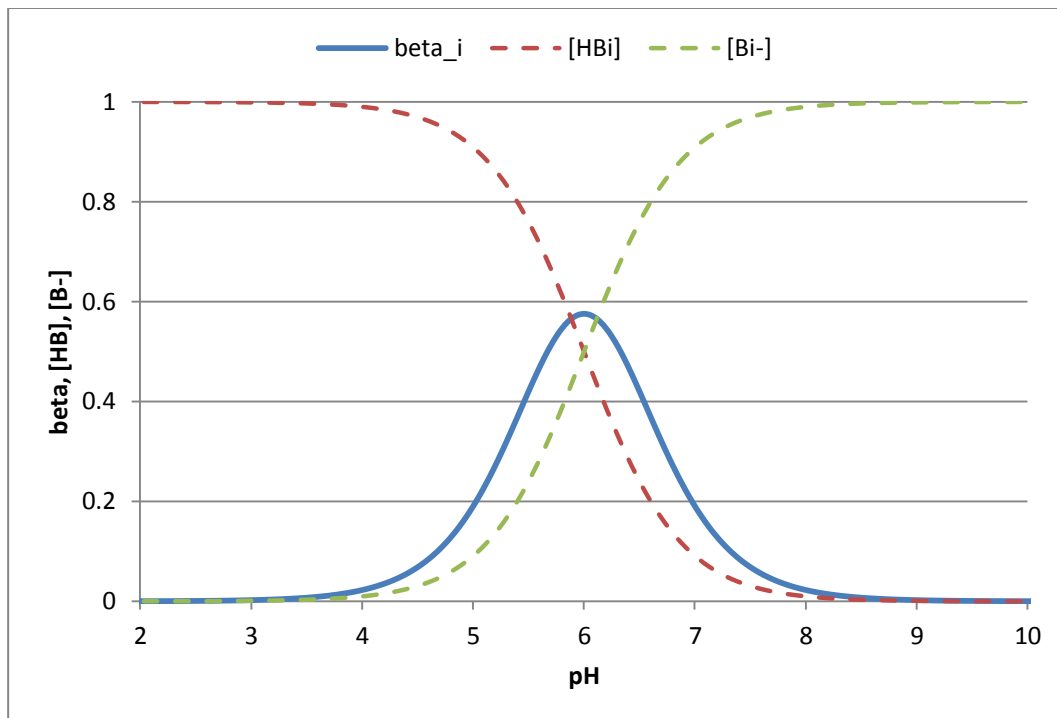

Figure 4: Relationship between buffer capacity and the concentration of acid form [HB_i] *and the base form* [B_i^-] *of a buffer. Buffer is in unit concentration. The* [HB_i] *is therefore numerically equal to the average number of bound hydrogen ion per molecule,* $\bar{n}_i$ *, as defined in eq. (1.36) and* [B_i] *is equal to the average number of free hydrogen binding sites per molecule,* $\bar{e}_i$ *, as defined in eq. (1.37). Note that when pH of interest is sufficiently large (e.g. above 9),* B_i^- *behaves as a strong ion, its charge being practically constant.*

Besides the strong ions that originate from strong acids and bases, another class of substances is treated as strong ions in physiology even though their pK_A is not as extreme as to call a substance "strong" in general chemistry. This is because the pH of the most interest in physiology i.e. that of either extracellular or intracellular fluid is quite close to 7, being fairly limited range as compared to the general chemistry. Physiological pH of plasma is tightly regulated to be between 7.36 - 7.44 under physiological circumstances. Even in the most extreme pathologies, plasmatic pH does not get outside 6.9 – 7.9, this being a life compatible range. Intracellular pH is generally slightly below 7, its variations being comparable to those of plasma. Only physiological fluids with pH outside this tight range are fluids of the digestive tract (stomach, duodenal fluid) and excretions (urine, sweat). For instance, urine pH varies between 4.6 and 8.

pH	$pK_A - 4$	$pK_A - 3.5$	$pK_A - 3$	$pK_A - 2.5$	$pK_A - 2.0$	$pK_A - 1.5$	$pK_A -1$	$pK_A -0.5$	pK_A
[HB$_i$] ($\overline{n}_i$)	99.99%	99.97%	99.9%	99.7%	99%	97%	91%	76%	50%
[B$_i^-$] ($\overline{e}_i$)	0.010%	0.032%	0.10%	0.32%	1.0%	3.1%	9.1%	24%	50%

pH	$pK_A +0.3$	$pK_A +0.5$	$pK_A +1$	$pK_A + 1.5$	$pK_A + 2$	$pK_A + 2.5$	$pK_A +3$	$pK_A +3.5$	$pK_A +4$
[HB$_i$] ($\overline{n}_i$)	34%	24%	9.1%	3.1%	1.0%	0.32%	0.10%	0.032%	0.010%
[B$_i^-$] ($\overline{e}_i$)	66%	76%	91%	97%	99%	99.7%	99.9%	99.97%	99.99%

Table 4: *Percentage of a substance i in the acid form and in the base form as a function of pH. When |pK$_A$-pH| is larger then 3, substance is 99.9 % in the acid form (acidic side) or the base form (alkalotic side).*

When the substance pK_A is lower by 3 than the pH of interest, 99.9% of it is going to be in its base form, as can be seen in table 4. Buffer capacity is at 0.4% of its maximum value (table 3), while for the true strong ion, it should be zero. These differences are negligible even in most research, not to mention clinical practice. Similarly, substances with pK_A higher by 3 than the pH of interest are going to be 99.9% in their acid form, buffer capacity being at 0.4% of its maximum value. This again differs negligibly from the true strong ion behaviour (that is if the acid form charge is non-zero). This type of thinking is going to be used repeatedly in this work, being called pH - pK_A criterion.

Even with the pH - pK_A criterion, the division between buffer ions and strong ions is not completely clear cut. When chemists want to use a single substance as a buffer, the advised pH range of effective buffering is only about $pK_A \pm 1$ or ±1.5 (16). Constable uses pK_A being ±1.5 away from the pH of interest as the division criterion between buffer ions and strong ions (2), but I believe that this is not enough, as substances still possess 12 % of their maximum buffer capacity around this value (table 3). It is true, though, that buffers becomes gradually weaker when its pK_A is in between 1 and 2 units away from the pH of interest. pK_A between 2 and 3 units away from the pH of interest constitutes a boundary zone, where strong ion behaviour gradually predominates over the buffer behaviour. Inclusion in each category can be discussed, for instance depending on buffer

concentration. When pK_A is more than 3 units away from the pH of interest, strong ion behaviour can be assumed (17). However, the division has to be reconsidered when pH of interest changes or broadens.

Given the physiological pH range of 6.9 to 8.0, one can generally assign strong ion behaviour to any species or buffer group with pK_A below 4 or above 10.5. A typical species where this approach has been widely accepted is lactate, pK_A =3.84, which is generally considered a strong anion in physiology (18), (19), (7), (20), (21) even though, generally speaking, it is a (weak) base form of weak acid. So far, the principle has not been widely accepted for amino acid residues on proteins, although some researchers have already pointed out to it (22). Modelling of albumin properties in chapter 2 is going to show that if the pK_A − pH criterion is well fulfilled, its application even to proteins may be substantiated.

Mixture of more monovalent substances

Let's now consider a situation quite typical of biology of having a mixture of several or even many substances that can function as buffers. For the sake of simplicity, let's just consider each substance monovalent for now, i.e. each substance can dissociate just once. When more than one substance is present in the solution, the balance is going to establish, where mass action equation (1.10) is satisfied for all substances. If we cause a disturbance to this established balance, for instance by adding strong acid or by adding a new buffer, hydrogen and hydroxide ions are going to redistribute in the system. General mixture of substances has to satisfy the following set of equations, based on eq. (1.11) and (1.12) for each of them.

$$pH = pK_{A(1)} + \log_{10} \frac{[\text{B}_1^-]}{[\text{HB}_1]} \qquad (1.27)$$

$$pH = pK_{A(2)} + \log_{10} \frac{[\text{B}_2^-]}{[\text{HB}_2]}$$

$$\vdots$$

$$pH = pK_{A(\text{n})} + \log_{10} \frac{[\text{B}_n^-]}{[\text{HB}_n]}$$

$$C_1 = [B_1^-] + [HB_1] \qquad (1.28)$$

$$C_2 = [B_2^-] + [HB_2]$$

$$\vdots$$

$$C_n = [B_n^-] + [HB_n]$$

In order to solve this system, all the $pK_{A(i)}$ and C_i should be known. Unknown entities include all the $[HB_i]$, $[B_i^-]$ and pH (for the sake of simplicity, we write HB and B⁻, although other charges are possible). Thus, we have a system of 2n equations with 2n+1 unknown variables. One equation is missing, one that would account for the total hydrogen ion concentration in all its forms. This is it

$$C_H \stackrel{\text{def}}{=} [\text{H}^+] - [\text{OH}^-] + [\text{HB}_1] + [\text{HB}_2] + \cdots + [\text{HB}_n] \qquad (1.29)$$

New variable C_H is defined here; called total titratable acid, it includes the total concentration of hydrogen ions, both free and bound. In order to solve for pH, C_H has to be known (alternatively we could solve for C_H, knowing pH e.g. from measurement). Unfortunately, two more variables, $[\text{H}^+]$ and [OH⁻], appear in equation (1.29), now necessitating two more equations to make the system

complete. These two equations are the definition equation of pH (1.5) and the ionic product of water (1.4).

The logic of the total titratable acid definition equation (1.29) is as follows. Imagine that we have added strong acid to the system, for instance 1 mmol/l. Before the acid (i.e. added H^+) starts to react with other substances present in the solution, C_H changes. There is now 1 mmol/l of H^+ more, increasing C_H by 1 mmol/l. However, system is now in disequilibrium. There are three possibilities of what can happen with the added $[H^+]$ until new equilibrium is established:

1) Part of the H^+ reacts away with the OH^-, let's call this part $\Delta[H^+]_1$. Because of the 1:1 reaction ratio, there is an equal decrease of OH^- concentration, i.e.

$$\Delta[OH^-]_1 = \Delta[H^+]_1 \tag{1.30}$$

This reaction proceeds until equilibrium is established based on eq. (1.4). Obviously, the amount of $[H^+]$ that has "disappeared" has to be smaller than the original concentration of OH^-. This is why the amount that has reacted away this way depends on the pH: The higher the pH, the bigger is the concentration of $[OH^-]$ available for the reaction. Nevertheless, whatever the case, C_H stays constant during the process, because the change in $[H^+]$ is offset by the equal change in $[OH^-]$, and the two entities are subtracted from one another in the C_H definition equation (1.29). This is the only process that causes a "disappearance" of hydrogen ion; however, C_H is still conserved by the virtue of subtracting less $[OH^-]$ that accounts for the lost $[H^+]$.

2) Part of the H^+ binds to the base forms of the dissolved substances. The decrease of free $[H^+]$ is offset by the concomitant increase in $[HB_i]$ forms of the present buffer species. This process does not cause any change in total H^+ concentration, H^+ merely moves from the free form to the bound form, i.e.

$$\Delta[H^+]_2 = -\sum_i \Delta[HB_i]_2 \tag{1.31}$$

This second process continues until the equilibrium is reached for all substances. Only the free form is accessible to direct measurement by pH meters.

3) The remaining part of H^+ ions just stays in solution without reacting with anything.

Let's call the C_H value before the addition of strong acid $C_{H(Old)}$, the amount of added acid $[H^+]_{Add}$, value of C_H after the addition of strong acid $C_{H(PreEq)}$ and its value after equilibration $C_{H(PostEq)}$. The

analysis that we have just carried through can be written in a more formalized fashion, using equations (1.29) to (1.31) in the equation (1.33)

$$C_{H(Old)} + [H^+]_{Add} = C_{H(PreEq)} \tag{1.32}$$

$$C_{H(PostEq)} = C_{H(PreEq)} + \Delta[H^+]_1 - \Delta[OH^-]_1 + \Delta[H^+]_2 + \sum_i \Delta[HB_i]_2 = C_{H(PreEq)} \tag{1.33}$$

We have just shown that for addition of strong acid, C_H changes by the same amount as is the amount of acid added and that it stays constant during internal equilibrations of the system. Similar analysis could be done for addition of strong base (OH^-) or a buffer (conjugate weak acid and weak base together, in Brønsted and Lowry sense). When adding a buffer, we would have to account the HB form of the buffer as an added acid as well, but otherwise, we would arrive at same conclusion: C_H only changes when acid or base is added from outside[3] and it stays constant during internal rearrangements of the system taking place before new equilibrium is established: Another words, C_H is a conservative quantity that changes by an interval of added acid or base and behaves as an *invariant* during internal equilibrations.

Instead of total titratable acid, C_H, we can use the complementary value, total titratable base C_B

$$C_B \ \overset{def}{=} \ [OH^-] - [H^+] + [B_1^-] + [B_2^-] + \cdots + [B_n^-] \tag{1.34}$$

This is a mirror equation to (1.29). C_B is also a conservative quantity, similar to C_H. A question might be asked, as to which bases should be included into (1.34). Should the base forms of strong acids (=very weak bases) also be included or should only the base forms of buffers (i.e. weak acids) be part of the formula? Analogically, should the very weak acids (i.e. acid forms of strong bases) be included into the equation (1.29)? The answer is straightforward: Species that do interact with hydrogen ion in the pH of interest have to be included, because otherwise the interaction is missed. These are essentially buffers. Species that do not interact with hydrogen ion either releasing it (Cl^- ion) or binding it permanently (arginine side chain, pK_A =12.0) can be omitted or included in the sum. If included, they are only going to move the resulting value of C_H (acid forms of strong bases) or C_B (base forms of strong acids) by a constant equal to their concentration. However, omission or inclusion has to be consistent throughout the calculation. I think it is more reasonable to omit the species that can really be considered strong in order to simplify the calculation. However, given the uncertain nature of the division between "strong" species and buffer species discussed in previous section, it is good to know that the decision of including strong species into calculations is sometimes arbitrary.

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[3] Actually, there is one more way to change C_H: Changing the amount of solvent substance, i.e. water. This causes a so called dilution or concentration disturbance, covered in chapter 6.

When C_H increases due to the addition of strong acid, C_B decreases by the same amount, mirroring its change (i.e. exactly when the base form of strong acid is not counted in the calculation of C_B, as just discussed). Similarly, when C_B increases due to addition of strong base (hydroxide), C_H decreases by the same amount. When a weak acid or base (in Arrhenius sense) is added to the system, one should first determine the concentration of its base form and the acid form using its measured pH and equations (1.11) and (1.12). [HB] is then added to the C_H and [B⁻] is added to the C_B together with the free [H⁺] and [OH⁻].

One more property of C_B and C_H can be derived if we sum the two definition equations (1.29) and (1.34) and the conservation of mass equations (1.28)

$$C_H + C_B = \sum_{i=1}^{n} C_i \tag{1.35}$$

Recently, an elegant formalism was developed by Guenter (23). Let $\bar{n}_i$ be the average number of bound hydrogen ions per molecule of substance i. Since we only work with monovalent substances, $\bar{n}_i$ is going to be equal to the ratio of the substance that is in the acid form, designated α_1. If, for instance, 40% of the substance i is in the acid form HB (also sometimes called protonated form, because H⁺ ion consists of single proton nucleus), the corresponding α_1 would be 0.4. Let $\bar{e}_i$ be the average number of free hydrogen ion binding sites per molecule of substance i. In a monovalent case, $\bar{e}_i$ is equal to the ratio of the substance that is in the base form, designated α_0. For our substance i, 60% is in the unprotonated form B⁻, the corresponding α_0 would be 0.6. Written in formula

Monovalent

$$\bar{n}_i = \alpha_1 = \frac{[\text{HB}_i]}{C_i} \tag{1.36}$$

substance:

$$\bar{e}_i = \alpha_0 = \frac{[\text{B}_i^-]}{C_i} \tag{1.37}$$

$$\bar{n}_i + \bar{e}_i = 1 \tag{1.38}$$

The variables $\bar{n}_i$ and $\bar{e}_i$ are pH dependent, but the equation (1.38), which is a consequence of (1.12) and the previous equations, is true for any pH. General definitions of $\bar{n}_i$ and $\bar{e}_i$ are given in the next section, equations (1.54) and (1.55).

Using these definitions, equations (1.29) and (1.34) can be written as

$$C_H = [\text{H}^+] - [\text{OH}^-] + C_1\bar{n}_1 + C_2\bar{n}_2 + \cdots + C_n n_n \tag{1.39}$$

$$C_B = [\text{OH}^-] - [\text{H}^+] + C_1 \bar{e}_1 + C_2 \bar{e}_2 + \cdots + C_n e_n \tag{1.40}$$

This can also be put down more concisely using standard mathematical notation for sums

$$C_H = [\text{H}^+] - [\text{OH}^-] + \sum_{i=1}^{n} C_i \bar{n}_i \tag{1.41}$$

$$C_B = [\text{OH}^-] - [\text{H}^+] + \sum_{i=1}^{n} C_i \bar{e}_i \tag{1.42}$$

For any substance of known K_A, pH (or $[\text{H}^+]$) dependence of $\bar{n}$ and $\bar{e}$ can be written down, using their definition (1.36), (1.37) and the mass action equation (1.10)

$$\bar{n} = \alpha_1 = \frac{[\text{HB}]}{[\text{HB}] + [\text{B}^-]} = \frac{[\text{HB}]}{[\text{HB}] + \dfrac{[\text{HB}]K_A}{[\text{H}^+]}} = \frac{[\text{H}^+]}{[\text{H}^+] + K_A} \tag{1.43}$$

$$\bar{e} = \alpha_0 = \frac{[\text{B}^-]}{[\text{HB}] + [\text{B}^-]} = \frac{[\text{B}^-]}{\dfrac{[\text{H}^+][\text{B}^-]}{K_A} + [\text{B}^-]} = \frac{K_A}{[\text{H}^+] + K_A} \tag{1.44}$$
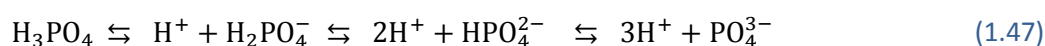
Using the equation (1.36), (1.37) and (1.6), these formulas can serve as a basis for calculating the species concentrations $[\text{B}_i^-]$ and $[\text{HB}_i]$, supposing that C_i is known.

$$[\text{HB}_i] = C_i * \frac{[\text{H}^+]}{[\text{H}^+] + K_A} = C_i * \frac{10^{-pH}}{10^{-pH} + 10^{-pK_A}} \tag{1.45}$$

$$[\text{B}_i^-] = C_i * \frac{K_A}{[\text{H}^+] + K_A} = C_i * \frac{10^{-pK_A}}{10^{-pH} + 10^{-pK_A}} \tag{1.46}$$

Substances with more than one dissociation step

The situation becomes somewhat more complicated for substances with more dissociation steps. An example of such substance is phosphoric acid with three dissociation steps

$$\text{H}_3\text{PO}_4 \leftrightarrows \text{H}^+ + \text{H}_2\text{PO}_4^- \leftrightarrows 2\text{H}^+ + \text{HPO}_4^{2-} \leftrightarrows 3\text{H}^+ + \text{PO}_4^{3-} \tag{1.47}$$

Let us first define its total concentration as the sum of all its forms

$$C_P = [\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}] \tag{1.48}$$

Fraction of phosphate/phosphoric acid system with j hydrogen ions bound can be defined as

$$\alpha_{j(P)} = \frac{[H_jPO_4^{(3-j)-}]}{C_P} \tag{1.49}$$

i.e.

$$\alpha_{0(P)} = \frac{[PO_4^{3-}]}{C_P}; \quad \alpha_{1(P)} = \frac{[HPO_4^{2-}]}{C_P}; \quad \alpha_{2(P)} = \frac{[H_2PO_4^-]}{C_P}; \quad \alpha_{3(P)} = \frac{[H_3PO_4]}{C_P} \tag{1.50}$$

If the phosphate/phosphoric acid were present, it has to be in one of its four species, it is thus elementary to see that the sum of all fractions equals 1. This is also consequence of the definition equations (1.48) and (1.50):

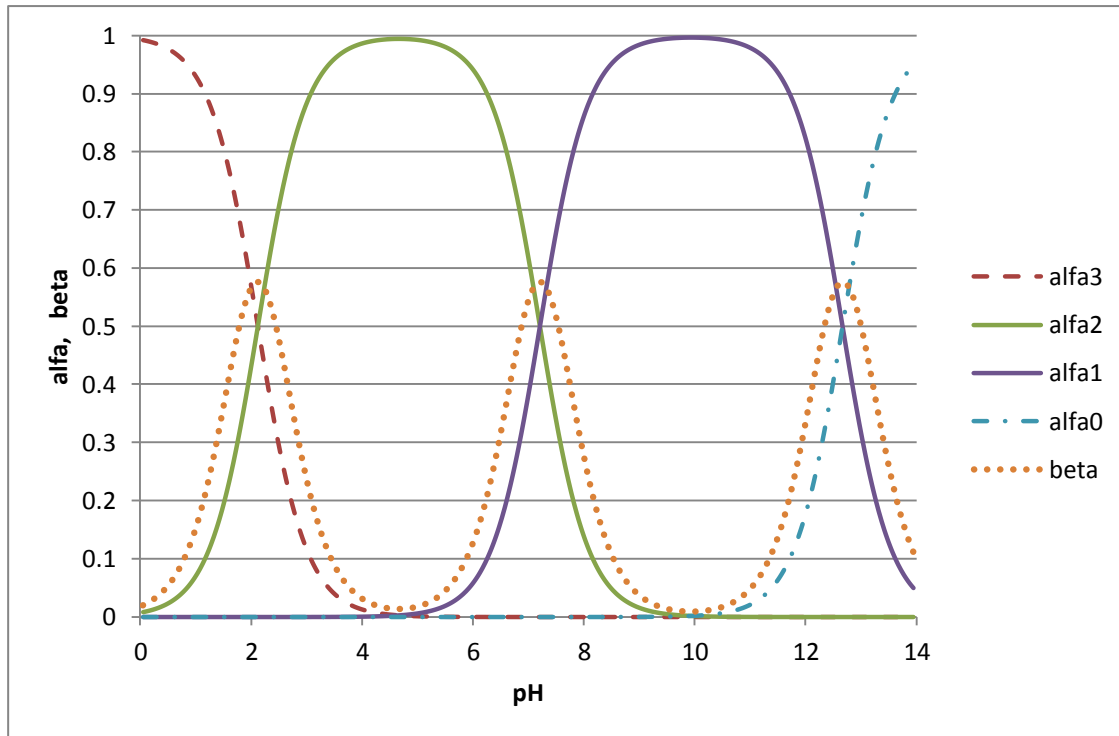$$\sum_{j(P)=0}^{3} \alpha_{j(P)} = 1 \tag{1.51}$$

Figure 5: *Relative concentration of the four species of the phosphoric acid/ phosphate system as a function of pH. As the pH goes down, concentration of species with more hydrogen ions bound increases. Alfa3 represents relative concentration of H_3PO_4 (red dashed line), alfa2 relative concentration of $H_2PO_4^-$ (green line), alfa1 relative concentration of HPO_4^{2-} (dark blue line) and alfa0 relative concentration of PO_4^{3-} (light blue dot-and-dashed line). The sum of relative concentrations of all forms is equal 1 at any pH. Note that the pH bands at which transition from one form to the next*

one occurs are quite distinctive. This results in three peaks of phosphate buffer capacity (beta, orange dotted line). β_i drops almost all the way to zero in between these peaks.

When calculating $\bar{n}$ - the average number of bound hydrogen ions per molecule, we have to multiply each fraction by the number of hydrogen ions bound to that fraction

$$\bar{n}_P = 3\alpha_{3(P)} + 2\alpha_{2(P)} + 1\alpha_{1(P)} + 0\alpha_{0(P)} \tag{1.52}$$

When calculating $\bar{e}$ - the average number of free hydrogen ion binding sites per molecule - one multiplies each fraction by the number of free hydrogen ion binding sites present at that fraction

$$\bar{e}_P = 0\alpha_{3(P)} + 1\alpha_{2(P)} + 2\alpha_{1(P)} + 3\alpha_{0(P)} \tag{1.53}$$

Written in a more formal notation

$$\bar{n}_P = \sum_{j(P)=0}^{3} j(P) * \alpha_{j(P)} \tag{1.54}$$

$$\bar{e}_P = \sum_{j(P)=0}^{3} (3 - j(P)) * \alpha_{j(P)} \tag{1.55}$$

Equation (1.55) can be also written as

$$\bar{e}_P = \sum_{j(P)=0}^{3} (3 - j(P)) * \alpha_{j(P)} = 3\sum_{j(P)=0}^{3} \alpha_{j(P)} - \sum_{j(P)=0}^{3} j(P) * \alpha_{j(P)} = 3 - \sum_{j(P)=0}^{3} j(P) * \alpha_{j(P)} \tag{1.56}$$

We have used the equation (1.51) in the derivation of (1.56).

Note that combining (1.54) and (1.56) also proves that

$$\bar{n}_P + \bar{e}_P = 3 \tag{1.57}$$

, which is what was expected for phosphate/phosphoric acid system.

Thus, when definitions of $\bar{n}$ and $\bar{e}$ are broadened as shown on the phosphate example, equations (1.39) and (1.40) also hold for systems of substances with more than one dissociation step. For simple phosphate/phosphoric acid system, one can compute its total titratable acid C_H or the total titratable base C_B as

$$C_H = [\text{H}^+] - [\text{OH}^-] + C_P\bar{n}_P \tag{1.58}$$

$$C_B = [\text{OH}^-] - [\text{H}^+] + C_P\bar{e}_P \tag{1.59}$$

Given the relationships between $\bar{n}_P$ ($\bar{e}_P$) and $\alpha_{j(P)}$ (1.52),(1.53), and the definitions of $\alpha_{j(P)}$ (1.50), these concise relationships mean same as

$$C_H = [\text{H}^+] - [\text{OH}^-] + 3[\text{H}_3\text{PO}_4] + 2[\text{H}_2\text{PO}_4^-] + 1[\text{HPO}_4^{2-}] \tag{1.60}$$

$$C_B = [\text{OH}^-] - [\text{H}^+] + 1[\text{H}_2\text{PO}_4^-] + 2[\text{HPO}_4^{2-}] + 3[\text{PO}_4^{3-}] \tag{1.61}$$

Dissociation constants of phosphate/phosphoric acid vary substantially with temperature and ionic strength and should be carefully checked when important (24), (25), (26). Here, only the general values were taken (pK$_{1(P)}$ = 2.15, pK$_{2(P)}$ = 7.20 and pK$_{3(P)}$ = 12.32) (27), as the matter is only of theoretical interest. So to continue, the only question remaining is how to calculate the fractions $\alpha_{j(P)}$ given the three equilibrium constants of the mass action equations

$$K_{1(P)} = \frac{[\text{H}^+][\text{H}_2\text{PO}_4^-]}{[\text{H}_3\text{PO}_4]} \quad ; \quad K_{2(P)} = \frac{[\text{H}^+][\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \quad ; \quad K_{3(P)} = \frac{[\text{H}^+][\text{PO}_4^{3-}]}{[\text{HPO}_4^{2-}]} \tag{1.62}$$

For the shortness of notation in the following derivation, PO$_4{}^{3-}$ group will be written as P and its charge omitted, as well as subscript (P) in dissociation constants

$$K_1 = \frac{[\text{H}^+][\text{H}_2\text{P}]}{[\text{H}_3\text{P}]} \quad ; \quad K_2 = \frac{[\text{H}^+][\text{HP}]}{[\text{H}_2\text{P}]} \quad ; \quad K_3 = \frac{[\text{H}^+][\text{P}]}{[\text{HP}]} \tag{1.63}$$

Using these mass action equations and the definition equations for $\alpha_{j(P)}$ (1.50), we can calculate individual $\alpha_{j(P)}$

$$\alpha_{0(P)} = \frac{[\text{P}]}{[\text{P}] + [\text{HP}] + [\text{H}_2\text{P}] + [\text{H}_3\text{P}]} = \frac{[\text{P}]}{[\text{P}] + \dfrac{[\text{H}^+][\text{P}]}{K_3} + \dfrac{[\text{H}^+]^2[\text{P}]}{K_2 K_3} + \dfrac{[\text{H}^+]^3[\text{P}]}{K_1 K_2 K_3}} =$$

$$= \frac{K_1 K_2 K_3}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.64}$$

In the last step, the [P] is first reduced from both numerator and denominator and then they are expanded by $K_1 K_2 K_3$. Similarly for the dissociation fraction $\alpha_{1(P)}$

$$\alpha_{1(P)} = \frac{[\text{HP}]}{[\text{P}] + [\text{HP}] + [\text{H}_2\text{P}] + [\text{H}_3\text{P}]} = \frac{\dfrac{[\text{H}^+][\text{P}]}{K_3}}{[\text{P}] + \dfrac{[\text{H}^+][\text{P}]}{K_3} + \dfrac{[\text{H}^+]^2[\text{P}]}{K_2 K_3} + \dfrac{[\text{H}^+]^3[\text{P}]}{K_1 K_2 K_3}} =$$

$$= \frac{[\text{H}^+]K_1 K_2}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.65}$$

And using the same procedure for the remaining fractions

$$\alpha_{2(P)} = \frac{[\text{H}^+]^2 K_1}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.66}$$

$$\alpha_{3(P)} = \frac{[\text{H}^+]^3}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.67}$$

These formulas can be written more concisely using mathematical notation for sums and products. In order to do that, we need to define K_0 as 1 (a neutral element in the product). For instance, the formulas (1.64) for $\alpha_{0(P)}$ and (1.67) for $\alpha_{3(P)}$ can be written as

$$\alpha_{0(P)} = \frac{\prod_{l=1}^{3} K_l}{\sum_{k=0}^{3}[\text{H}^+]^k \prod_{m=0}^{3-k} K_m} \tag{1.68}$$

$$\alpha_{3(P)} = \frac{[\text{H}^+]^3}{\sum_{k=0}^{3}[\text{H}^+]^k \prod_{m=0}^{3-k} K_m} \tag{1.69}$$

This expression can further be generalized for arbitrary $\alpha_{j(P)}$ as

$$\alpha_{j(P)} = \frac{[\text{H}^+]^j \prod_{l=0}^{3-j} K_l}{\sum_{k=0}^{3}[\text{H}^+]^k \prod_{m=0}^{3-k} K_m} \tag{1.70}$$

Given the expressions for calculating $\bar{n}_P$ () and $\bar{e}_P$ () from $\alpha_{j(P)}$, we can now calculate $\bar{n}_P$ and $\bar{e}_P$, of course given that we know $[\text{H}^+]$ (pH)

$$\bar{n}_P = \frac{\sum_{j=0}^{3} j[\text{H}^+]^j \prod_{l=0}^{3-j} K_l}{\sum_{k=0}^{3}[\text{H}^+]^k \prod_{m=0}^{3-k} K_m} \tag{1.71}$$

$$\bar{e}_P = \frac{\sum_{j=0}^{3} (3-j)[\text{H}^+]^j \prod_{l=0}^{3-j} K_l}{\sum_{k=0}^{3}[\text{H}^+]^k \prod_{m=0}^{3-k} K_m} \tag{1.72}$$

These formulas, although concise, might be more legible written without sums and products

$$\bar{n}_P = \frac{1[\text{H}^+]K_1 K_2 + 2[\text{H}^+]^2 K_1 + 3[\text{H}^+]^3}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.73}$$

$$\bar{e}_P = \frac{3K_1 K_2 K_3 + 2[\text{H}^+]K_1 K_2 + 1[\text{H}^+]^2 K_1}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.74}$$

Thus, the relationship between total titratable acid and concentration of free hydrogen ion for the phosphate/phosphoric acid system is

$$C_H = [\text{H}^+] - [\text{OH}^-] + C_P * \frac{1[\text{H}^+]K_1 K_2 + 2[\text{H}^+]^2 K_1 + 3[\text{H}^+]^3}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.75}$$

And the complementary relationship between total titratable base and concentration of free hydrogen ion is

$$C_B = [\text{OH}^-] - [\text{H}^+] + C_P * \frac{3K_1 K_2 K_3 + 2[\text{H}^+]K_1 K_2 + 1[\text{H}^+]^2 K_1}{K_1 K_2 K_3 + [\text{H}^+]K_1 K_2 + [\text{H}^+]^2 K_1 + [\text{H}^+]^3} \tag{1.76}$$

Last two equations can be used, together with the equation (1.6), to plot the titration curve of phosphate/ phosphoric acid system seen at figure 6.
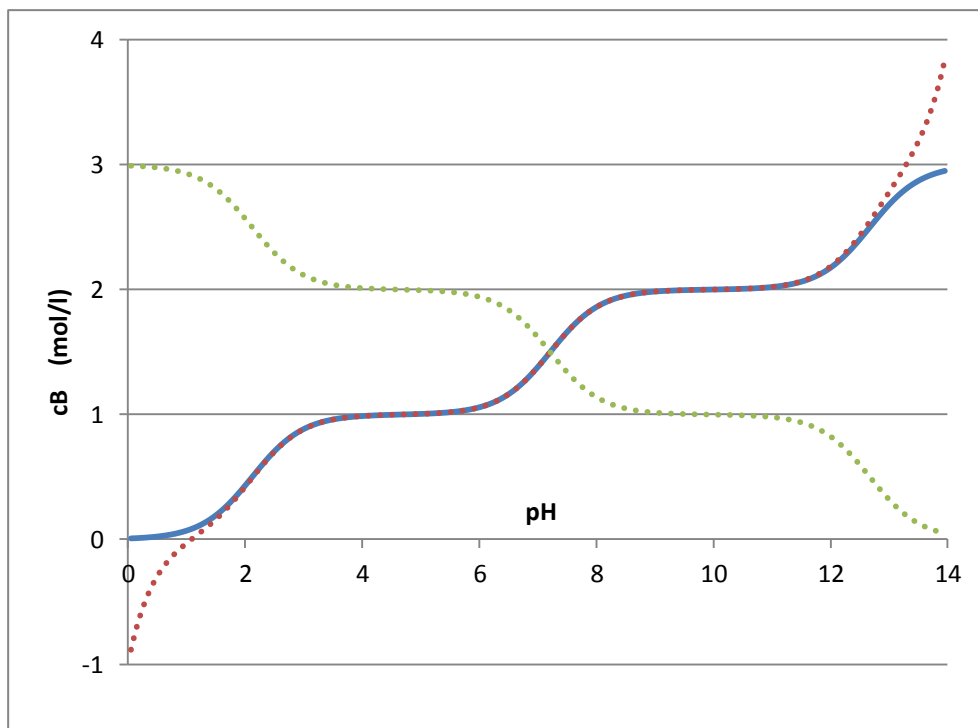
Figure 6: *Titration curve of phosphate / phosphoric acid system. Total concentration of the phosphoric acid is 1 mol/l, which is equivalent to 98 g of H_3PO_4 per liter of solution. This is much more concentrated then the histidine buffer of figure 3; therefore the overall titration curve (red dotted line) starts to differ from the internal buffer curve of phosphoric acid (blue line) only at more extreme values of pH. Because of the unit concentration, blue line also represents $\bar{e}_P$ (eq. 1.74), while the green dotted line represents its complementary variable $\bar{n}_P$ (eq. 1.73). $\bar{n}_P$ is the average number of bound H^+ ions per molecule, being almost at 3 at very low pH and gradually decreasing to zero. $\bar{e}_P$ is the average number of free hydrogen binding sites per molecule, being almost at 0 at very low pH level and gradually increasing to 3. The overall titration curve was calculated using equation (1.76).*

It has hopefully become clear that calculating titration curves of a multivalent substance is more complex than the same task performed for the monovalent case. Given all these complexities, it can be nice to recognize that a simpler approach can also be used for phosphate calculations based on the pH - pK_A criterion. Looking at the K_1 = 2.15, K_2 = 7.20 and K_3 = 12.32, one can see that the difference between pH and pK_1 is larger than 3 at pH > 5.15. The difference between pH and K_{A3} is larger than 3 at pH < 9.38. Thus, if the pH is between 6 and 9, one can consider only the second dissociation and the introduced error will be very small
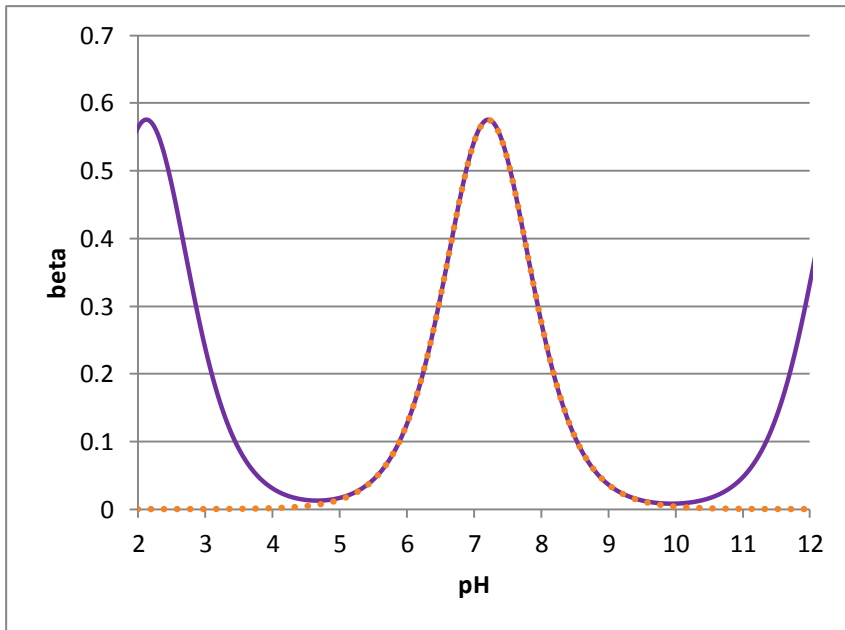
$$H_2PO_4^- \; \leftrightarrows \; H^+ + HPO_4^{2-} \tag{1.77}$$

Figure 7: *Buffer capacity of full phosphate / phosphoric acid system (violet line) compared to the buffer capacity of the system simplified to the middle dissociation step (orange dotted line). In the pH range from 6-9, simplified model provides excellent fit, being practically indistinguishable from the complete model.*
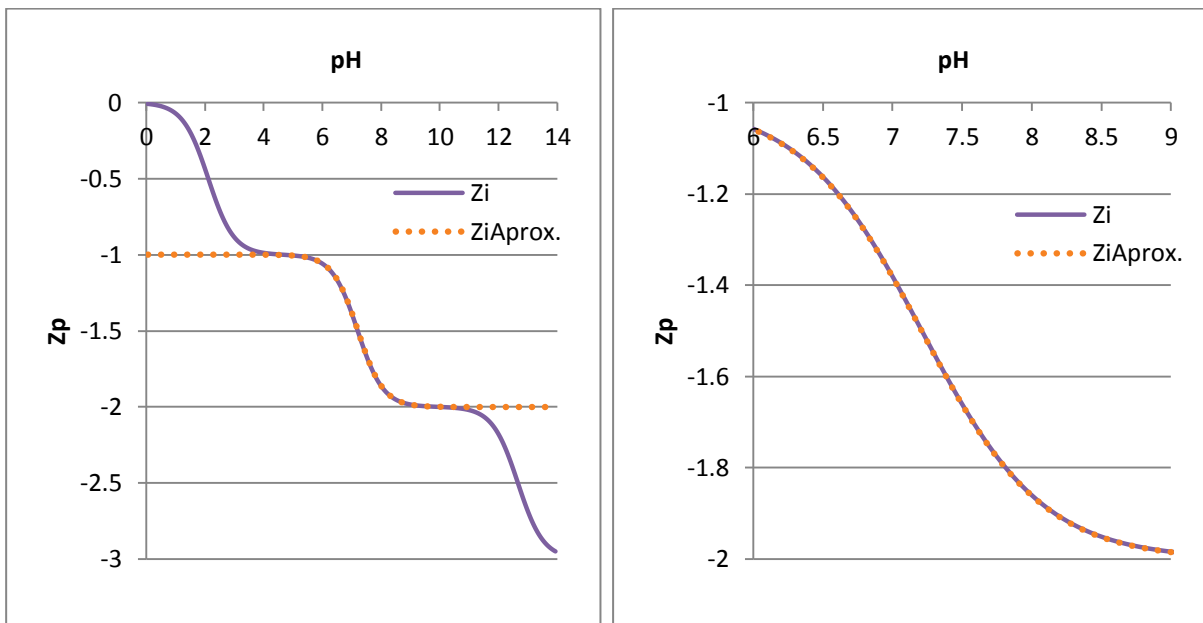

Figure 8: *Average charge $\overline{Z}_P$ of the phosphate / phosphoric acid system (in unity concentration) plotted as a function of pH. The relationship between average charge of a substance and other measures of its buffering properties is described in chapter 2, section concerned with the relationship between buffering and charge. Orange dotted line shows the charge of the system simplified to a monovalent case. The right detail shows excellent fit of this simplification in the pH range 6.0 -9.0*

If we do this, equations (1.73) to (1.76) simplify back to (1.43) to (1.46). Using this simplified approach provides excellent estimation of phosphate buffering properties at physiological range of pH (figure 7). In this simplified model, $H_2PO_4^-$ does not become further hydrogen ion acceptor and can be considered a strong ion. If we want to have the charge of the system correct as well, we have to add this maximum charge (being -1) to the charge calculated according to the buffer properties of the second dissociation (see equation 2.29). Simplified model provides excellent fit, as can be seen in figure 7 and 8.

General formulation for a mixture of monovalent and polyvalent substances

Equation (1.70) derived on the phosphate example can be generalized for any substance i with $\bar{n}_{max}$ dissociation steps

$$\alpha_{j(i)} = \frac{[H^+]^j \prod_{l=0}^{\bar{n}_{max}-j} K_l}{\sum_{k=0}^{\bar{n}_{max}} [H^+]^k \prod_{m=0}^{\bar{n}_{max}-k} K_m} \tag{1.78}$$

The corresponding equations for $\bar{n}_i$ and $\bar{e}_i$ (generalized forms of equations (1.71) and (1.72)) are

$$\bar{n}_i = \frac{\sum_{j=0}^{\bar{n}_{max}} j[H^+]^j \prod_{l=0}^{\bar{n}_{max}-j} K_l}{\sum_{k=0}^{\bar{n}_{max}} [H^+]^k \prod_{m=0}^{\bar{n}_{max}-k} K_m} \tag{1.79}$$

$$\bar{e}_i = \frac{\sum_{j=0}^{\bar{n}_{max}} (\bar{n}_{max} - j)[H^+]^j \prod_{l=0}^{\bar{n}_{max}-j} K_l}{\sum_{k=0}^{\bar{n}_{max}} [H^+]^k \prod_{m=0}^{\bar{n}_{max}-k} K_m} \tag{1.80}$$

This might be a good place to recall the equations of total titratable acid (1.41) and total titratable base (1.42)

$$C_H = [H^+] - [OH^-] + \sum_{i=1}^{n} C_i \bar{n}_i \tag{1.81}$$

$$C_B = [OH^-] - [H^+] + \sum_{i=1}^{n} C_i \bar{e}_i \tag{1.82}$$

By substituting in the derived expressions for $\bar{n}_i$ and $\bar{e}_i$, we get the **master equations** that bind total titratable acid () and buffer base () to the free hydrogen ion concentration in a complex system of n titratable substances, where i-th species has $\bar{n}_{max\,(i)}$ dissociation steps

$$C_H = [H^+] - \frac{K_W}{[H^+]} + \sum_{i=1}^{n} C_i \sum_{j(i)=0}^{\bar{n}_{\max(i)}} j(i) \frac{[H^+]^{j(i)} \prod_{l(i)=0}^{\bar{n}_{\max(i)}-j(i)} K_{l(i)}}{\sum_{k=0}^{\bar{n}_{\max(i)}} [H^+]^k \prod_{m(i)=0}^{\bar{n}_{\max(i)}-k} K_{m(i)}} \tag{1.83}$$

$$C_B = \frac{K_W}{[H^+]} - [H^+] + \sum_{i=1}^{n} C_i \sum_{j(i)=0}^{\bar{n}_{\max(i)}} \left(\bar{n}_{\max(i)} - j(i)\right) \frac{[H^+]^{j(i)} \prod_{l(i)=0}^{\bar{n}_{\max(i)}-j(i)} K_{l(i)}}{\sum_{k=0}^{\bar{n}_{\max(i)}} [H^+]^k \prod_{m(i)=0}^{\bar{n}_{\max(i)}-k} K_{m(i)}} \tag{1.84}$$

Fortunately, these complicated equations simplify again and thus would be rarely used in modelling acid-base chemistry of biological systems. Hence, the main reason for including them lies in impressing revered opponents. The logic that allows their simplification is following: The most important polyvalent substances functioning as physiological buffers are proteins. In the case of blood, these include for instance hemoglobin and albumin. The buffering groups in these molecules include $-NH_3^+$ and COO^- termini, as well as numerous side chains of various amino acids. In physiology of buffering, the most important side chains are those of amino acid histidine because their pK_A is generally scattered in the range of pH of physiological interest (around 6.0). However, because each of these buffer groups is distinguishable and generally functions independently[4], they could and should be mathematically treated as separate monovalent acids that are only tied together by fixed concentration of the whole protein. This simplifies the calculations significantly: In case of a mixture of monovalent acids, formulas (1.83) and (1.84) simplify back to monovalent case (1.41) to (1.46). The master equations can then be written in their simpler form as

$$C_H = [H^+] - [OH^-] + \sum_{i=1}^{n} C_i \frac{[H^+]}{[H^+] + K_i} \tag{1.85}$$

$$C_B = [OH^-] - [H^+] + \sum_{i=1}^{n} C_i \frac{K_i}{[H^+] + K_i} \tag{1.86}$$

In the case of protein buffers, it also makes sense to group the buffering side chains according to the protein they are on to account for the same concentration of each buffer residue of the given protein

$$C_H = [H^+] - [OH^-] + C_A \sum_{prot\ A} \frac{[H^+]}{K_{j(A)} + [H^+]} + \cdots + C_Z \sum_{prot\ Z} \frac{[H^+]}{K_{j(Z)} + [H^+]} \tag{1.87}$$

$$C_B = [OH^-] - [H^+] + C_A \sum_{prot\ A} \frac{K_{A,j}}{K_{j(A)} + [H^+]} + C_B \sum_{prot\ B} \frac{K_{B,j}}{K_{j(B)} + [H^+]} + \cdots \tag{1.88}$$

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[4] An exception to this rule is represented by structural transitions of tertiary protein structure due to changes of pH. These transitions can cause a change of pK_A of the amino-acid residues whose chemical neighborhood undergoes conformational change.

$$\dots + C_Z \sum_{prot\ Z} \frac{K_{Z,j}}{K_{j(Z)} + [\text{H}^+]}$$

Each of these sums is, according to their definition, the $\bar{n}_i$ and $\bar{e}_i$ of the given protein i. Thus, in case of protein buffers, equations (1.79) and (1.80) that give the *pH* dependence of the $\bar{n}_i$ and $\bar{e}_i$ simplify into the following expressions

$$\bar{n}_i = \sum_{prot\ i} \frac{[\text{H}^+]}{K_{j(i)} + [\text{H}^+]} \tag{1.89}$$

$$\bar{e}_i = \sum_{prot\ i} \frac{K_{j(i)}}{K_{j(i)} + [\text{H}^+]} \tag{1.90}$$

Chapter 2

Acid-base chemistry in the context of human physiology

Chapter one covered the general description of acid-base chemistry, applicable to any field. Topics covered include the dissociation of water and definition of pH; description of acids, bases and buffers and general description of mixture solutions containing more than one substance with possibly more than 1 dissociation step. Finally, two master equations that summarize the acid-base behavior of complex mixture solutions in a nicely concise form are given, one for total titratable acid (C_H) and the other for total titratable base (C_B).

Chapter two is concerned with the notions of acid-base chemistry pertinent to human (and animal) physiology. These include special role of carbon dioxide – bicarbonate buffer, concept of linearization in describing buffering properties of proteins, lumping of all the buffers other than bicarbonate under the term non-bicarbonate buffer, relationship between charge of complex buffers and their buffering properties, modelling of albumin properties as an example protein substance illustrating some of the typical pitfalls of using concepts like strong ion difference and buffer base; and finally, the electroneutrality equation in blood plasma.

Carbon dioxide – bicarbonate buffer as a volatile acid buffer system

The bicarbonate buffer is one of the most important physiological buffers. When it is considered open at the CO_2 end, it is the strongest buffer of the extracellular fluid. Its buffering reaction is

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \tag{2.1}$$
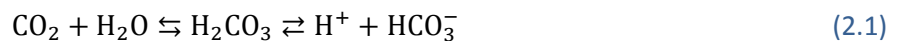
Acidic form of this buffer is volatile; the acid spontaneously dehydrates, forming CO_2 and water. Carbon dioxide dissolves well in water and aqueous solutions; the amount of dissolved CO_2 is directly proportionate to its partial pressure – pCO_2. This fact can be used in the formulation of the buffer equilibrium (mass action) equation, also known as the Henderson-Hasselbalch equation.

$$pH = pK_c + \log_{10} \frac{[HCO_3^-]}{S * pCO_2} \tag{2.2}$$

pKc is a dissociation constant of the bicarbonate buffer and S is solubility of carbon dioxide. For human plasma, the value of pK_c equals 6.10 and S at 37°C is 0.0306 mmol/l/mmHg (28), (29; 30) .

Although Henderson-Hasselbalch equation is a general relationship that can be, with certain limitations, used for any buffer (31), (32), (33), (34), bicarbonate buffer plays a prominent role in its formulation. Carbonic acid was described as a buffer with its help by Lawrence J. Henderson (31) and Karl A. Hasselbalch used the formula (in its logarithmic form) to further explore the role of the buffer in blood physiology (32).

The role of bicarbonate buffer in maintaining the acid-base equilibrium of human body is summarized in figure 9. It shows the coupling of bicarbonate with other buffers through the hydrogen ion concentration, as well as its connection to the other electrolytes by the electroneutrality equation. Regulation of the bicarbonate buffer components is the primary means of maintaining constant plasmatic pH. CO_2 side has a much higher turnover, some 20,000 mmol are produced every day (35). The rate of its production in metabolism is fairly constant, but it can increase up to several times for instance in physical exercise. Elimination of CO_2 is regulated to maintain its partial pressure in (arterial) blood around 40 mmHg. The regulatory circuit includes peripheral and central chemoreceptors, respiratory center in medulla, chest wall with the respiratory muscles and lungs.

Base form of the buffer is bicarbonate, its normal value being around 24 mmol/l. Turnover on this side is much slower than on the acidic side. Typically, bicarbonate is lost, when it buffers acid produced in the metabolism. Typically, some 100 mmol of these acids are released to the blood-stream each day. The acid that is produced this way causes shift in pH to the acidic side, causing formation of CO_2 from the bicarbonate (larger arrow). Such an acid can be organic, as is the case of lactate produced during anaerobic exercise. Once the lactate is metabolized, the hydrogen ion it once dissociated is consumed with its metabolism, shifting pH back to the alkalotic side and favoring reverse formation of bicarbonate from CO_2. This is summarized in figure 10. When the acid that has dissociated the hydrogen ion is inorganic, it cannot be metabolized back. It has to be excreted by the kidneys together with its hydrogen ion, generally bound to kidney buffers NH_4 and HPO_4^{2-} (figure 9). Bicarbonate that was used during buffering of the inorganic acid is regenerated by the kidneys during the process. This is also done in a regulated fashion as to keep the pH constant. Thus, kidney is the second, much slower, but equally important regulator of the bicarbonate buffer.

Since the acidic side of the bicarbonate buffer is in such a high turnover and regulated fast by the pulmonary regulator, the bicarbonate buffer can be sometimes regarded as an open system with constant value of pCO_2 (13). This concept can be very helpful simplification when describing the physiological behaviour of the bicarbonate buffer system. Its limits lie in the fact that the buffer is only open when blood flows through the lungs.

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When looking at the Henderson-Hassselbalch equation (2.2), one should not miss one fact. The equation binds together pCO_2, [HCO_3^-] and pH. When any two of these variables are known, the third one can be calculated. For instance, pCO_2 and [HCO_3^-] uniquely determines pH. This can be used in the basic diagnostics of acid-base disorders. Traditionally these have been divided into respiratory, where the change in the pCO_2 is the primary problem, and metabolic disorders, where the change in [HCO_3^-] is the primary cause. When one side of the buffer brings about a pH disturbance, the regulator of the other side tries to compensate. For instance, when an increased production of metabolic acids in diabetes brings about metabolic acidosis (i.e. decrease of HCO_3^-), respiratory regulator reacts to the decrease in pH by increasing ventilation. The increased ventilation brings about a decrease of pCO_2, which has a compensatory tendency to bring pH back to normal. In order to learn more about the causes of each disturbance, more detailed discussion of its pathophysiology and the diagnostics, see any standard textbook, for instance (36).

Metabolic disorders are compensated almost immediately, although the maximum compensation may take about 12 hours to develop. Respiratory disorders are compensated by kidneys, which increase or decrease bicarbonate concentration in response to the change of pH brought about by increase or decrease of pCO_2. Renal compensation takes about 2 and half days to develop. Thus, respiratory disorders have a well demarcated acute phase and a compensated chronic phase.

Diagnostics of acute and chronic disorders can be made either using some of the compensation diagrams or the so called Boston rules. Perhaps the most common compensation diagram was designed by Siggaard-Andersen in 1970's; it is shown on figure 11. Although it operates with the term of base excess as the marker of the metabolic disorders, the base excess it shows is only the average value for normal concentration of hemoglobin and albumin. On the contrary, it can show an exact value of [HCO_3^-], because each point of the diagram is a coordinate of pH and pCO_2 thus uniquely determining [HCO_3^-] as well (equation 2.2). Boston rules, the empirical formulas that exist in various versions (37), (30), (38) are shown in table 5. The Boston rules can be plotted into the compensation diagram of Siggaard Andersen, falling into the respective bands of acute and compensated disorders.

Bicarbonate can also dissociate the second hydrogen ion

$$HCO_3^- \rightleftarrows H^+ + CO_3^{2-} \tag{2.3}$$

The pK_A of this reaction is quite high, equaling to 10.3. Given the physiological concentration of bicarbonate 24 mmol/l and the Henderson-Hasselbalch equation (1.11), the concentration of carbonate would normally be around 0.03 mmol/l, possibly rising up to 0.1 mmol/l in severe cases of metabolic alkalosis. Both normal and the extreme number can be neglected as insignificant in clinical

practice. Another minor form of the carbonate system is CO_2 bound to $-NH_2$ termini of proteins (e.g. hemoglobin) forming so called carbamino compounds. A detailed treatment of the bicarbonate and its possible transitions is given for instance in (28).

Metabolic	Acidosis		$(pCO_2)_{EXPECTED} = 1.5 * [HCO_3^-] + 8$ or $\Delta pCO_2 = 1.2 * \Delta[HCO_3^-]$
	Alkalosis		$(pCO_2)_{EXPECTED} = 0.7 * [HCO_3^-] + 20$ or $\Delta pCO_2 = 0.6 * \Delta[HCO_3^-]$
Respiratory	Acidosis	Acute	$[HCO_3^-]_{EXPECTED} = 24 + 1\left(\dfrac{pCO_2 - 40}{10}\right)$
		Chronic	$[HCO_3^-]_{EXPECTED} = 24 + 3.5\left(\dfrac{pCO_2 - 40}{10}\right)$
	Alkalosis	Acute	$[HCO_3^-]_{EXPECTED} = 24 + 2\left(\dfrac{pCO_2 - 40}{10}\right)$
		Chronic	$[HCO_3^-]_{EXPECTED} = 24 + 5\left(\dfrac{pCO_2 - 40}{10}\right)$

Table 5: So called Boston rules for diagnostics of acute and compensated acid-base disorders. These rules are based primarily on the work of Narins and Emmett (37). There are more versions, the one given here is from (30). Bicarbonate concentration is in mmol/l, pCO_2 in mmHg. The rules use bicarbonate buffer as the only measure of acid-base disturbances. Generally, bicarbonate should fall within ±2 mmol/l of the expected value for the given disorder. The range is wider for pCO_2, especially metabolic alkalosis (±5 mmHg), where respiratory compensation may be problematic due to hypoxia.
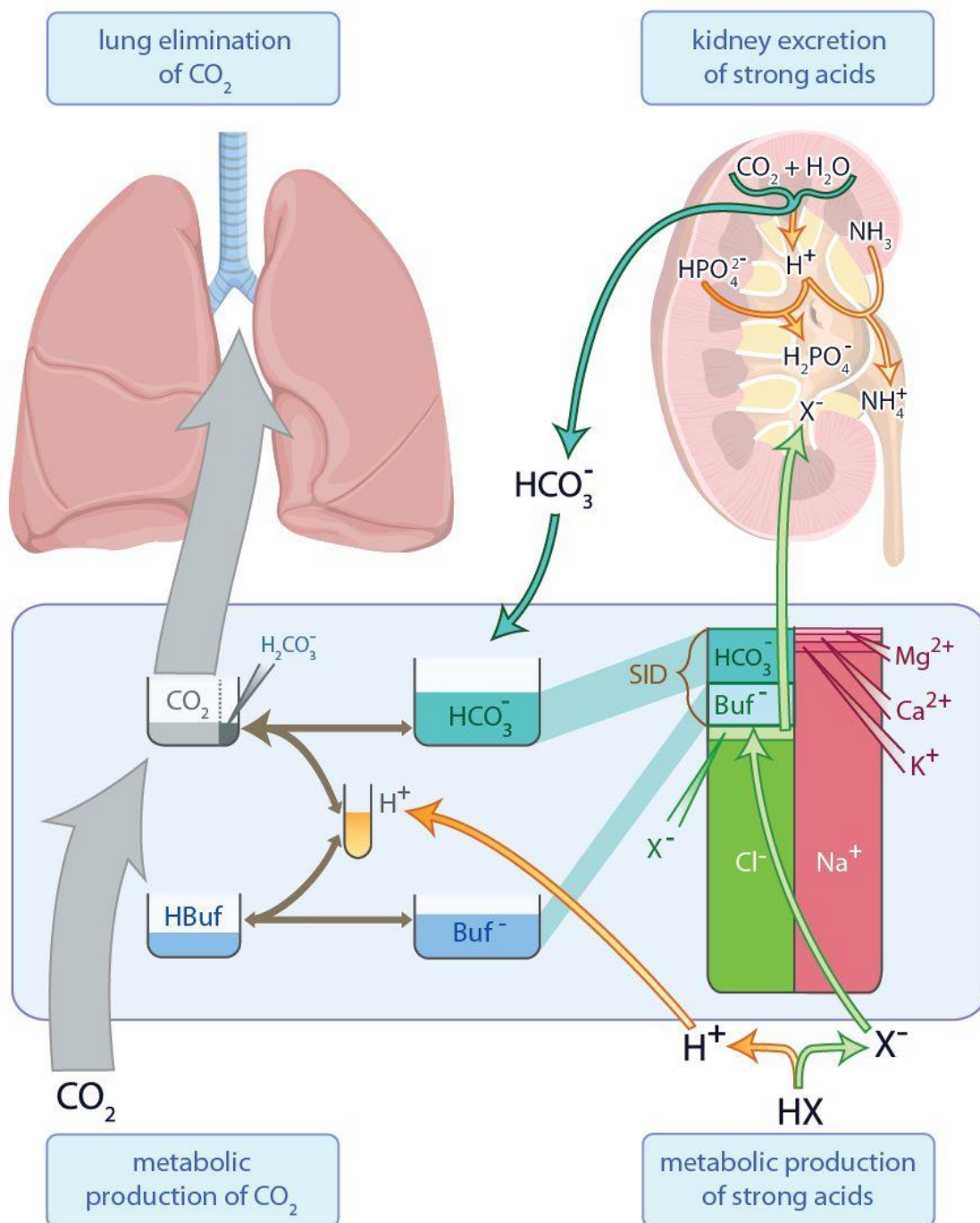
Figure 9: *Central role of bicarbonate buffer in maintaining the acid-base equilibrium of human body. Since all buffer systems are connected through the hydrogen ion concentration, only carbon dioxide – bicarbonate system needs to be regulated in order to regulate pH. CO_2 side is basically a flow-through system, level of pCO_2 depending primarily on the rate of production and the regulated rate of pulmonary elimination. Bicarbonate side changes much slower; it is connected to the electrolyte balance. Primary site of bicarbonate regulation are kidneys.*

1) Lactic acid produced in the metabolism
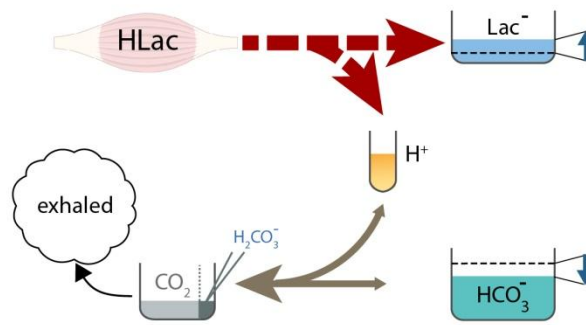
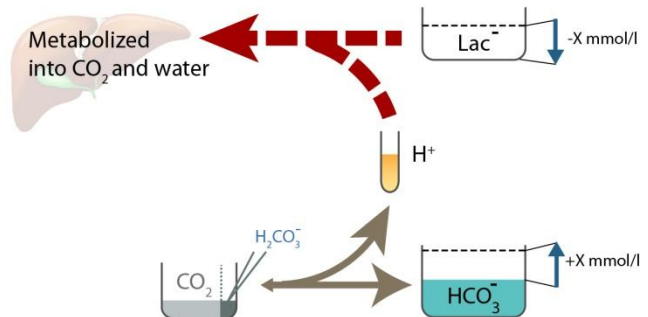2) Metabolism of lactate in liver
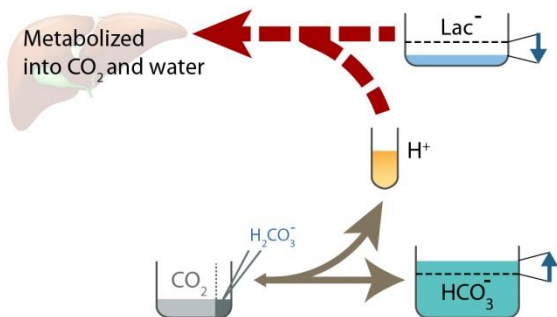
2b) Metabolism of infused lactate

Figure 10: *Role of lactic acid and lactate in acid-base equilibria. Lactic acid produced in metabolism, as in exercise or ischemia, can produce metabolic acidosis as seen on the upper picture (1). Dissociated hydrogen ion reacts with bicarbonate and causes a decrease of its concentration. Similarly, it can react with non-bicarbonate buffers, which is not shown. After exercise, lactate is typically released back to blood and metabolized in liver (lower left, 2). This process replenishes lost bicarbonate. Similar process happens when lactate anion is part of infused solution, for instance Ringer's lactate. Lactate is metabolized and same amount of HCO_3^- is created from constant suply of CO_2 during the process The increase of blood buffer base concentration is equivalent to the infused concentration of lactate, as was recently experimentally confirmed by Carlesso et al. (39). Note that in this process, lactate does not behave as a strong ion not because its pK_A would be too high, but because its role in the metabolism.*
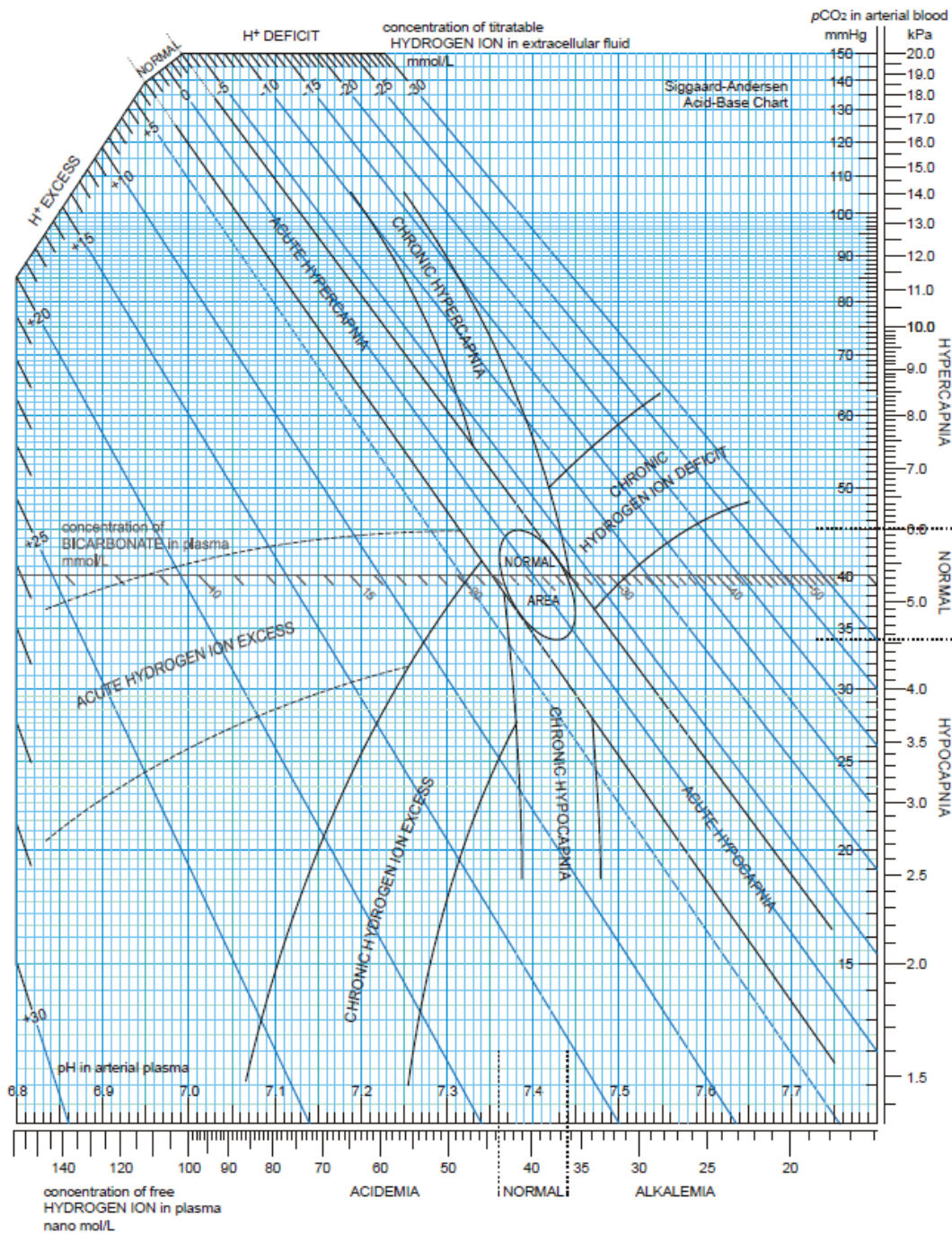
Figure 11: *pH – pCO₂ diagram with the bands of acute and compensated disorders, as designed by Siggaard-Andersen. pCO₂ scale is logarithmic; it was experimentally proven, that when the pH is plotted as a function of varying pCO₂ in this coordinate system, the resulting function is a straight line going from upper left corner to the lower right corner. The bands Acute hypocapnia (=acute respiratory alkalosis) and Acute hypercapnia (=acute respiratory acidosis) are around this line. Note that the kidney compensation of the respiratory disorders keeps the pH closer to 7.4 even in marked hypo- or hypercapnia. Metabolic disorders are mainly chronic, because most of respiratory compensation develops fast (chronic hydrogen ion excess or deficit = metabolic acidosis or alkalosis)*

Concept of linearization in describing titration curve of protein buffers

Besides bicarbonate buffer and phosphates, significant portion of buffering is done by proteins in physiological fluids. In these substances, side chains (residues) of some amino acids can behave as individual buffers of varying pK_A. Although side chain of each dissociable amino acid has its typical average pK_A (as is already mentioned 6.0 for histidine), the actual pK_A can be substantially different due to the amino acid environment of each moiety. Thus, for instance the Figge-Mydosh-Fencl model of albumin works with the following pK_A of 16 histidine residues:

pK_9	pK_{15}	pK_{10}	pK_{13}	pK_{11}	pK_8	pK_{12}	pK_7	pK_5	pK_3	pK_1	pK_2	pK_{14}	pK_{16}	pK_6	pK_4
4.85	5.2	5.75	5.82	6.17	6.35	6.73	6.75	7.01	7.10	7.12	7.22	7.3	7.3	7.31	7.49

Table 6: *Dissociation constants of 16 histidine residues found on albumin, as given by Figge Mydosh and Fencl* (40) *and sorted by increasing value of pK_A .*

These pK_A's range from 4.85 to 7.49. pK_A's of other amino acid residues can be scattered the same way. Thus, in the broader perspective, it could be a reasonable concept to regard the pK_A's of an arbitrary protein buffers as essentially random numbers. It might be interesting to get an better insight in what would the titration curves of such "random" protein look like.

The titration curves that result from this random concept were simulated by solving the master equations (1.85) and (1.86). A random number generator of Microsoft Excell was to choose 10 numbers between 5 and 9. These numbers represent individual pK_A of 10 amino acid residues buffering in the vicinity of pH 7, each being assigned concentration of 1 mmol/l. Titration curve of this hypothetical protein was plotted between pH 6 and 8. The procedure was repeated 3 times to find titration curves of three different hypothetical proteins. For comparison, a titration curve of a single species with pK_A of 7 (concentration of 10 mmol/l) was also plotted. The random sets of pK_A's are given in the table 7 and the results of the simulation can be seen in on figures 12 to 16.

i	pK$_{1(i)}$	pK$_{2(i)}$	pK$_{3(i)}$	pK$_{4(i)}$	pK$_{5(i)}$	pK$_{6(i)}$	pK$_{7(i)}$	pK$_{8(i)}$	pK$_{9(i)}$	pK$_{10(i)}$
1	5.05	5.26	5.67	5.97	6.11	6.74	7.19	7.73	8.00	8.02
2	5.14	5.17	5.3	5.65	6.91	7.78	8.13	8.33	8.45	8.68
3	5.05	5.52	6.16	7.03	7.44	7.78	7.79	7.8	7.96	8.27

Table 7: *3 sets of 10 dissociation constants, randomly generated in the range 5 to 9. The numbers were sorted according to the increasing value of pK$_A$.*
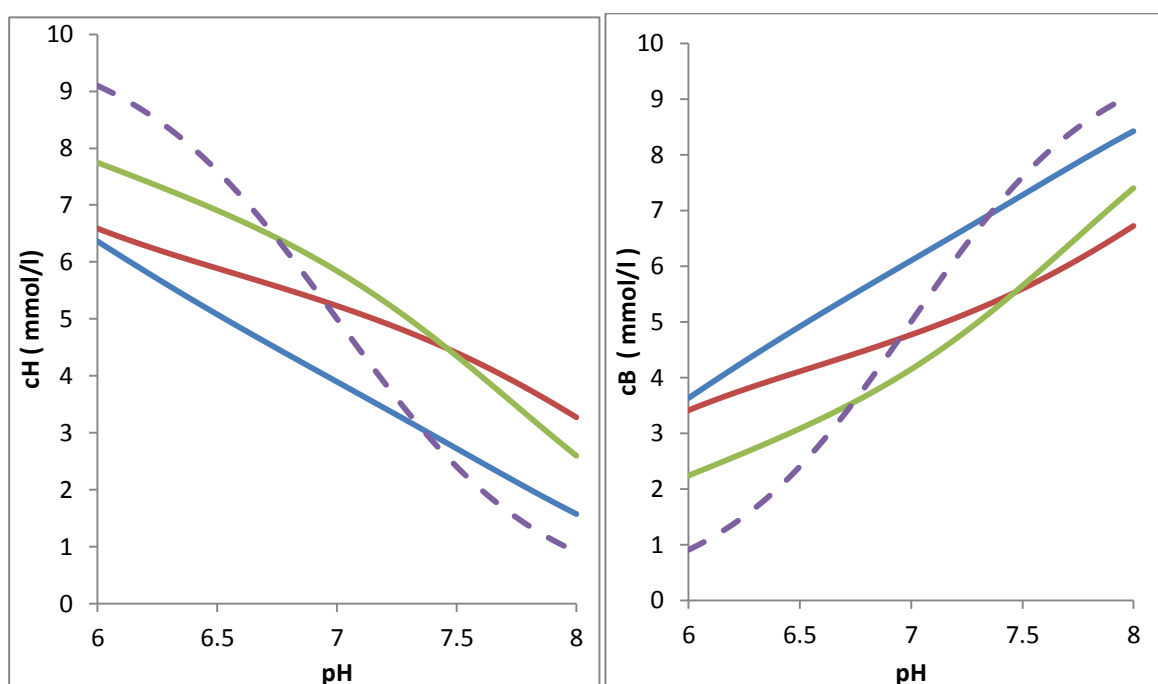

Figure 12 and 13: *Titration curves of 3 random proteins of pK$_A$ between 5 and 9, each of concentration 1 mmol/l (blue, red and green lines). Dashed purple line is a titration curve single buffer with pK$_A$ = 7. Note the mirroring nature of plotting C_H and C_B, whose sum is 10 mmol/l for each buffer and pH. The steeper the line is, the higher the buffer capacity of the buffer (i.e. better the buffer is).*
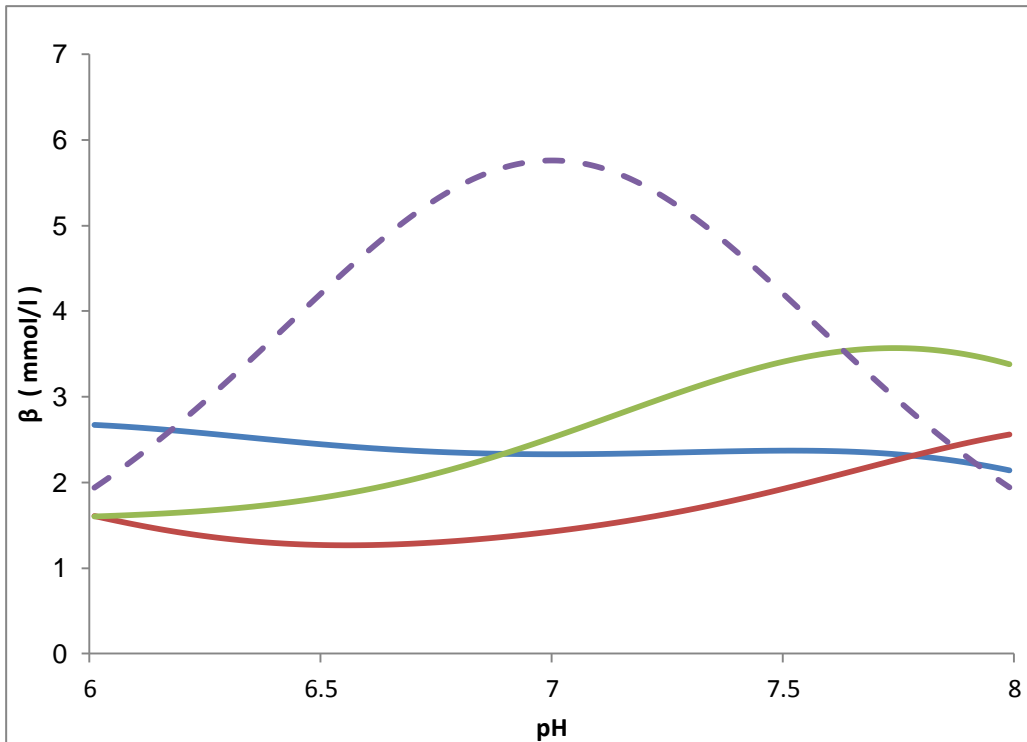
Figure 14: *Buffer capacities of three random protein buffers as a function of pH. The more constant the buffer capacities are, the more linear the buffering curve is. Dashed line is a single buffer case.*
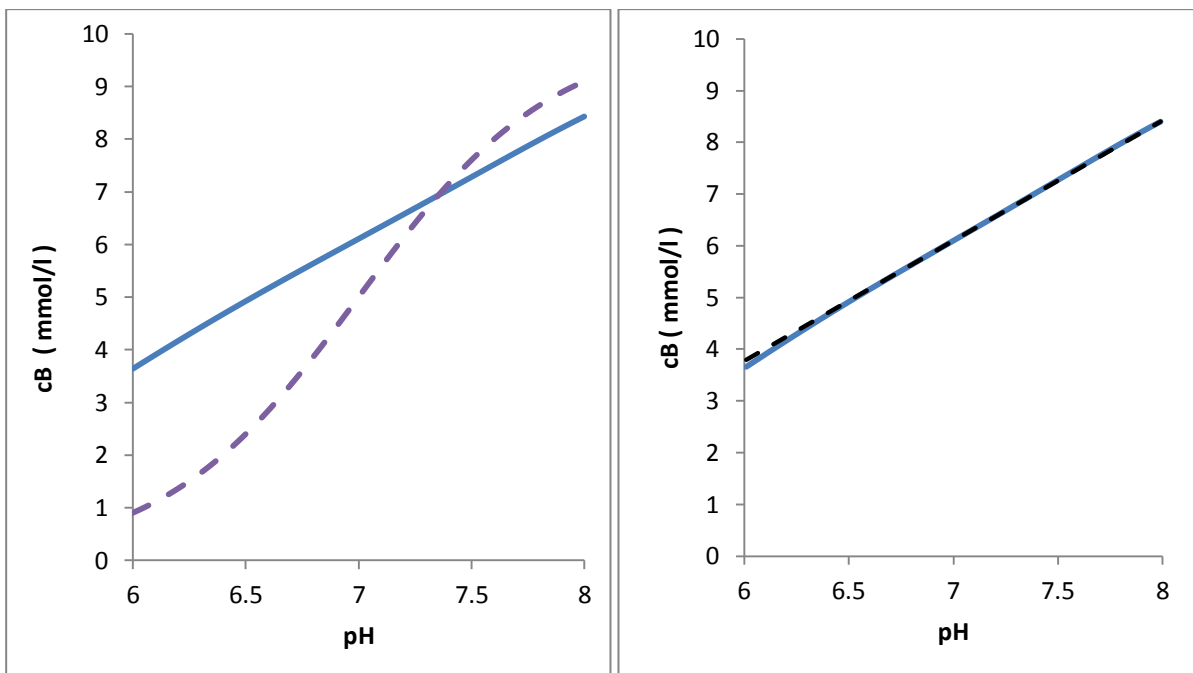
Figures 15 and 16: *The buffer capacity of a random protein buffer (number one) compared to single pK$_A$ buffer (left) and to its linear approximation (right). Note that the random protein buffer cannot be approximated well by a single pK$_A$ buffer. On the contrary, linearized approximation (black dashed line) produces excellent fit (in No 1. random protein at least).*

The results of this simulation clearly show one thing. Titration curves of a random mix of 10 buffers mimicking protein buffering are generally much closer to a straight line then to a buffering curve of a single buffer. Hence, it might be reasonable to simplify the calculations by linearization. One way of doing that is Taylor series, which gives approximation of a k times differentiable function around a given point a by a k-th order Taylor polynomial

$$f(x) \approx f(a) + f'(a) * (x - a) + \frac{f''(a)}{2} * (x - a)^2 + \cdots + \frac{f^{(k)}(a)}{k!} (x - a)^k \qquad (2.4)$$

A curve that is sufficiently close to a line can be approximated by a line, i.e. only the first two terms of Taylor series

$$f(x) \approx f(a) + f'(a) * (x - a) \qquad (2.5)$$

In the case of the random protein No. 1, such an approximation is plotted on figure 16, together with the original curve. The equation of the linearized relationship in this case is

$$C_B = 7.900 + 2.105 * (pH - 7) \qquad (2.6)$$

Figure 16 shows that the fit is very good, even though we have used a set of only 10 different buffer residues over a pH range of 4. Looking at figures 12 and 13, one can see that first order Taylor series (a tangent line) would not be as suitable for the other random sets. For instance, set number two (green line) is quite curved and at least quadratic approximation would be more suitable in this case (or the linearized relationship could only be used over a smaller pH range). Have we used more than 10 residues, the curve would be more likely to approach straight line, which could probably be proved using the theory of probability.

There are other possible ways to linearize a curve. For instance, if we would like to get a best fit in an interval of pH (instead of around a given point), a function approximation using the idea of least squares could be used. A simple numerical solution using for instance Microsoft Excel to the problem is possible. The disadvantage lies in more complicated mathematical formalization of trying to find the analytical solution.

Overall, one can assume that the linear approximation is generally very fitting for protein buffers, especially if one only needs to describe them over relatively small life compatible range of pH (e.g. 6.9 – 7.9). In fact, the linearized description of protein buffers is so useful that even the proponents of Stewart approach advocate its use, for instance when describing the buffering properties of albumin (40), (17). The fitness of a given protein for a linearized relationship can be confirmed by directly measuring the titration curve, which has been done for instance for albumin, whole plasma, hemoglobin and erythrocyte fluid (28), (41).

Mixture of bicarbonate and protein buffers

So far, we have only considered the bicarbonate buffer alone or protein buffers alone. In biological fluids, these two types of buffers often occur together and influence each other. In case of blood plasma, full blood and interstitial fluid, one could even say that these two types of buffers are absolutely dominant. As was demonstrated in the bicarbonate buffer section, one could easily disregard the other buffers and diagnose acid-base disturbances from the bicarbonate buffer alone. However, this poses two problems: Firstly, information about all the other buffers is lost and thus we don't how much buffering has been done in case of pH disturbance. A potential correction might be more difficult due to the lack of this knowledge. And secondly, bicarbonate concentration is not such a precise measure of metabolic disturbances, i.e. those that result from addition of strong acid or base to the body, once non-bicarbonate buffers are present. When pCO_2 changes and there are non-bicarbonate buffers present, $[HCO_3^-]$ changes as well. For instance, when acute respiratory acidosis develops, being caused by an increase of pCO_2 from 40 mmHg to 80 mmHg, concentration of bicarbonate increases as well, typically from 24 mmol/l to 28 mmol/l (see rules in table 5).

This change in bicarbonate is not due to kidney compensation, but due to redistribution between bicarbonate and non-bicarbonate buffers. Nature of this effect is going to be further covered in chapter 6. For now, it suffices to say that the magnitude of bicarbonate concentration change depends on ΔpH and non-bicarbonate buffer capacity β_{NonB}: The higher the β_{NonB}, the bigger the change in $[HCO_3^-]$. One important thing concerning the redistributions during CO_2 variations is that the sum of bicarbonate and non-bicarbonate buffer base stays constant. This was first described by Singer and Hastings in their famous article from 1948 (42). For the sum of bicarbonate and non-bicarbonate buffers of the blood, they used the term buffer base, abbreviated *BB.* It makes sense to equate the *BB* of Singer and Hastings with the total titratable base, C_B, defined in equation (1.34).

 This can be done by excluding the strong bases and very weak bases (originating from strong acids) from the C_B (if they were included), potentially offsetting its value, but not interfering with the solution of the equation system (1.27),(1.28) and (1.34). By excluding them, we suppose that the titration determining the value of *BB* does not go up or down into very alkalotic or very acidic *pH* and stays fairly close to neutral *pH.* In that case, we can also drop the $[H^+]$ and $[OH^-]$ terms from the equation (1.86) because these are several orders of magnitude lower than the rest. Finally, we can group together all the buffer residues of each protein as we did in eq. (1.88). Each of these sums represents concentration of buffer base on a particular protein. Only non-protein buffer base is bicarbonate, being expressed independently. Equation (1.86) then becomes

$$BB =$$

$$[HCO_3^-] + C_A \sum_{prot\,A} \frac{K_{j(A)}}{K_{j(A)} + [H^+]} + C_B \sum_{prot\,B} \frac{K_{j(B)}}{K_{j(B)} + [H^+]} + \cdots + C_Z \sum_{prot\,Z} \frac{K_{j(Z)}}{K_{j(Z)} + [H^+]} \qquad (2.7)$$

Each sum is equal to $\bar{e}_i$ of the given protein, average number of free hydrogen binding sites per molecule of protein i (eq. 1.90). Thus, we can also write.

$$BB = [HCO_3^-] + C_A * \bar{e}_A(\text{pH}) + C_B * \bar{e}_B(\text{pH}) + \cdots + C_Z * \bar{e}_Z(\text{pH}) \qquad (2.8)$$

As became apparent in the section about linearization, it generally makes sense to use linear approximation of $\bar{e}_i$ as a function of pH in case of proteins ($\bar{e}_i$ is equal to the buffering curve of the protein in unit concentration). The linearization can be done using Taylor series at point N, which is commonly taken at physiologically normal "neutral" pH = 7.4 (further referred to as pHn). Linearization by using the first two terms of the Taylor series makes the following substitution

$$\bar{e}_i(pH) = \bar{e}_{N\,(i)} + \frac{d\bar{e}_i}{d\,pH} * \Delta pH \qquad (2.9)$$

Where $\bar{e}_{N\,(i)}$ is the value of $\bar{e}_i(pH)$ at the normal pH (pHn), ΔpH equals (pH - pHn) and derivative of $\bar{e}_i$ with respect to pH is in fact the molar buffer value ($\beta_{M(i)}$) of the protein (species) i, that is

$$\frac{d\bar{e}_i}{d\,pH} = \beta_{M(i)} \qquad (2.10)$$

Substituting (2.10) and (2.9) into the equation (2.8), we get

$$BB = [HCO_3^-] + C_A\big(\bar{e}_{N(A)} + \beta_{M(A)} * \Delta pH\big) + C_B\big(\bar{e}_{N(B)} + \beta_{M(B)} * \Delta pH\big) + \cdots \\ + C_Z\big(\bar{e}_{N(Z)} + \beta_{M(Z)} * \Delta pH\big) \qquad (2.11)$$

This equation can be rearranged

$$BB = [HCO_3^-] + C_A * \bar{e}_{N(A)} + C_B * \bar{e}_{N(B)} + \cdots + C_Z * \bar{e}_{N(Z)} \\ + \big(C_A * \beta_{M(A)} + C_B * \beta_{M(B)} \ldots + C_Z * \beta_{M(Z)}\big)\Delta pH \qquad (2.12)$$

Written with the mathematical symbols for sums, the rearrangement takes a tidier form

$$BB = [HCO_3^-] + \sum_{i=A}^{Z} C_i * \bar{e}_{N(i)} + \Delta pH * \sum_{i=A}^{Z} C_i * \beta_{M(i)} \qquad (2.13)$$

These two sums can be used as definition terms of two new entities, normal buffer base of non-bicarbonate buffers (NBB_{NonB}) and buffer value of the non-bicarbonate component of the biological fluid (β_{NonB})

$$NBB_{NonB} \stackrel{\text{def}}{=} \sum_{i=A}^{Z} C_i * \bar{e}_{N(i)} \tag{2.14}$$

$$\beta_{NonB} = \sum_{i=A}^{Z} C_i * \beta_{M(i)} \tag{2.15}$$

Equation (2.13) then takes nice and concise form

$$BB = [HCO_3^-] + NBB_{NonB} + \beta_{NonB} * \Delta pH \tag{2.16}$$

Equation (2.16) will be used in the chapter 3, when describing the classical approach of Ole Siggaard-Andersen and comparing it to the modern approach of Peter Stewart.

Relationship between buffering, charge of multivalent substance and strong ions

Let's consider again the random protein from the linearization section with 10 buffering residues of pK_A's between 5 and 9. Let`s imagine, to simplify the situation, that there is no buffering outside this range and all other residues of the protein are electroneutral. The protein will be in concentration 2 mmol/l in a solution of NaCl (physiological solution). Titration curves are going to be determined by titrating with HCl to the acidic side and NaOH to the alkalotic side. Let's suppose that mixing is fast and the protein does not denaturize in contact with the titration agent.

Finally, let's divide the buffering residues of the protein i into two groups. First group (group A) of four residues has negative charge as a base and it becomes electroneutral in its acidic form. This behaviour corresponds to the side chain of cysteine or of a glutamic acid[5], as can be seen on figure 17. The buffering reaction of this group can be expressed by the general formula, in which the charge is now explicitly set.

$$\text{HA} \rightleftarrows \text{H}^+ + \text{A}^- \tag{2.17}$$

The second group (group B) of six remaining buffer moieties has different behaviour. These moieties are electroneutral in its base form and become positively charged as they change into conjugate acid. Such behaviour would correspond to the side chain of histidine or amino terminus of the protein. Their buffering reaction can be expressed by the formula

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[5] pK_A of the side chain of glutamic acid is generally below 5, around 4.15. However, in our example, we suppose that it has been sufficiently altered by the neighbouring protein moieties to fit into our pH range 5-9.
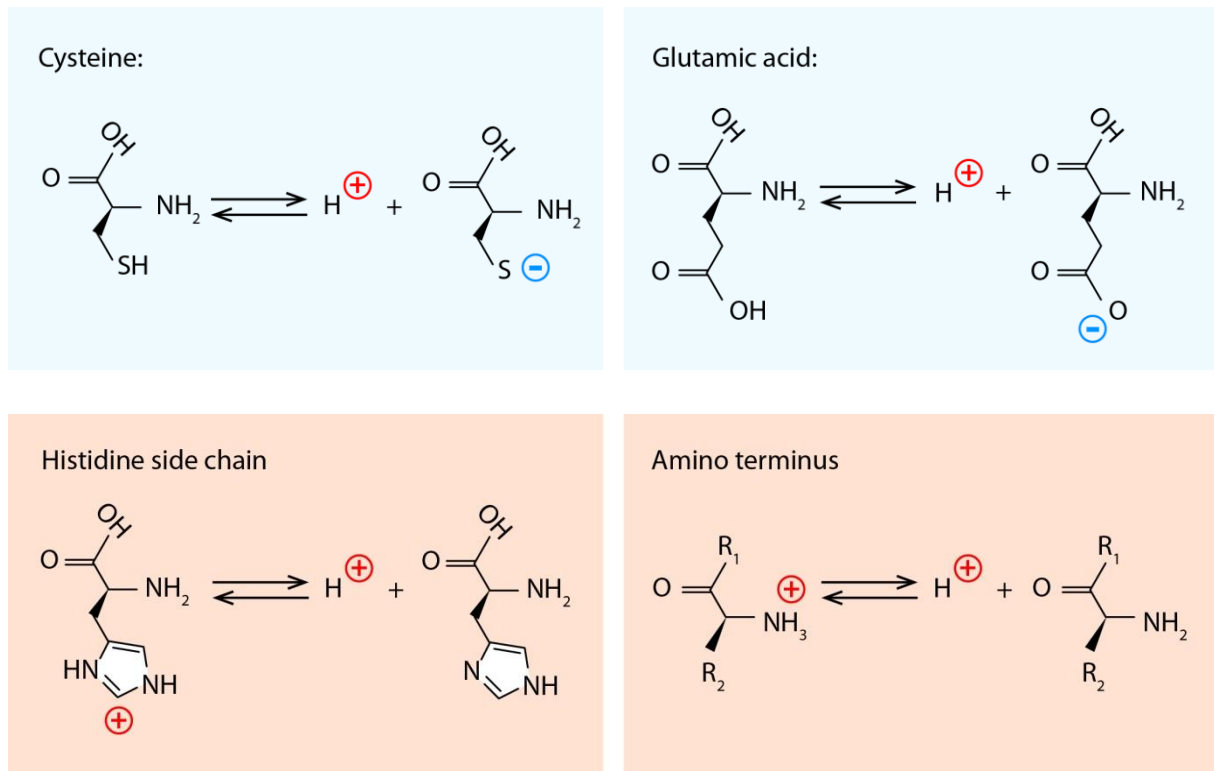
Figure 17: *Charge of the acid and base form of different amino-acid buffer residues. Conjugate acid of cysteine and glutamic acid side chain is electroneutral, while the conjugate base carries a single negative charge. Acid form of histidine side chain and amino terminus of protein is positively charged, while the base form is electroneutral. Upper row is called group A in the text, lower row group B.*

$$\text{HB}^+ \rightleftarrows \text{H}^+ + \text{B} \qquad\qquad (2.18)$$

Again this behaviour can be seen on figure 17.

When the protein *i* is in very acidic environment, let's say around pH=2.5, essentially all its buffer moieties bind hydrogen ion and turn into their acidic form. This means that the four moieties of the group A have charge zero now and the six moieties of the group B should have charge +1 mmol/l each, i.e +6 mmol/l all together. This charge is also called maximum charge per mole of the protein *i*, $\bar{z}_{\max(i)}$.

When the protein *i* is in very alkalotic environment, let's say around pH 11.5, essentially all its buffer moieties dissociate hydrogen ion and turn into their base form. This means that the four moieties of the group A have charge -1 now, i.e. -4 all together and the six moieties of the group B should have charge 0 mmol/l. The resulting charge, -4 mmol/l, is called the minimum charge per mole of the protein *i*, $\bar{z}_{\min(i)}$. Thus, we have

$$\bar{z}_{\min(i)} = \bar{z}_{\min(A)} + \bar{z}_{\min(B)} = -4 + 0 \tag{2.19}$$

$$\bar{z}_{\max(i)} = \bar{z}_{\max(A)} + \bar{z}_{\max(B)} = 0 + 6 \tag{2.20}$$

The charge of the protein at arbitrary pH can be calculated, according to the same logic, as a sum of the charge of group A, $\bar{z}_A$ (which is on their base form), and that of group B, $\bar{z}_B$ (which is on their acid form).

$$\bar{z}_i = \sum_{j(i)} -[A^-_{j(i)}] + [HB_{j(i)}] \tag{2.21}$$

Thus, for the group A, the charge per mole (or relative charge per molecule) is negative and equal in size to the average number of the "sites in the base form", i.e. free hydrogen ion binding sites per molecule, $\bar{e}_A$. For the group B, the charge per mole is positive and equal in size to the average number of the "sites in the acid form", i.e. average number of bound hydrogen ions per molecule, $\bar{n}_B$. We have

$$\bar{z}_A = \sum_{j(i)} -[A^-_{j(i)}] = -\bar{e}_A = \bar{z}_{\max(A)} - \bar{e}_A \tag{2.22}$$

$$\bar{z}_B = \sum_{j(i)} [HB_{j(i)}] = \bar{n}_B = \bar{z}_{\min(B)} + \bar{n}_B \tag{2.23}$$

The last identity is possible, because both $\bar{z}_{\max(A)}$ and $\bar{z}_{\min(B)}$ equal zero.

Alternatively, we could take the minimum charge per molecule of the group A (which is -4) and add to it the average number of the residues that are in the alternative state, i.e. occupied by hydrogen ion, this being $\bar{n}_A$. For the group B, we could take the maximum charge (which is 6) of the group B and subtract from it the average number of residues that do not bear this charge anymore, i.e. which are in their base form $\bar{e}_B$

$$\bar{z}_A = \bar{z}_{\min(A)} + \bar{n}_A = -4 + \bar{n}_A \tag{2.24}$$

$$\bar{z}_B = \bar{z}_{\max(B)} - \bar{e}_B = 6 - \bar{e}_B \tag{2.25}$$

Note that the equations (2.22) and (2.25) are identical in their general form, as well as the equations (2.23) and (2.24). Adding these couples together, we get

$$\bar{z}_i = \bar{z}_A + \bar{z}_B = \bar{z}_{\min(A)} + \bar{z}_{\min(B)} + \bar{n}_A + \bar{n}_B \tag{2.26}$$

$$\bar{z}_i = \bar{z}_A + \bar{z}_B = \bar{z}_{\max(A)} + \bar{z}_{\max(B)} - \bar{e}_A - \bar{e}_B \tag{2.27}$$

Of course, the A group terms and the B group terms can be summed together to get the value of the whole protein, thus getting the final formulas

$$\bar{z}_i = \bar{z}_{\min(i)} + \bar{n}_i \tag{2.28}$$

$$\bar{z}_i = \bar{z}_{\max(i)} - \bar{e}_i \tag{2.29}$$

These two formulas give an explicit relationship to calculate charge of the protein as a function of pH. $\bar{z}_{\min(i)}$ and $\bar{z}_{\max(i)}$ are constants that characterize the given protein and their value depends on the type of buffer residues of the protein (the two differ exactly by $\bar{n}_{\max(i)}$, which is 10 in our case). The calculation of $\bar{n}_i$ and $\bar{e}_i$ was covered extensively in chapter 1. For the protein buffers, one can calculate their value using the equations (1.89) and (1.90), getting

$$\bar{z}_i = \bar{z}_{\min(i)} + \sum_{j(i)} \frac{[H^+]}{K_{j(i)} + [H^+]} \tag{2.30}$$

$$\bar{z}_i = \bar{z}_{\max(i)} - \sum_{j(i)} \frac{K_{j(i)}}{K_{j(i)} + [H^+]} \tag{2.31}$$

Let's now define a new parameter, called strong ion difference, or SID, as a sum of all charges on strong ions, i.e. those that do not participate in acid-base reactions

$$SID \stackrel{\text{def}}{=} \sum_k Z_k * [I_k^{Z_k}] \tag{2.32}$$

where I_k is a strong ion of the charge Z_k. In our example, the only strong ions present are the Na^+ and Cl^- and strong ion difference equals

$$SID = [Na^+] - [Cl^-] \tag{2.33}$$

At each moment, the concentration of positively charged species has to match the concentration of negatively charged species. Therefore, the charge of the cations minus the charge of the anions equals 0 in every solution, this being called the principle of electroneutrality. There are only five charged species in our example solution. The charge of the example protein can be calculated as its charge per molecule $\bar{z}_i$ times its concentration C_i. The charge of the protein $\bar{z}_i$ can be either a positive or negative number (depending on pH) and the term that includes it is thus added with a plus sign

$$[Na^+] - [Cl^-] + C_i * \bar{z}_i + [H^+] - [OH^-] = 0 \tag{2.34}$$

Substituting (2.33) into (2.34), we get another expression for SID

$$SID = -C_i * \bar{z}_i + [OH^-] - [H^+] \tag{2.35}$$

If we switch back to the general formulation, we have to include the charge of all buffers

$$SID = [OH^-] - [H^+] - \sum_i C_i * \bar{z}_i \tag{2.36}$$

Substituting in for the $\bar{z}_i$ from the equation (2.31), we get

$$SID = [OH^-] - [H^+] - \sum_i C_i * \left(\bar{z}_{\max(i)} - \sum_{j(i)} \frac{K_{j(i)}}{K_{j(i)} + [H^+]} \right) \tag{2.37}$$

This can be rearranged as

$$SID = [OH^-] - [H^+] + \sum_i C_i * \sum_{j(i)} \frac{K_{j(i)}}{K_{j(i)} + [H^+]} - \sum_i C_i * \bar{z}_{\max(i)} \tag{2.38}$$

Note that most of the right-hand side is the expression total titratable base (equation 1.86 and 1.88) This gives us formula that relates strong ion difference and total titratable base

$$SID = C_B - \sum_i C_i * \bar{z}_{\max(i)} \tag{2.39}$$

Noting that C_B is equal to the buffer base (BB), more traditional term in physiology, we can also write

$$SID = BB - \sum_i C_i * \bar{z}_{\max(i)} \tag{2.40}$$

This last equation was first pointed out to the acid-base physiologists by Wooten in 1999 (7). It might be surprising for some acid-base experts to note that SID and BB are not necessarily same. They have been considered same since the days of Singer and Hastings. The original formulation of the Stewart's approach does not mention buffer base, but it implicitly sets $\bar{z}_{\max(i)}$ as zero by setting the charge of weak acid (HA) zero. This was later taken for granted by the followers of Stewart (2), (4). Even the critique of the Stewart's approach by Siggard-Andersen and Fogh-Andersen (6) focuses on this original formulation and thus equates SID and BB.

In order to give this rather formal discussion a bit more substance, $\bar{z}_A$, $\bar{z}_B$ and $\bar{z}_i$ of the hypothetical protein i are plotted as function of pH on the figure 18. The $\bar{z}_i$, $\bar{n}_i$ and $\bar{e}_i$ are plotted as function of pH on figure 19 and BB and SID are plotted as function of pH in figure 20. The last curves are, in fact, the titration curves of the random protein no.1 of the linearization section, when it is in concentration 2 mmol/l. The titration curves of protein no.1 (i.e. C_B and C_H vs. pH curves) were already plotted on figures 12 and 13 (blue line), albeit over a smaller range of pH.
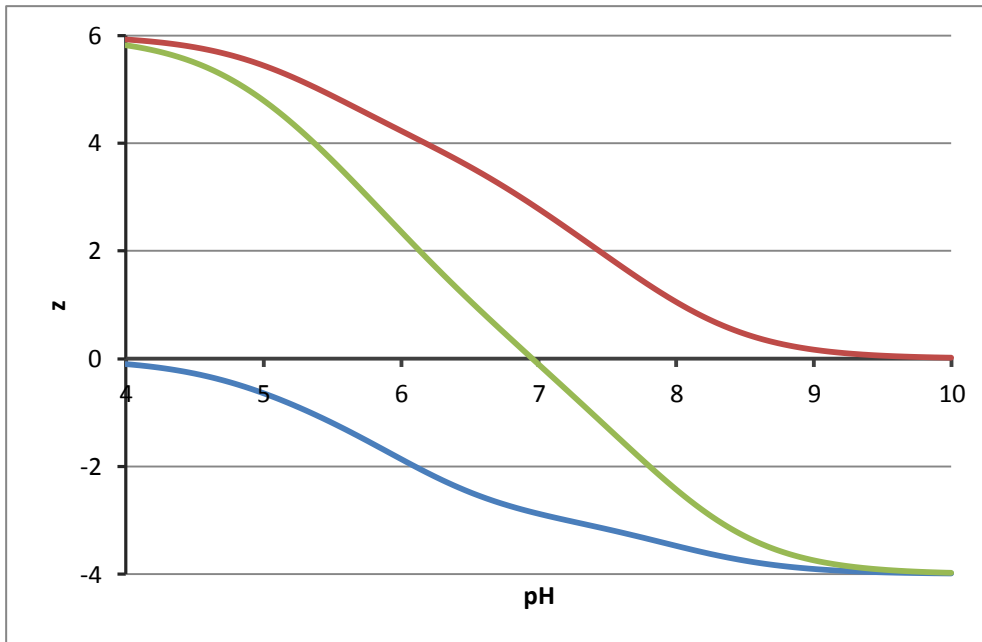
Figure 18: *Total charge of protein i as a sum of the charge of positively and negatively charged residues, plotted as a function of pH. Charge on the negatively charged buffer residues $\bar{z}_A$ of the protein i - blue line; charge of the positively charged buffer residues $\bar{z}_B$ - red line; total charge of the protein $\bar{z}_i$ green line. $\bar{z}_i = \bar{z}_A + \bar{z}_B$ for any pH. As discussed in the text, $\bar{z}_{\min(i)} = -4$ and $\bar{z}_{\max(i)} = 6$.*

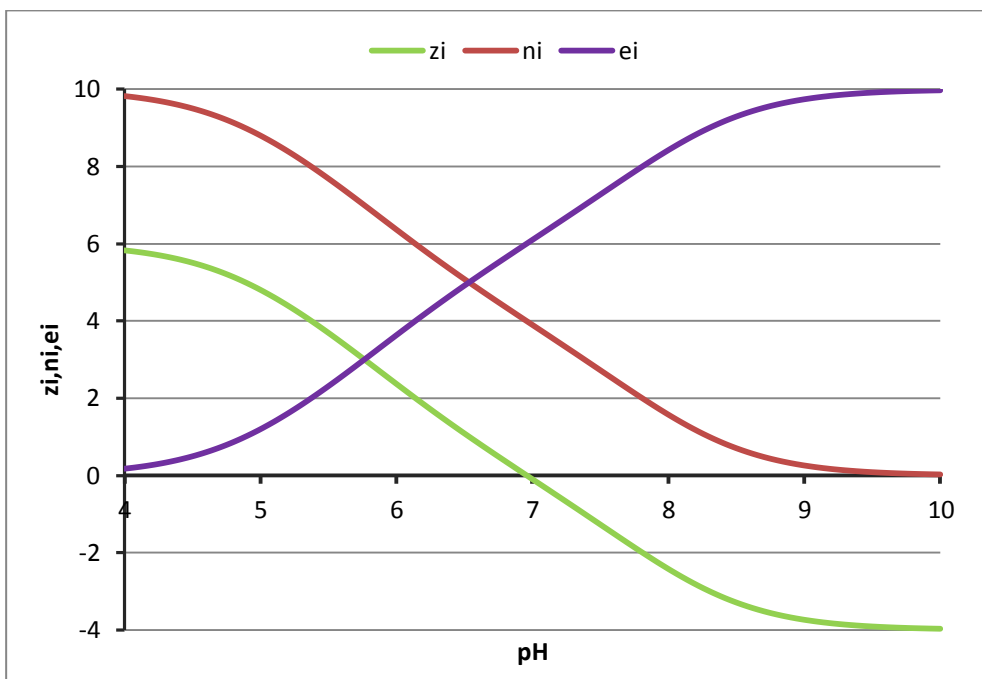**Figure 19:** *Relationship between $\bar{n}_i$, $\bar{e}_i$ and $\bar{z}_i$ plotted over a range of pH. Average number of bound hydrogen ions per molecule $\bar{n}_i$ - red line; average number of free hydrogen ion binding sites per molecule $\bar{e}_i$ - blue line. Total charge of the protein i $\bar{z}_i$ - green line. Notice that charge of the protein changes in concordance with the bound hydrogen ions (equation 2.28).*

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According to eq. (2.40), *SID* changes simultaneously with *BB* during buffering. This can be seen on figure 20 and can be understood also intuitively. Imagine that you add let's say 0.5 mmol of HCl per liter of resulting solution. HCl is a strong acid and dissociates completely. H^+ mostly binds to the hypothetical protein and 0.5 mmol/l of its buffering amino-acid side chains turn from their base form into the acid form. This is equivalent to C_H increasing by 0.5 mmol/l and BB decreasing by 0.5 mmol/l. SID changes simultaneously: It decreases by 0.5 mmol/l, because there is now 0.5 mmol/l of free Cl^- ions (strong anion) more. Analogical, but opposite changes happen when we titrate by NaOH.
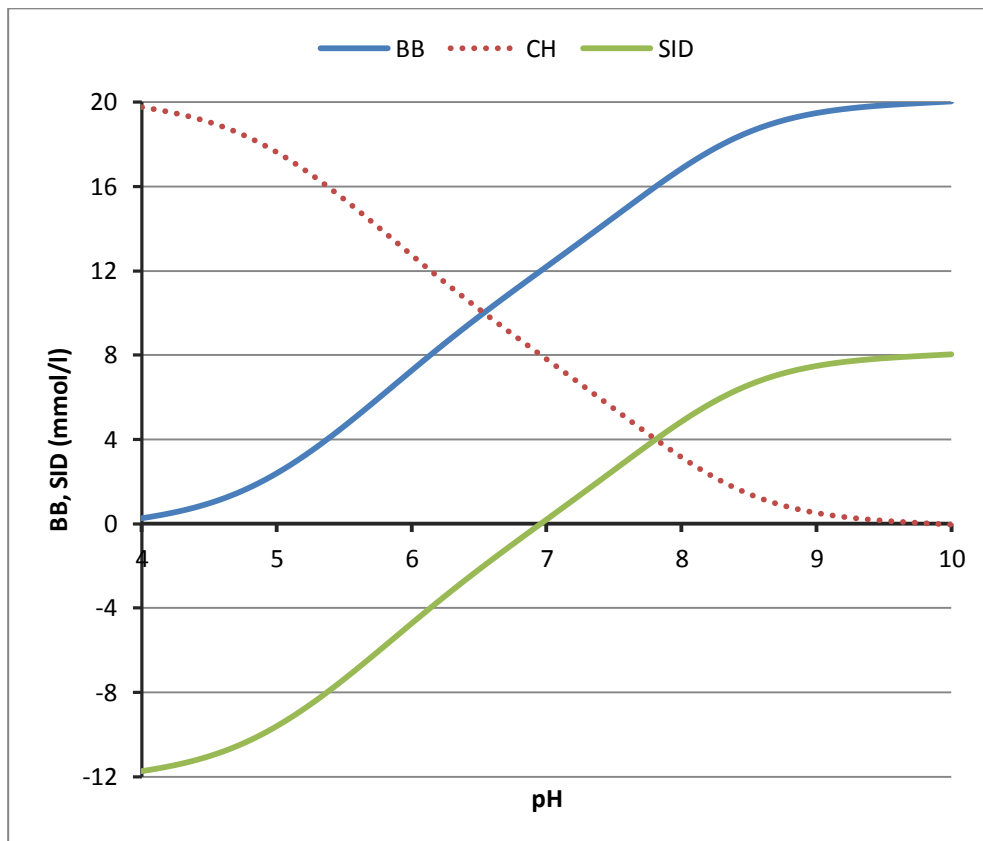
Figure 20: *Relationship between total buffer base BB, total titratable acid C_H and strong ion difference (SID) of a solution containing 2 mmol/l of the protein i (see text) as a function of pH. Blue line represents BB, red dotted line C_H and green line SID of the same system (random protein no.1). Note that BB and SID are not the same, but ΔBB equals ΔSID. Also note that the value of SID basically opposes the charge on the protein $\bar{z}_i$, by which means the electroneutrality of the solution is kept, This is the reason why BB changes concordantly with SID, while $\bar{z}_i$ changes concordantly with $\bar{n}_i$ ($\bar{n}_i$ is related to C_H and not C_B/BB)*

Modelling albumin charge and buffering properties, iSID and A_{tot} uncertainty

When we introduced the hypothetical protein *i* of previous section, we included one simplifying assumption for the sake of clarity. All the charged residues were buffers with their pK_A between 5 and 9. Such a situation would be extremely rare for real world proteins, because significant number of polar amino-acid side chains have pK_A's outside this range. Average pK_A's of different amino-acid residues are shown in figure 21. Note that the pK_A of an individual residue in a given protein can be altered significantly by its particular microenvironment, as is shown for 16 histidine side chains of albumin molecule (table 6).
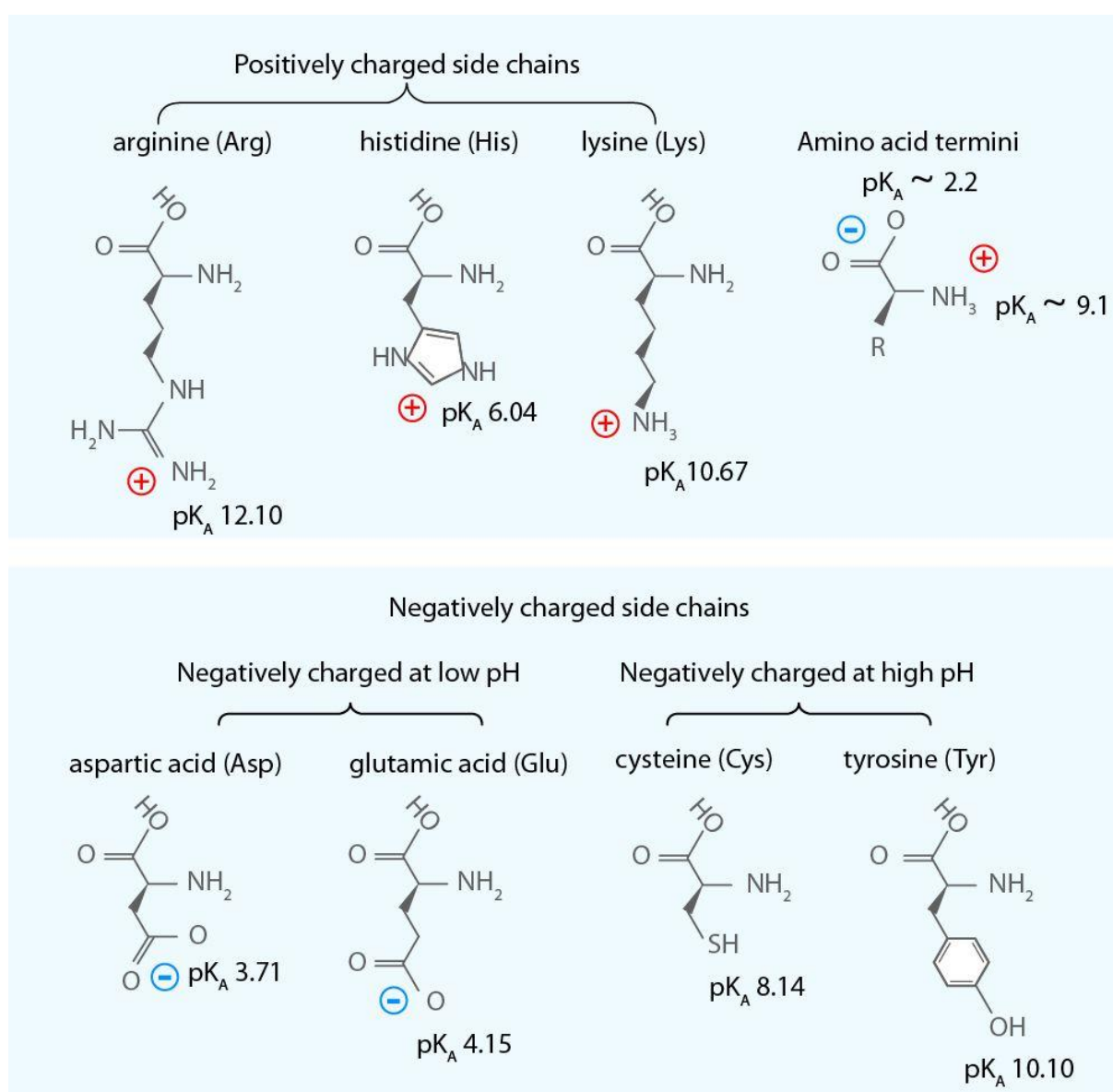

Figure 21: *The most common charged amino acid residues with their respective average pK_A's. The pK_A in a protein can be altered significantly by residue's microenvironment (as seen in table 6).*

Notice that aspartic acid side chain's pK_A (and to a lesser degree glutamic acid's), as well as the acidic terminus of the protein's pK_A are so low, that 99.9% of it are in its negatively charged base form at life compatible range of pH. Therefore, these residues could be considered strong anions in acid-base physiology (see discussion at the end of the section Elementary concepts of acid-base chemistry of Chapter 1 for details). Similarly, pK_A's of the side chain of arginine (and to a lesser degree of lysine) are so high, that 99.9% of these residues are in their positively charged acid form and their charge stays practically constant at life compatible range of pH. These residues can be considered strong cations for the sake of acid-base physiology.

However, probably due to impracticality of measurement, I have no knowledge of a published version of the electroneutrality equation that would include these charges in the SID, even though, strictly chemically speaking, this would make sense. Closest to this is a published model of albumin by Watson (22), which uses the Stewart approach, but divides the charges on albumin into fixed charges, behaving the same way as any other strong ion and attributable to the amino acid residues of very high or very low pK_A, and the variable charges of the buffering residues, mainly histidine. Variable charges are treated as a single pK_A system, while the fixed charges (called A^-_{fix} by Watson) are added to the SID of the surrounding plasma in the electroneutrality equation. Of course, Watson's model, although being a version of the modern approach, requires to be identified to different values of albumin K_A and A_{tot} then the original model of Stewart.

In order to emphasize the similarity between the fixed charge of these protein residues and the fixed charge of the plasmatic ions (be it inorganic or organic), I prefer to call the fixed charges on the protein i internal strong ion difference of the protein i ($iSID_{(i)}$). The term $iSID_{(i)}$ is going to be used at any protein concentration, the charge of the strong ions per mole is going to be designed $Z_{Fix(i)}$. With this terminology, the definition equation of $iSID$ takes a similar form to the equation 2.32

$$iSID_{(i)} \stackrel{\text{def}}{=} C_i * Z_{Fix(i)} \tag{2.41}$$

In order to see how sound this concept of internal SID is, several models of albumin buffering were constructed and compared to the up to date most detailed model of albumin buffering properties by Figge Mydosh and Fencl (40).

Titration curve of albumin, constructed according to the Figge-Mydosh-Fencl model (40) (abbreviated FMF model), is shown on figure 22. The graph includes a separate curve that shows buffering done by the residues effective at very low pK_A and high pK_A, i.e. those that are considered fixed in the Watson model. Ideally, their buffering curve should approach constant value over the physiological range of pH.
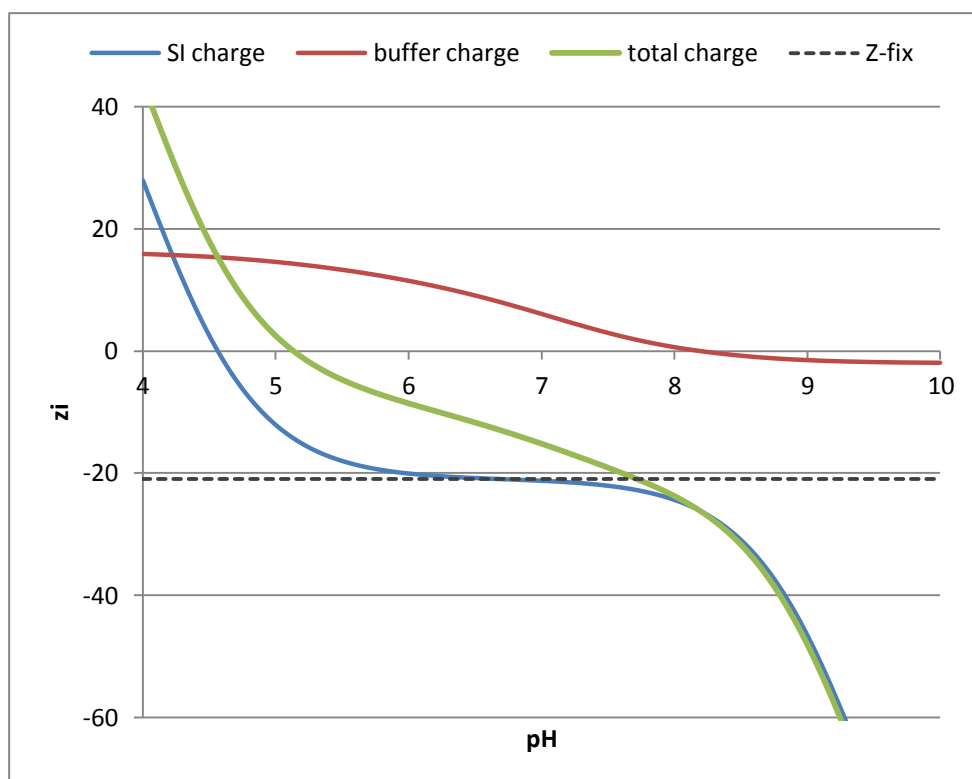
Figure 22: *Average charge of albumin molecule as a function of pH, according to Figge-Mydosh-Fencl model (green curve). The charge is composed of the charge of "strong ion" residues and the charge of buffer residues. The charge of very acidic residues (mainly Asp and Glu side chains) and the charge of the very basic species (mainly Arg and Lys side chains) are taken together and called the SI charge (blue curve). This charge should be completely constant over physiological range of pH (6.5 – 8) and equal to -21 elementary charges per molecule (or equivalents per mole), as reported by Watson (black dashed line). Obviously, this is not completely true; the reasons are discussed in the text. Buffer residues (mainly His side chains) are those that have their pK_A's within the physiological range (red curve). The curve was experimentally validated by titration data only little beyond the physiological range of pH, i.e. for the interval of pH (6.6 -8.2); the rest is extrapolation based only on the known amino acid composition of albumin.*

As can be seen on figure 22, the charge of the amino-acids that are considered fixed in the Watson model is not that fixed after all even at the pH of physiological interest. This is because the difference between the pH of the interest and the pK_A of the amino acid residues that we want to consider fixed is not at least 3, as would be required by the pH - pK_A criterion (see the end of section Elementary concepts of acid-base chemistry in chapter 1 for details). The pH-pK_A criterion is basically fulfilled at the low pH end, because 98 residues of aspartic and glutamic acid are assigned pK_A = 4 in the model, being 3 units lower then 7. However, there are 77 arginine and lysine residues in Figge-Mydosh-Fencl model that are assigned a pK_A of 9.4 (significantly lower than average value given in figure 21) and

further 16 tyrosine residues that are assigned a pK_A of 9.6. Taken together, there are almost 100 residues with their pK_A only 2 units higher than 7.4. This 2 unit difference means that about 10^{-2} = 1% of these residues (see table 4) are in their more negatively charged base form that is not accounted for in the *iSID* model. Since there are about 100 of these residues, this 1% difference in behaviour makes a difference of full 1 elementary charge per molecule at pH = 7.4 and more than 2 elementary charges per molecule at pH = 7.7 and beyond. This unsatisfactory behaviour can be seen together with (equally unsatisfactory) behaviour of Watson's original model in figure 23, plotted over the pH of physiological interest.
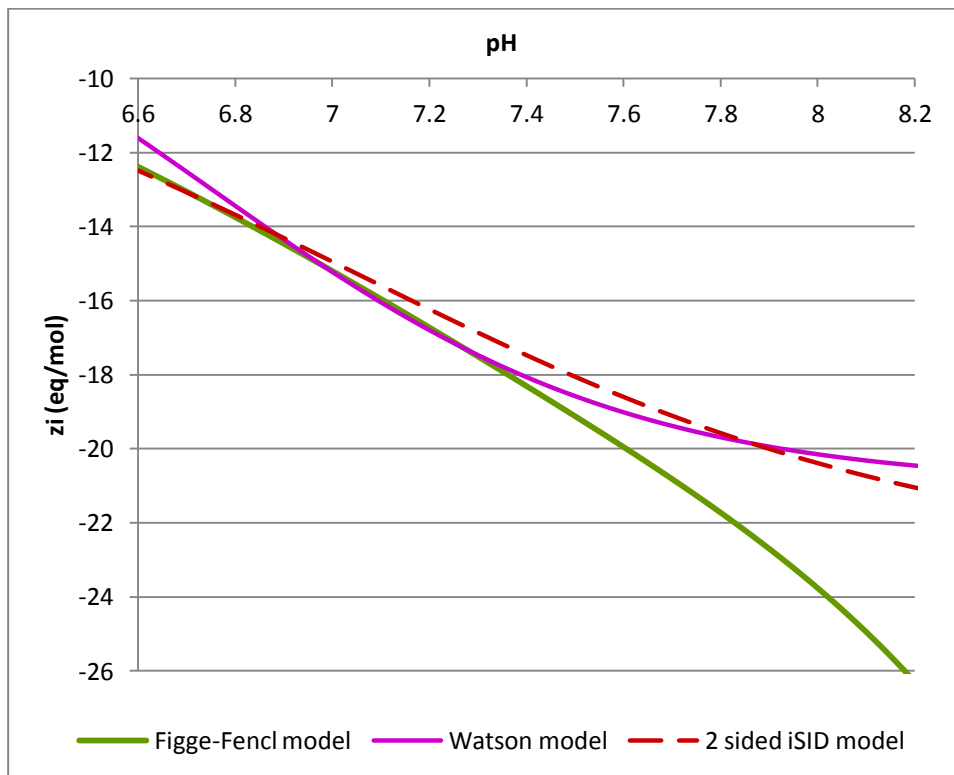
Figure 23: *Charge of albumin as a function of pH, plotted over pH range of physiological interest. The green line corresponds to the Figge-Mydosh-Fencl model of albumin charge. The red dashed line corresponds to the iSID model, where charged ions were divided into those that buffer at physiological range of pH (their pK_A being close to the pH of interest) and those that behave as strong ions, because their pK_A is far from the pH of interest. This curve is same as the red buffer curve on figure 22, shifted by the fixed charge of the "strong ion" residues. The curve of this model does not fit well the actual buffering curve of the Figge-Fencl model especially at alkalotic pH side, as is discussed in the text. The magenta curve represents Watson's model. In the area of physiologic acidemia (pH lower than 7.4), it gives good fit both in terms of buffering (slope of the curve) and the actual albumin charge, but it behaves poorly at alkalotic pH side, underestimating significantly especially the albumin buffer capacity (slope of the curve).*

As is apparent from both the analysis and the figure 23, residues of high pK_A (arginine, lysine, tyrosine and amino terminus) cannot be considered fixed charge in Figge-Mydosh-Fencl model because their pK_A is too close to the pH of physiological interest. However, the acidic residues glutamic acid and aspartic acid are ascribed pK_A of 4, which could be considered to fulfill the pH-pK_A criterion. Model that considers these acidic residues as fixed charge, but still treats the base residues as buffer residues, can be seen on figures 24 and 25. Note how well the model behaves in the pH of physiological interest – there is only a slight deviation to a more complex Figge-Mydosh-Fencl model at pH below 7.
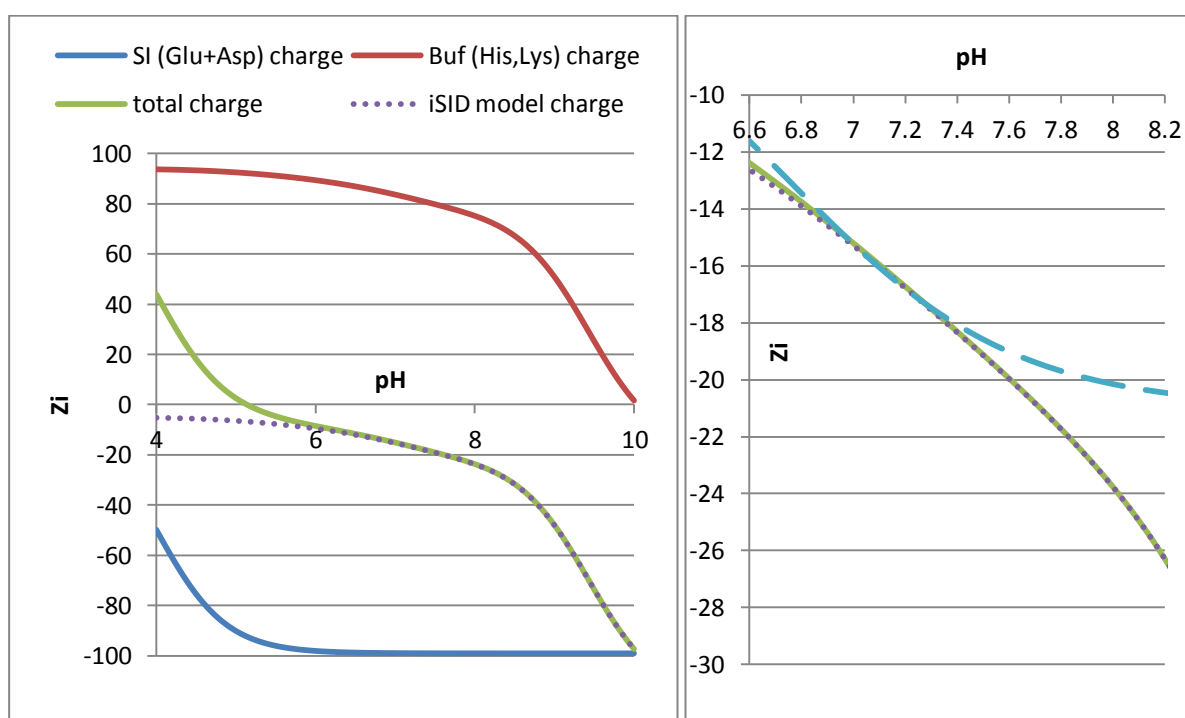
Figures 24 and 25: 1 sided iSID model of albumin based on Figge-Mydosh-Fencl model compared to the original model and Watson's model (right). Base (high pK_A) residues (Lys, Arg) are assigned unusually low pK_A values in FMF model. Thus, their pK_A is too close to the pH of interest and their inclusion into internal strong ion difference results in significant error (as seen on figure 23). These figures show that in the FMF model only the acidic (low pK_A) residues (Glu, Asp) can be treated as strong ions. The resulting model is called 1 sided iSID model. Total charge of albumin as predicted by FMF model is shown as a green curve. This curve is a sum of the charge of the acidic residues, i.e. charge that is almost fixed at physiological pH (blue full line, strong ion (SI) charge) and the charge of the buffer residues (His, Lys, Arg, Tyr). Only the charge of buffer residues changes at physiological range of pH (red line). Purple dotted line is the albumin charge as predicted by "1 sided iSID model". 1 sided iSID model treats acidic residues as fixed charge (-99 in this case) and gives excellent predictions for pH > 6.5. Watson's model (22) (blue dashed line) is shown for comparison on the right.

So, to summarize these results, when modeling protein buffers, amino-acid residues can be assigned two types of behaviour in terms of their charge: Residues that retain their fixed charge $Z_{Fix(i)}$ over the pH of interest and contribute to the charge of other strong ions in the solution and residues that behave as buffers. Buffer residues contribute variable charge $\bar{z}_i$ that is a function of pH in the range of interest. Buffer residues can be quantified by their total number per molecule of protein i: $N_{Buf(i)}$. When multiplied by protein concentration, this should give total concentration of monovalent buffer residues, i.e. what is called weak acid (A_{tot}) in Stewart's original formulation

$$A_{tot(i)} = C_i * N_{Buf(i)} \tag{2.42}$$

However, as will be shown later, A_{tot} ends up being determined differently to its name and original (simplified) meaning in practice. Furthermore, as became clear from the previous discussion and the discussion over the pH-pK$_A$ criterion in chapter one, the division between strong ions and buffers (or strong ion residues and buffer residues in case of proteins) is not at all clear-cut, but rather fuzzy. Residues that can truly be treated as strong ion residues by the pH-pK$_A$ criterion can be also treated as buffers with their respective pK$_A$, causing the model to become more complex and slightly more precise in the pH of interest. Residues that have pK$_A$ very close to the pH of interest can only be considered buffer ions, as their average charge always changes significantly with pH.

	Figge-Mydosh-Fencl model 1992	Figge-Fencl model 2012	1 sided iSID model based on F.M.F.	Watson model	2 sided iSID model based on F.M.F.	2 sided iSID model based on F.F.2012
Precision	reference	reference	precise	imprecise	imprecise	precise
Z_{Fix} (iSID)	0	0	-99	-21	- 22	-25
N_{Buff} (A_{tot})	212	218	95	16	18	27

Table 8: *Comparison of various models of albumin and their parameters. All models give albumin charge as a function of pH, i.e. also its buffering properties. Both Figge-Mydosh-Fencl published 1992 and Figge-Fencl model version 3.0 (published 2012) have been regarded as reference, although the newer model matches experimental data outside the physiological pH much better. The other four models are derived from these two as described in the text. These four models treat the charge of the very acidic or very alkalic amino acid residues as fixed, resulting in a total fixed charge per molecule Z_{Fix}. The remaining residues contribute to the albumin buffering properties, their number per molecule being N_{Buff}. These quantities are either without unit (number of elemental charges or residues per molecule) or they can be expressed in meq/mmol.*

Residues that have pK_A somewhat close to the pH of interest, let's say higher or lower by 2 combine the properties of the two groups, their charge is fairly constant yet it changes measurably (with slope around $\sim$2.3% in case of pH - pK_A difference of 2 (table 3)). Including these residues into the strong ion group is possible if there are only few of them and will always be somewhat a matter of personal preference. The decision changes the values of Z_{Fix} and $N_{Buf(i)}$ (and thus the protein contribution to iSID and A_{tot}). When pH-pK_A criterion is applied correctly, the decision does not really change the charge and buffering properties of the given protein. Various values of Z_{Fix} (iSID) and N_{Buf} (A_{tot}) that can be used in case of albumin are given in table 8.

Update: Version 3.0 of the Figge-Fencl model was published on-line in October 2012 (43). Previous version of the model (40) explained extra albumin buffering capacity observed at the regions of physiological alkalosis by artificially lowering pK_A's of about 95 arginine, lysine and tyrosine residues during the process of model fitting. However, such a lump difference to the average pK_A value of these amino-acid side chains (table 9) is highly improbable. Furthermore, the previous model did not fit well more recent experimental data outside the range of normal physiological interest (41).

		Asp +Glu	Tyr	Arg	Lys	Carboxy term.	Amino term.	Cys	Lys1	Lys2	Lys3	Lys4	Lys5
FMF 1992	pK_A	4	9.6	11	9.4	3.1	8	8.5					
	N	98	18	0	77	1	1	1					
F.F. 2012	pK_A	3.9	11.7	12.5	10.3	3.1	8	8.5	5.8	6.15	7.51	7.685	7.86
	N	98	18	24	50	1	1	1	2	2	2	2	1
	Z_{max}	0	0	1	1	0	1	0	1	1	1	1	1

Table 9: Comparison of the structures of albumin models of Figge-Mydosh-Fencl (version 2.0, 1992) and Figge-Fencl (version 3.0, 2012). N indicates total number of each amino acid residue per molecule. The most important buffer residues, 16 histidine side chains are not included, as their pK_A's were already given in table 6. These stay same in both models. The new model version uses higher values of pK_A's for tyrosine, arginine and most lysine residues, this being more consistent with the generally accepted values (e.g. given in (44)). The new version includes 9 so called low titrating lysine residues to explain steeper slope of the titration curve around pH 7.7, this being based on experimental evidence as given in (43). It also includes the effect of a measured transition in tertiary structure with the change of pH from neutral to alkalotic. Z_{max} value indicates whether the maximum charge of residue's acid form is 0 (group A) or 1 (group B).
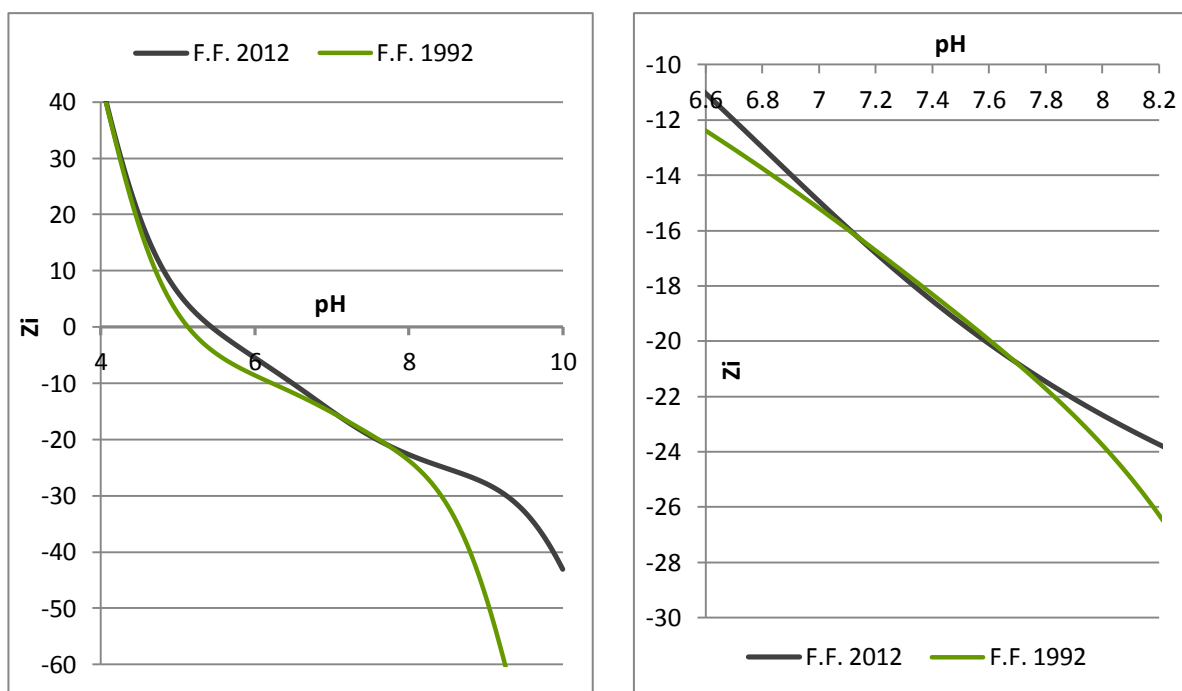
Figures 26 and 27: *Charge of albumin molecule as a function of pH according to the Figge-Mydosh-Fencl model, published in 1992, and Figge Fencl model version 3.0, published online in 2012. Note that the two models predictions match well in pH range 7.0 – 7.8, while differing substantially everywhere else. The new model includes newly established structural data, for instance structural transition of albumin when moving from neutral to alkalic pH. The new model reproduces very well (ref) the titration curves measured by Tanford (ref) and Fogh-Andersen, Bjerrum and Siggaard-Andersen (ref) together covering range of pH from 4 to 10.*

Figge-Fencl model version 3.0 assigns more realistic behaviour to the base residues Lys, Arg and Tyr, while maintaining correct slope of albumin titration curve around pH 7.7. This is done by assigning 9 lysine residues unusually low pK_A's, a step for which some experimental evidence exists (43), including tryptophan and tyrosine fluorescence (45). The logic behind this step is following: In the FMF model version 2.0, there were about 100 residues of pK_A around 9.4 (higher by 2 as compared to the pH of interest around 7.4). At pH around 7.4, this model structure is going to behave very similarly to 10 residues of pK_A around 8.4 (higher by 1 from the pH of interest) taken together with 90 fixed charges resulting from moving the other residues further away. This is because at pH = 7.4, each pK_A 9.4 residue is about 1% in its H^+ bound (protonated) form, while each pK_A 8.4 residue is about 10% in its protonated form. 1 residue 10% protonated is going to behave similarly as 10 residues 1% protonated. Figge-Fencl model version 3.0 assigns Arg, Lys and Tyr their normal average

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values (43; 2), (44), except for 9 lysine residues of unusually low pK_A, given in table 9. James Figge assigns 5 different pK_A's (5.8, 6.15, 7.51, 7.685 and 7.86) to these residues, fitting the model parameters to the known titration curve. The titration curve of the new model as compared to the previous version is shown on the figures 26 and 27. Figure 28 and 29 show behaviour of the simplified model as compared to the original. The simplified 2 sided *iSID* model now shows very good fit over physiological range of pH (figure 29), contrary to the situation with Figge-Mydosh-Fencl model version 2.0. The resulting values of Z_{Fix} and N_{Buf} are added to the table 8. This is because assigning their normal high pK_A's to the Tyr, Arg and most Lys residues results in their much better fulfillment of the pH - pK_A criterion, allowing them to be assigned strong ion behaviour a discussed previously.

Figure 28: Charge of albumin molecule as a function of pH according to the Figge-Fencl model version 3.0, published online in 2012 (black line), the linear approximation of the Figge-Mydosh-Fencl model, published in 1992 (gray dashed line) and 2 sided iSID model based on Figge-Fencl model version 3.0. The models are plotted over wide range of pH, which allows us to see that the approximations only work well in the physiological range of pH.
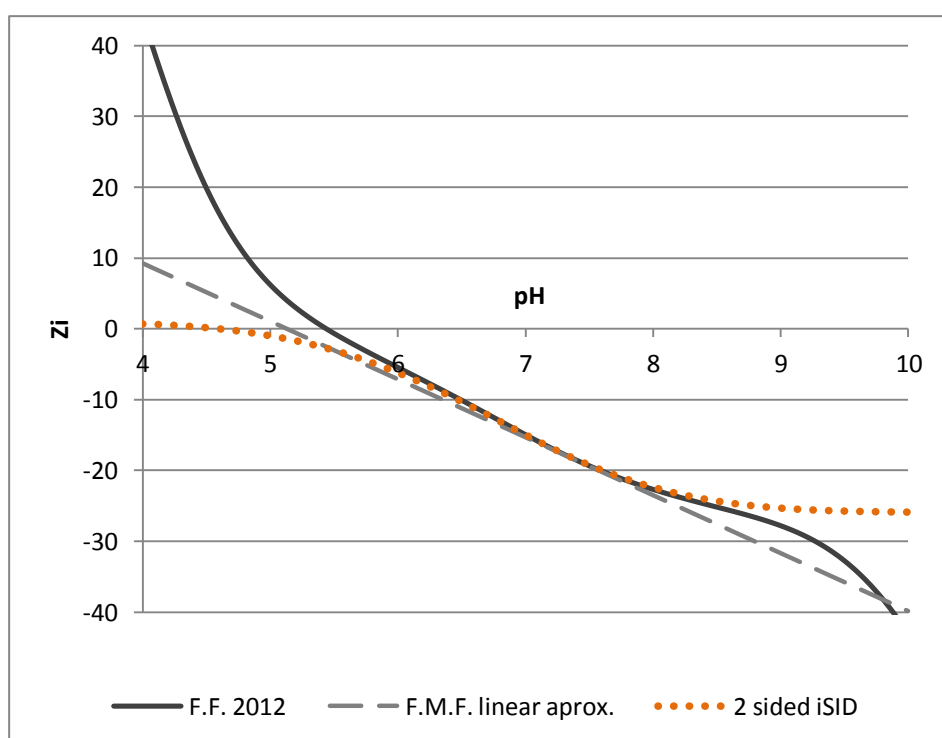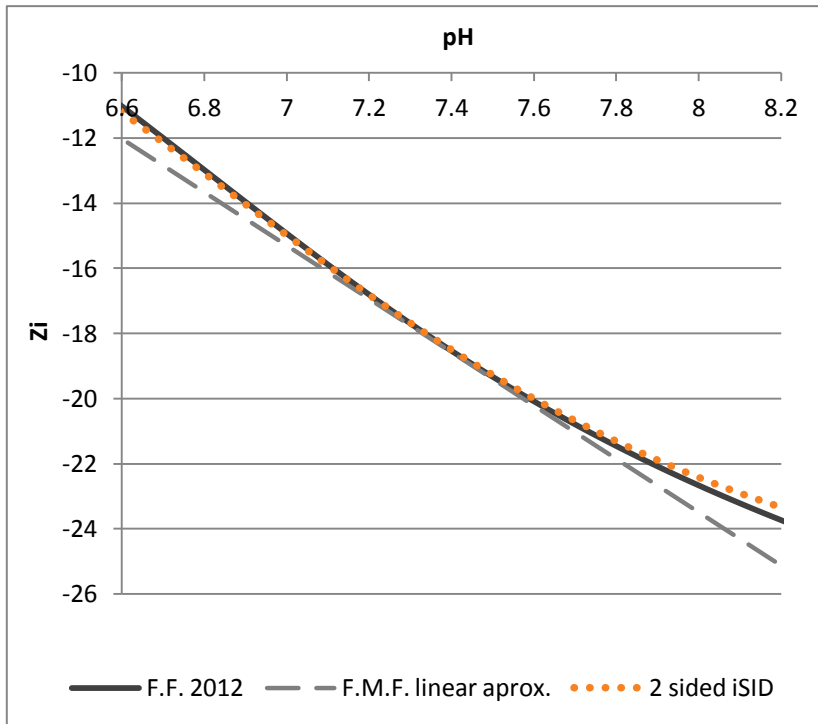
Figure 29: *Charge of albumin molecule as a function of pH according to the Figge-Fencl model version 3.0, published online in 2012 (black line), the linear approximation of the Figge-Mydosh-Fencl model, published in 1992 (gray dashed line) and 2 sided iSID model based on Figge-Fencl model version 3.0 – physiological range of pH. The iSID model follows the albumin buffering curve really well in this case. The formula of the linear approximation did not change in the model version 2.0 and 3.0, being equal to -66.55*(0.123*pH – 0.631).*

Electroneutrality in plasma, strong ion and buffer ion uncertainty

Electroneutrality has to be kept in any solution, including solutions where acid-base reactions take place. The solution of particular interest in physiology is blood plasma, where the general equation (2.36) takes following form

$$[Na^+] + [K^+] + [Ca^{++}] + [Mg^{++}] - [Cl^-] - [X^-] = [HCO_3^-] + [Alb^-] + [Glb^-] + [P_i^-] \qquad (2.43)$$

The terms [H⁺] and [OH⁻], representing free concentration of these ions, are omitted from the right hand side, because they are about four orders of magnitude lower than the other terms. However, they are still present implicitly, because their concentration influences charge of the buffers of the right hand side. Average physiological concentration of cations and anions in plasma is shown on figure 30, plotted in proportion.

The terms on the left hand side represent what is traditionally known as the strong ion difference, a term coined by Peter Stewart (13)

$$SID = [Na^+] + [K^+] + [Ca^{++}] + [Mg^{++}] - [Cl^-] - [X^-] \qquad (2.44)$$

SID includes species whose charge depends only of their concentrations and not of pH. However, in practice, *SID* is difficult to measure because it contains the [X⁻] term, standing for unknown anions.
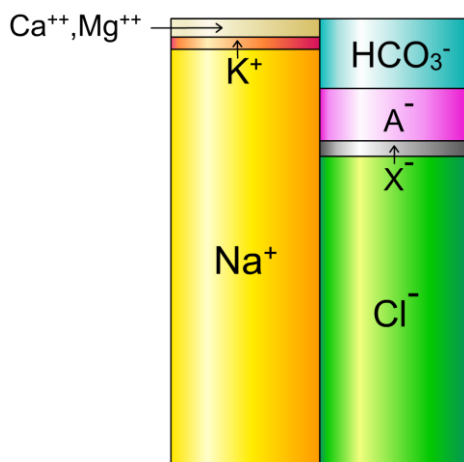

Figure 30: *Average charge of cations and anions in plasma, plotted in proportion. Due to the principle of electroneutrality, two columns have to equal one another. Most of the terms are standard chemical notation of elements and compounds, which the exception of [A⁻], term that signifies concentration of negative charge on albumin, (as well as globulins and phosphates) and [X-] that signifies the concentration of the negative charge on all other substances possibly present in serum (lactate, anions of keto-acids, etc.).*

These anions are generally difficult to measure as they include all the charged substances of plasma outside those explicitly mentioned in equation (2.43). Mentioning only the most important, we can include the most important inorganic anions of plasma such as SO_4^{2-} (normally about 1.5 meq/l), as well as the most important organic anions like lactate and less than 1 meq/l of lactate, fatty acid carboxylates, and keto-acid carboxylates (46). These organic anions already pose a theoretical problem: Although they are fully dissociated and can be considered strong anions according to the pH-pK$_A$ criterion, they can be also quite easily metabolized. As is shown in the figure 10 for the lactate example their metabolism leads to equivalent increase of HCO_3^- (or any other buffer base). This is why, when already looking at the result state after their metabolism, it is practical not to include lactate or similar substances among strong ions. This is exactly what was done by Carlesso et al in their study on the effects of lactate containing infusions of crystalloids on pH (39). However, on other occasions, such as in sepsis, where the increased concentrations of these anions are more long-lasting, their inclusion into strong anions is customary.

Normally X⁻ would also include charge of substances that are not strong ions at all, for instance some amino acids freely dissolved in plasma (see figure 21 for their pK_A's). In fact, getting into more and more detailed description, there should be two X⁻, one on the strong ion side of the equation (2.43) and the other one (smaller in size) on the buffer side (i.e. the right hand side).

This gets us to the buffer terms on the right hand side. The sum of these terms is traditionally known as plasma buffer base (*BB*)

$$BB = [HCO_3^-] + [Alb^-] + [Glb^-] + [P_i^-] \tag{2.45}$$

By far, the most important term here is bicarbonate, being followed by albumin. If the definitions are as clear cut as in (2.45) and (2.44), then the equation (2.43) effectively means same as

$$SID = BB \tag{2.46}$$

However, as for the term X⁻, which, on the close inspection, contained not only strong ions, the terms of the equations (2.45) also start to get more complicated and less clear when taking a closer look. One of the entities that might actually be questioned as a true charge of buffer base is the charge of albumin, as might be already anticipated from the previous section. Traditionally, albumin charge has been regarded as buffer charge, or a charge due to the base forms of amino acids. This would be in accordance with the original approach of Stewart. However, we have shown in the previous section that parts of the [Alb⁻] charge could be considered a fixed charge similar to *SID*. Furthermore, the buffer residues of albumin are mostly positively charged in their acid form. Hence, the value of Z_{max} of equation (2.40) is not going to be zero, meaning that, for albumin *SID* is not necessarily equal to *BB,* when the latter one is defined rigorously according to equation (1.86)). Table 10 shows some possible values of albumin Z_{Fix} (or iSID), Z_{Max} and *BB* at pH 7.4.

Table 10 clearly shows that the problem of agreeing upon what is buffer base and what is strong ion difference might not be as simple as it first appeared even when dealing with a simple molecule of known structure. The point I would like to make here is that there might be various opinions and none of them is necessarily wrong. Even the simplistic black-box approach can be clinically justified as sufficient and convenient, although the measured $\bar{z}_i$ is obviously not equal to albumin , although the other approaches seem more correct theoretically. The issue is going to be developed further when discussing problems of identifiability of Stewart's approach parameters.

	C_i	Z_{Fix}	iSID	Z_{Max}	$Z_{Max}*C_i$	$\bar{e}_i$	C_B / BB	$\bar{z}_{Alb}$	SID_{Alb}
Simplistic black-box model	1	-		-		18.5		-18.5	
	0.66		-		-		12.2		12.2
iSID model based on F.F. 3.0	1	-25		26		19.5		-18.5	
	0.66		-16.5		17.2		12.9		- 4.2
iSID model based on F.F. 3.0	1	-		1		19.5		-18.5	
	0.66		-		0.7		12.9		12.2
Figge-Fencl model version 3.0	1	-		100		118.5		-18.5	
	0.66		-		66		78.3		12.2

Table 10: *Possible values of SID and BB attributable to albumin at pH = 7.4 based on Figge-Fencl model version 3.0. First two rows describe the simplistic approach, which implicitly attributes all the negative charge on albumin to the negatively charged buffer base (i.e. base forms of buffer residues). This would be the most commonly used approach in clinical practice. As a black box model, it was the only possibility before the structure of albumin buffer was known. Second two rows are based on the 2 sided iSID model described in the previous section (figure 28). This model assigns the amino-acid residues of very high or very low pK_A strong ion behaviour, because their charge stays nearly constant at physiological range of pH. This charge is being equal to -25 per molecule. At average physiological concentration of albumin (0.66 mmol/l or 44 g/l), this is equivalent to internal SID charge of -16.5 mmol/l. Most of the buffer residues of albumin are histidine and low titrating lysine residues, whose acid form charge is +1. Thus, the Z_{max} of the whole molecule is 26; out of 27 buffer residues, average of 19.5 are in their deprotonated base form at pH = 7.4, leading to physiological C_B of 12.9 (eq.1.86). SID is different in this case, because it is calculated as external SID (= - $C_i * \bar{Z}_{Alb}$) plus the internal SID; Its meaning is the charge needed to oppose the charge of albumin buffer residues. Third two rows are calculated using the same model, only this time, the maximum charge of the buffer residues is taken together with the fixed charge of the "strong ion" residues as the maximum charge of albumin molecule. The last two rows are derived from the Figge-Fencl model, where all the residues on albumin are taken as buffer residues. Note that the equation (2.40) that relates BB, Z_{max} and SID stays valid for any decomposition.*

Other two non-bicarbonate buffers of plasma include globulins and phosphates. Globulins are a complex mixture of substances that can vary in concentration and charge. This makes accounting of individual amino acid residues impossible, together with any realistic description of their Z_{fix}, Z_{Max} and

BB. Simplistic black box approach does not seem to work well here either. Figge, Mydosh and Fencl's data show that the average charge of globulins is probably around zero (40), (47), but their buffer capacity has been measured by Siggaard-Andersen as significant (28), (6). Given the general theory of protein buffers, as was discussed on the albumin example, this is perfectly possible, when the sum of Z_{Max} and Z_{Fix} of the globulin mixture equals the value of C_B/BB (equation 1.40). However, according to the original Stewart's approach, zero charge of globulins would automatically mean they also have zero buffer capacity (equations 3.41 and 3.36).

Phosphates, their concentration generally being around 1 mmol/l, play a minor role in plasmatic buffering, both in terms of their buffer capacity and charge. Yet they receive extraordinary coverage in physiological acid-base literature, probably due to psychological reasons. Here, phosphoric acid - phoshate systems was also covered extensively as a suitable example for clarification of the general theory in chapter 1. In terms of strong ion charge and buffer charge, there are several possible approaches to the phosphate system. Either the whole average charge $\bar{Z}_i$ of phosphates is taken as a buffer charge, which is commonly done in literature, or the -1 charge of $H_2PO_4^-$ is taken as Z_{Max} (Z_{Fix}) of the second dissociation (figure 8) and the first dissociation is disregarded, its pK_A being too low to matter in physiology. This approach would be more according to the pH-pK_A criterion. When the -1 charge is treated as Z_{Max}, it constitutes part of the independent C_i*Z_{Max} term in the equation (2.40), one that causes a difference between *BB* and *SID*. Alternatively, it can be regarded as Z_{Fix} of the phosphate system and included into *iSID*. The last option was used in the article of Matousek et al (9), because the article worked with several simplifying assumptions, one of them being that $Z_{Max} = 0$.

Chapter 3

Comparison of the traditional and the modern approach

Chapter one covered the general concepts of acid-base chemistry, providing basis needed for mathematical description of complex solutions with known individual components. Its applicability is in any field both outside and inside physiology. Chapter two was concerned with those notions of acid-base chemistry pertinent to human physiology. These include special role of carbon dioxide – bicarbonate buffer, concept of linearization in describing buffering properties of proteins, relationship between charge of complex buffers and their buffering properties (i.e. relationship between BB and SID), uncertainty of defining the values of BB and SID in complex buffers and a typical form of electroneutrality equation in blood plasma.

Chapter three describes and compares two most popular approaches to the mathematical description of acid-base chemistry of plasma and blood; so called traditional approach, based mostly on the work of Ole Siggaard-Andersen and so called modern approach, based mostly on the work of Peter Stewart. First, both approaches are introduced, including historical perspectives of their development. Then, major weaknesses of one of the approaches are covered. This is needed in order to get a reunified description of plasma acid-base chemistry. Finally, detailed transformation between the variables and equations of the modern and the traditional approach is described in detail.

There is general notion that traditional approach is two dimensional, describing acid base disorders with two principal parameters (pCO2, BE), while modern approach is three dimensional (its principal parameters being pCO2, SID and A_{tot}). The modern approach is thus considered to provide extra information missed in the traditional approach. It is shown in this thesis that both approaches can be considered three dimensional; this is part of the mapping that exists between the approaches, i.e. there is a direct mathematical transformation from the parameters of one approach to the parameters of the other. The reason why the third parameter is generally not mentioned in the traditional approach is because it is not considered a clinically relevant acid-base parameter. However, existence of a transformation or a mapping between two descriptions does not necessarily mean that the information is structured equally well in terms of its usefulness for direct interpretation of patient data.

Introduction

This work uses the terms traditional approach for the concepts that come mainly from the work of Ole Siggaard-Andersen and the term modern approach for the concepts arising from the work of Peter Stewart. However, even this terminology could be considered controversial and misleading. Stewart used the title Modern quantitative acid-base chemistry for one of his articles (13), partially because his method required use of then not so common computers for calculations and partially because he probably saw some of the principles he proposed as new and revolutionary. Other authors also used the terms Stewart's approach, Stewart method, strong ion model or even physicochemical model for the concepts that reference themselves back to the original work of Stewart (20), (48), (17), (22), (21), (49). Consequently, the use of base excess and related concepts has often been called traditional approach ((50), (9), (4), (20)). This terminology has never been accepted by Ole Siggaard-Andersen and others, who see base excess centered diagnostics as fully adequate description of acid-base status and the use of strong ion difference as an anachronism. I do share their resentment about an alternative approach proclaiming itself as modern, which might indeed be perceived as bit of unjustified marketing. However, I believe that there is now an understanding in the scientific community that this is just a terminology without any necessary underlying meaning other then referring to the time when the major concepts were first published. Newer is not necessarily better. Since there are more contributors to the development and promulgation of each theory, I have chosen to adopt this terminology rather than calling each approach by the name of its main proponent.

As covered in chapter 2, the acid-base disturbances can be categorized solely according to the changes of the bicarbonate buffer parameters, i.e. using the Henderson-Hasselbalch equation. This divides acid-base disturbances into respiratory, where pCO_2 changes, and metabolic, where $[HCO_3^-]$ changes. This approach is still widely used for clinical diagnostics, for instance in the United States of America (30) and will be called bicarbonate centered approach here. Indeed, its accuracy can mostly be considered sufficient for clinical work, especially if non-bicarbonate parameters help to accurately diagnose the cause of the acid-base disturbance.

However, as was discussed in the section about mixture of bicarbonate and protein buffers (chapter 2), $[HCO_3^-]$ is not only reflective of metabolic disturbances, but also changes with variations of pCO_2 in human blood and plasma. This variation is caused by presence of non-bicarbonate buffers and can be as high as 15% in vivo. A parameter other then $[HCO_3^-]$ and independent of pCO_2 changes was

searched for; Singer and Hastings found it in 1948 (42) and called it total buffer base. The problem with total buffer base was that its normal values differed among different people, depending mainly on hemoglobin concentration or hematocrit. Thus, a parameter based on buffer base concept was designed by Peter Astrup et al and Siggaard-Andersen and Engels in the 1960 (51), (52), and called base excess (BE). Base excess was constructed such that its value would always be zero at physiological pH = 7.4. The traditional approach to the acid-base chemistry uses pCO_2 as a measure of uncompensated respiratory disturbance and BE as a measure of uncompensated metabolic disturbances. When compensation occurs (which is almost immediately in the case of metabolic disturbances), the other parameter changes as well, although the change of the primary disturbing parameter is still more pronounced. For instance: The primary disturbing influence is characterized by high pCO_2 in acute respiratory acidosis; unlike $[HCO_3^-]$, which increases immediately due to buffer redistribution, BE stays constant first. If the disturbance lasts for more than 2 days, renal compensation develops, causing a gradual increase of BE and further increase of $[HCO_3^-]$, returning pH closer to norm. Nevertheless, the most pronounced change (in proportion to the normal value) is the original disturbance, i.e. increase of pCO_2.

 When the so called modern approach of Peter Stewart (53), (54), (53) emerged in the 1980's, it has come up with three so called independent variables, pCO_2, strong ion difference (SID) and total concentration of weak acid (i.e. buffer) (A_{tot}). The SID is often considered equivalent to buffer base (BB), parameter from which BE was derived, thus being considered a measure analogous to the metabolic disturbances of the traditional approach. A_{tot} is a new variable. Proponents of the modern approach claim that one important acid-base parameter (A_{tot}) is missed in the traditional approach and that it is an improvement in terms of its explicit representation of the link between electrolyte and acid-base physiology.

Classical approach of Ole Siggaard-Andersen

Ole Siggaard-Andersen has worked in the field of acid-base chemistry since early 1960's. In the 1970's, he has compiled and enriched the existing knowledge in a monumental monograph The acid-base status of the blood (28), originally his doctoral thesis. This has been amended subsequently (55), (6).

The key term in the diagnostics of metabolic disturbances of acid-base chemistry is so called base excess (BE) - a parameter that generally changes in the same direction as bicarbonate. Base excess is

defined as the amount of strong acid, needed to titrate 1 liter of solution (blood, plasma, extracellular fluid) back to pH = 7.4, when pCO_2 is moved back to the normal value 40 mmHg (5.3 kPa). When we need strong base to titrate back to 7.4 (i.e. pH was originally acidotic), the BE is negative. At pH = 7.4 (and pCO_2 equal to 5.3 kPa), BE is zero by definition.

As already mentioned, BE is a parameter derived from the concentration of buffer base (BB). The definition relationship (52) with regard to buffer base can be set as

$$BE \overset{\text{def}}{=} BB - BB_{\text{pHn}}$$ (3.1)

Where BB is total concentration of buffer base and BB_{pHn} is the normal concentration of buffer base as it is understood in the traditional approach, i.e. its concentration at pH = 7.4 and pCO2 = 40 mmHg = 5.3 kPa.

$$BB_{\text{pHn}} = BB|_{\text{pH=7.4}, \text{pCO}_2\text{=5.3 kPa}}$$ (3.2)

BB_{pHn} can differ between different blood plasmas (or full bloods) being dependent on the total non-bicarbonate buffer concentration of the particular plasma. Thus, BB_{pHn} of the eq. 3.1 is that of the same blood as BB of the eq. 3.1. The only difference is that it has been brought to pCO_2 = 40 mmHg and titrated back to pH = 7.4 by addition of strong acid or base, i.e. without changing total concentration of non-bicarbonate buffers.
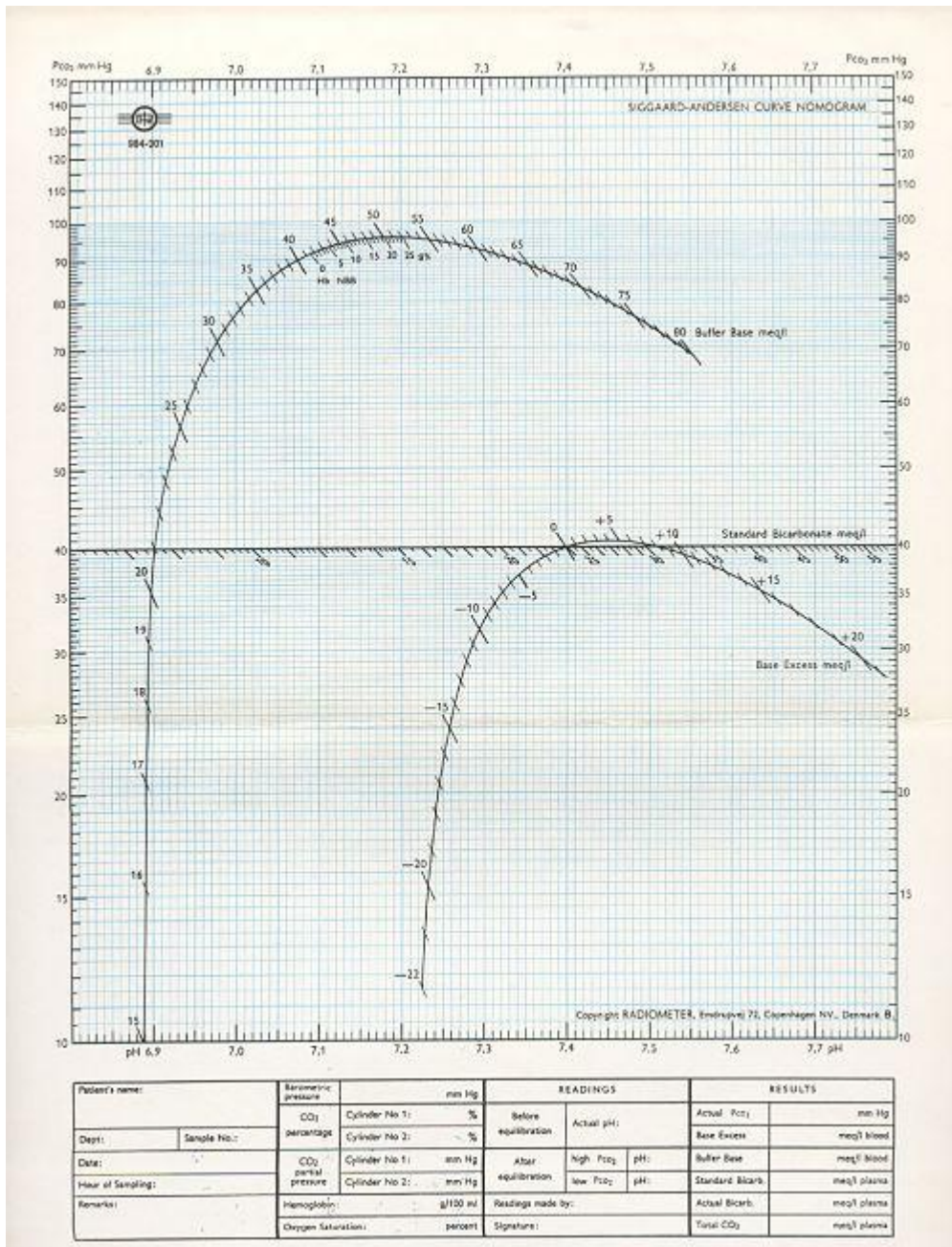
Figure 31: Original Siggaard-Andersen nomogram. The nomogram uses pH, pCO₂ coordinate system, with the scale of pCO₂ being logarithmic. It was used for diagnostics of acid-base disorders before widespread use of personal computers. When CO₂ is varied, blood moves on an (almost) straight line from upper-left corner to the lower right corner. This line is also called CO₂ titration line. The slopes of bloods with various hemoglobin concentration (various values of β_{NonB}) are slightly different. However, all the lines intersect at one point on the base excess curve (lower right), where the value of base excess can be read.

Obviously, the definition presented in the text and the one in equation (3.1) are connected: Titration by X mmol of strong acid per 1 liter of resulting solution decreases the concentration of buffer base

by X mmol/l (relationship analogical to eq. 1.31). When we need to add X mmol/l of acid to titrate back to 7.4, this also means that BB was originally by X mmol/l higher than normal.

Originally, before the widespread use of personal computers, the classical theory was used in form of so called Siggaard-Andersen nomogram (Figure 31). The nomogram was based on a set of precisely measured acid-base data yielded from blood samples of 4 Danish individuals. In order to be used in clinical practice, it required measurement of pH, pCO_2 and the concentration of hemoglobin (Hb); given these inputs[6], it permitted graphical reading of bicarbonate, buffer base and base excess.

In late 1970's, the development of the computational technology has lead Siggaard-Andersen to formulate so called van Slyke equation (24), which can be used, together with Henderson-Hasselbalch equation, as a substitute for the nomogram. Unlike Henderson-Hasselbalch equation, it includes both CO_2 – HCO_3^- buffer and non-bicarbonate buffers. Van Slyke equation was originally published for the whole blood and non-bicarbonate buffer capacity β depended only on concentration of hemoglobin; less important plasma buffer capacity was considered constant, equal to 7.7 mmol/l (it normally constitutes about 25% of blood non-bicarbonate buffering and about 9% of blood buffer capacity, open bicarbonate buffer being considered as well). Later, a version of van Slyke equation with variable plasma was published as well (6). Van Slyke equation for the whole blood will be discussed later in this chapter. Van Slyke equation for the plasma is quite straightforward and can be considered a direct consequence of the definition equation of the base excess (BE) and the equation (2.16) of chapter two, that essentially divides buffer base into bicarbonate and linearized non-bicarbonate buffers

$$BB = [HCO_3^-] + NBB_{NonB} + \beta_{NonB} * \Delta pH \qquad (3.3)$$

NBB_{NonB} is the normal level of buffer base concentration for non-bicarbonate buffers, generally taken as value at pH = 7.4 and pCO_2=40mmHg (although equation (2.16) was derived for any linearization point). Value of NBB_{NonB} depends on plasma protein and other buffer concentration as seen in equation (2.14); β_{NonB} is the buffer value of non-bicarbonate buffers, it also depends on plasma protein and other buffer concentration as seen in equation (2.15); ΔpH is the change of pH from normal value 7.4.

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[6] In fact, the Siggard-Andersen nomogram was a handy tool even before the widespread use of pCO_2 electrode that came in the 1960's. First, pH of the original blood sample was measured. Then the sample was equilibrated with gas of known pCO_2, let's say 80 mmHg (10.7 kPa) and pH1 was measured. Then the sample was equilibrated once more with a gas of different known pCO_2, let's say 20 mmHg (2.7 kPa), and pH2 was measured. The two points of known pCO2 and known pH (i.e. pH1 and pH2) were connected with a line on the nomogram and the point of original pH was found on that line. Original pCO_2 was now read as point's ordinate.

Essentially same equation holds for the value of total normal buffer base (N*BB*), except that ΔpH is now 0 (eq. 3.2) and the last term disappears

$$BB_{\text{pHn}} = [HCO_3^-]_N + NBB_{NonB} \tag{3.4}$$

Normal concentration of bicarbonate, $[HCO_3^-]_N$, is fully determined by Henderson-Hasselbalch equation (2.2) at pH = 7.4 and pCO_2 = 40 mmHg. Thus, it is same even in different blood or plasma samples, being equal to 24.4 mmol/l. BE can now be expressed by substituting equations (3.3) and (3.4) into the definition equation (3.1)

$$BE = BB - BB_{pHn} = ([HCO_3^-] - [HCO_3^-]_N) + (\cancel{NBB_{NonB}} - \cancel{NBB_{NonB}}) + \beta_{NonB} * \Delta pH \tag{3.5}$$

The N*BB*$_{NonB}$ terms are equal and cancel out, because plasma from equations (3.4) and (3.3) has same concentration of buffers, i.e. equation (2.14) contains same substances in same concentrations. The only difference between (3.4) and (3.3) is the added strong acid or strong base. Last formula can be written more concisely using Δ symbol for difference and omitting the subscript *NonB* (in physiology, beta without subscript generally denotes buffer capacity of non-bicarbonate buffers)

$$BE = \Delta HCO_3^- + \beta * \Delta pH \tag{3.6}$$

This formulation of the van Slyke equation is very similar to the form, in which the equation was published by Siggaard-Andersen[7] (6). In numerical values, this is same as

$$BE = (HCO_3^- - 24.4) + \beta * (pH - 7.4) \tag{3.7}$$

General theoretical treatment of non-bicarbonate buffer capacity β was performed in chapter 2 (equation 2.10 and 2.15). Here, it suffices to say that for practical purposes, all proteins other then albumin are collated into the term globulin and considered together. Phosphate buffering is also linearized, which is not completely correct theoretically, but given its minor contribution and relatively narrow range of life compatible pH (6.9-7.8), the difference to the true behaviour of the whole is practically negligible. Plasmatic β can be computed as

$$\beta_P = C_{Alb} * \beta_{M(Alb)} + C_{W,Glb} * \beta_{W(Glb)} + C_P * \beta_{M(P)} \tag{3.8}$$

, where C_{Alb} and C_P are substance (molar) concentrations of albumin and phosphates in mmol/l, normal concentration being 0.66 and 1, respectively. $C_{W,Glb}$ is mass concentration of globulins in g/l, norm being 26 − 46 g/l. β_M are molar buffer values, as defined in the equation (2.10). Siggaard-Andersen and Fogh-Andersen (6) give values 8.0 for $\beta_{M(Alb)}$ and 0.309 for $\beta_{M(Pi)}$. Same source gives value of 0.075 mmol/g for $\beta_{W(Glb)}$, average mass buffer value of globulins. Since concentration of

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[7] In the recent years, Siggaard-Andersen has switched to advocating use of of net titratable hydrogen ion concentration (ΔctH^+) instead of *BE,* where ΔctH^+ = - *BE.* I do not consider this change well thought through, as will yet be discussed in this chapter.

albumin is more commonly given in g/l, it might be handy to rewrite formula (3.8) in the following form

$$\beta_P = C_{W,Alb} * \beta_{W(Alb)} + C_{W,Glb} * \beta_{W(Glb)} + C_P * \beta_{M(P)} \tag{3.9}$$

$C_{W,Alb}$ is mass concentration of albumin, its normal value being around 44 g/l. $\beta_{W(Alb)}$ is mass buffer value of albumin, where Figge et al (40) give value of 0.123 mmol/g and recalculation from Siggaard-Andersen et al. (6) value of 0.120 mmol/g.

Generally speaking, 3 different values of *BE* can be used depending on the value of β. If plasmatic β is used, resulting base excess is the base excess of plasma. If we add apparent β of non-bicarbonate buffers in red blood cells (often mentioned just as β of hemoglobin), resulting base excess is that of full blood. In this case, we need to multiply terms of equation (3.6) by correction term accounting for uneven distribution of bicarbonate between plasma and erythrocytes. This model explains well large variations in [HCO$_3^-$] seen when pCO$_2$ is varied for the whole blood in vitro. However, smaller variations in [HCO$_3^-$] are seen when pCO$_2$ is varied in vivo. It was assumed that one should consider blood together with extracellular fluid as one system in vivo. Buffer value of extracellular fluid is similar to plasma, but larger volume results in smaller contribution of erythrocytes with their large β of hemoglobin. When base excess is calculated with β of plasma plus 1/3 of erythrocyte contribution, resulting smaller value of β leads to good predictions of [HCO$_3^-$] variations in vivo (these variations are proportional to β). This last base excess is called BE of extracellular fluid or standard base excess.

As already mentioned, disorders of acid-base chemistry are divided into metabolic and respiratory in the classical approach, which is similar to the simple use of Henderson-Hasselbalch equation. Respiratory disturbance can be divided to either acute or compensated. Compensation of metabolic disturbances develops fast and it is thus difficult to see uncompensated (acute) metabolic disturbance. Acute disorders only have one parameter changed, which is pCO$_2$ in case of respiratory disorders and (standard) BE in case of metabolic disorders. In compensated disorders, the situation is more complex and both pCO2 and BE change. Compensatory diagrams (figure 11) similar to the simple use of bicarbonate buffer were published to aid the diagnosis in these cases (56).

 Traditional approach does not place much emphasis on the link between electrolyte and acid-base physiology, although this link is definitely recognized. A parameter known as Anion gap (AG) can be used to differentiate between various causes of metabolic acidosis, which can be divided into those with normal and those with elevated AG. AG should always be corrected for abnormal values of albumin. However, at the time when the traditional approach was conceived, role of albumin levels in normal/abnormal values of AG was not yet appreciated and the corrections for abnormal levels of

albumin were suggested only later by authors associated with the modern approach (57). Also, authors of the traditional approach never completely appreciated that the values of buffer base (BB) equal to the strong ion difference (SID) only as a simplification (equation (2.46) vs. (2.40)). A more correct treatise distinguishing the two was published by Wren Wooten (7) and covered also in chapter 2. It should be noted, though, that this omission does not cause any discrepancy in the formulation of the traditional approach itself, as the approach is buffer centered rather than strong ion centered. It should only be considered when comparing the two approaches together.

Modern approach of Peter Stewart

The basis of this approach was formulated by Peter Stewart in late seventies and early eighties (53), (54), (13). Peter Stewart was a Canadian physiologist, who tried to simplify the generally used acid-base theory based on the underlying chemistry. Indeed, Stewart's formulation of acid-base equations is much closer to the physico-chemical structure of the problem than that of the traditional approach and the texts are generally nice to follow even without special mathematical (calculus) knowledge. However, not all notions of the modern approach can be agreed on. For instance Stewart's dislike of logarithms seems more a matter of personal taste than a well substantiated opinion. Furthermore, the simplification that Stewart brought about was often overdone, as will be shown in this chapter.

According to Stewart, the value of pH of plasma (or blood) depends solely on the values of three so called independent variables: pCO_2, strong ion difference (*SID*), and so called total concentration of weak acid A_{tot}. pCO_2 is used the same way as in the traditional approach or bicarbonate centered approach. Strong ion difference (SID) is defined according to the equation (2.32). For plasma, *SID* is generally taken to contain the terms listed in equation (2.44).

Stewart's term weak acid comes from Arrhenius definition of acids (chapter 1, page 7). Since the term A_{tot} includes substance concentration of both acid and base forms, a more suitable name would be total concentration of non-bicarbonate buffer. See pages 7 to 14 in chapter 1 for a more detailed discussion of the terminology and properties of buffers. In the original work of Stewart, all non-bicarbonate buffers are collated and treated as single K_A system. In this context, it is interesting to note that effectively, modern approach divides the buffering in biological fluid into bicarbonate and non-bicarbonate buffer contribution, just as the traditional approach does.

Modern approach is typically used only for blood plasma, but it has been broadened by several authors to include whole blood as well (58), (59), (60), (61). The last author has also provided

explanation to the variations of $[HCO_3^-]$ seen when pCO_2 is varied in vivo, similar to that provided by standard base excess.

There are 6 original equations of Stewart, which constitute a set with 4 constants (K_w, K_A, K_C and K_3) and 3 independent variables (pCO_2, A_{tot} and SID). When the values of these parameters are known, the system can be solved for 6 remaining unknowns, also known as dependent variables ($[A^-]$, $[HA]$, $[HCO_3^-]$, $[CO_3^{2-}]$, $[H^+]$ and $[OH^-]$). Out of these 6, $[H^+]$ is of course the most important, being a measure by which pH is defined (eq. 1.5). The equations of Stewart approach are given in figure 32.

Stewart's original model

Reaction Equations

$H^+ + OH^- \rightleftarrows H_2O$

$H^+ + A^- \rightleftarrows HA$

$H^+ + HCO_3^- \rightleftarrows H_2O + CO_2$

$H^+ + CO_3^{2-} \rightleftarrows HCO_3^-$

Mathematical Representation

1) $[H^+] * [OH^-] = K_W$

2) $[H^+] * [A^-] = K_A * [HA]$

3) $[A^-] + [HA] = A_{tot}$

4) $[H^+] * [HCO_3^-] = K_C * pCO_2$

5) $[H^+] * [CO_3^{2-}] = K_3 * [HCO_3^-]$

6) $SID + [H^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] = 0$

Simplified SID model

Reaction Equations

$H^+ + A^- \rightleftarrows HA$

$H^+ + HCO_3^- \rightleftarrows H_2O + CO_2$

Mathematical Representation

1) $[H^+] * [A^-] = K_A * [HA]$

2) $[A^-] + [HA] = A_{tot}$

3) $[H^+] * [HCO_3^-] = K_C * pCO_2$

4) $SID - [HCO_3^-] - [A^-] = 0$

Figure 32: Stewart's model of plasma acid base chemistry and simplified SID model, as first proposed by Constable (2). Terms shown in grey are not included in the simplified model. Solving the remaining equations without them does not cause any appreciable change to the resulting values, because the omitted terms are several orders of magnitude smaller than the rest. K_w is ionic product of water, K_A dissociation constant of non-bicarbonate buffers, K_C dissociation constant of bicarbonate and K_3 dissociation constant of carbonate.

These six equations can be solved as a system using numerical computer methods. Alternatively, [H⁺] can be expressed as a function of the independent variables by substituting into the last equation. This gives us 4ᵗʰ order polynomial

$$[H^+]^4 + (SID + K_A) * [H^+]^3 + (K_A * (SID - A_{tot}) - K_w - K_C * pCO_2) * [H^+]^2$$
$$- (K_A * (K_w + K_C * pCO_2) - K_3 K_C pCO_2) * [H^+] - K_A K_C K_3 pCO_2 = 0$$

(3.10)

This polynomial has to be solved numerically with use of computer methods as well, offering no true advantage to the equation system given in figure 32. However, it can be used for the same purpose as the equation (1.83) and (1.84) of chapter 1 and its mentioning has been popular in relevant literature.

Six equations of Stewart's original model can simplify to four equations without adversely affecting precision as follows. Equation (St. 1), concerning ionic product of water can be dropped completely, if we do not wish to know the concentration of OH⁻ as well (which we generally do not need). Equation (St. 4) describing mass action of bicarbonate – carbonate buffer can be dropped as well, because the pK_A of the bicarbonate-carbonate buffer equals about 10.3 (2) and thus, almost 99.9% is in the bicarbonate form at pH = 7.4. At this pH, the concentration of carbonate equals about 0.03 mmol/l. Given the imprecision of collating all non-bicarbonate buffers under one parameter A_{tot} (further discussed in [ref our article] and in the following section), the inclusion or exclusion of carbonate buffer is not going to make virtually any difference.

Finally, we can drop off some terms of the electroneutrality equation. Since the plasmatic pH is always close to neutral, concentrations of both H⁺ and OH⁻ are going to be several orders of magnitude lower (tenths of μmol/l vs. tens of mmol/l) then the remaining terms of the equation, whose imprecision of measurement is often in mmol/l. Thus, their inclusion, which would make numerical sense in very acidic (for H⁺) or very alkalotic (for OH⁻) environment, makes virtually no difference in the physiological range. The term [CO_3^{2-}], although being about 700 times higher than [H⁺] and 40 times higher than [OH⁻], is still 3 orders of magnitude lower than the remaining terms SID, [A⁻] and [HCO_3^-] and given the imprecision of assessment of SID and [A⁻], it can be omitted as well. Once the model has been reduced to 4 equations, it only yields four dependent variables that can be calculated, these variables being [A⁻],[HA],[HCO_3^-] and [H⁺], the last one acidity being measure of acidity analogous to pH. This simplification was first suggested by Constable (2), who also showed by carrying out the calculation in various cases that the difference between the original and simplified model is negligible. Constable also showed that when the original Stewart's model is solved for pH, the resulting solution is totally insensible to the variations of K_3 and K_w (being as large as ± 100% of the original value) (48), again pointing out to the fact that the two respective equations can be

dropped off completely. The resulting simplified model is comprised of a set of four equations. These equations can be, again, combined to solve for [H⁺], leading to a second order polynomial which, unlike fourth order polynomial, can be easily solved analytically.

It is interesting to note the form the electroneutrality equation (St.6) takes, once the terms [H⁺], [OH⁻] and [CO₃²⁻] are omitted

$$SID - A^- - HCO_3^- = 0 \tag{3.11}$$

This can be rearranged as

$$SID = A^- + HCO_3^- \tag{3.12}$$

SID on the left hand side is generally calculated according to the equation (2.44). The right hand side includes buffer base bicarbonate and the base form of all non-bicarbonate buffers considered together. Since there are no other bases considered to be present in the solution (13), it is equal to the total buffer base (BB = C_B) of eq. (1.34), i.e.

$$BB \stackrel{\text{def}}{=} [A^-] + [HCO_3^-] \tag{3.13}$$

The last two equations mean that in Stewart's original model

$$SID = BB \tag{3.14}$$

Section concerned with the relationship between buffering, charge of multivalent species and strong ions in chapter 2 dealt with the relationship between buffer base and strong ion difference in detail, leading to the conclusion (eq. 2.40) that generally

$$SID = BB - \sum_i C_i * \bar{z}_{\max\,(i)} \tag{3.15}$$

This shows that Stewart's theory is only simplified description of reality. In case of Stewart's original formulation, there are only two types of buffers considered: Carbon dioxide – bicarbonate buffer, whose maximum charge is zero and non-bicarbonate buffers that are considered to have two forms only, acid form HA and base form A⁻; maximum charge of a buffer that is defined this way is also zero. Substituting these values of $\bar{z}_{\max\,(i)}$ into equation (2.40 /3.15) gives

$$SID = BB + 0 \tag{3.16}$$

, which explains equation (3.14). There is a version of Stewart's model published by Watson, which considers the maximum charge on albumin (main plasma protein) higher than zero (20) leading to a more realistic model. Watson's model of albumin is covered in chapter 2 (figure 23 and 25).

In order to calculate values of the four dependent variables, one has to know the values of two dissociation constant and 3 independent variables: Dissociation constant of bicarbonate is given as K_c = $2.46*10^{-11}(Eq/L)^2$/mmHg by Stewart (13), which is basically identical to what can be obtained from the values reported by Siggaard-Andersen based on experimental data (given at equation 2.2). The value of the dissociation constant of non-bicarbonate buffers K_A has never been completely agreed on. Stewart originally used several values, most commonly $3.0*10^{-7}$. Table 11 gives summary of values used in various sources. Situation is similar for the normal values of independent variables. Only value that has been completely agreed on in the value of pCO_2 this parameter being shared with the traditional approach. Normal values of SID and A_{tot} were never completely agreed on. Reported values are again given in table 11.

		SID	*A_{tot}*	*K_A*	*pK_A*
1	*Stewart* (13)	42	19	$3*10^{-7}$	6.52
2	*Stewart* (53), (54)			$0.4*10^{-7}$	7.40
3				$2*10^{-7}$	6.70
4				$4*10^{-7}$	6.40
5	*Wilkes* (62)		17	$3*10^{-7}$	6.52
6	**Constable** (2)		20	$3*10^{-7}$	6.52
7	**Constable** (48)	41.7	24.1	$1.05*10^{-7}$	6.98
8	**Staempfli** (63)	46	23.3	$2.27*10^{-7}$	6.64
9		37.1	17.2	$0.80*10^{-7}$	7.10
10	**Fencl** (17)	39			
11	**Staempfli (Figge)** (63)		24.0	$0.42*10^{-7}$	7.38
12	**Matousek** (9)	46.3	20.5	$1.65*10^{-7}$	6.78

Table 11: Values assigned to K_A and pK_A of the modern approach by various authors, together with normal reported values of SID and A_{tot}. Only the first author is given in the left collumn. In case of Fencl et al (row 10), the value of SID was calculated as BB. Staempfli's values are based on data of Figge et al.

Clinical diagnostic system based on the modern approach was published by Fencl et al (17). It divides acid-base disturbances by the changes of the three independent variables and assigns clinical causes to each of them. This diagnostic system, taken directly from Fencl et al, is given in table 12.

CLASSIFICATION OF PRIMARY ACID-BASE DISTURBANCES

	Acidosis	Alkalosis
I. Respiratory	$\uparrow$ P_{CO_2}	$\downarrow$ P_{CO_2}
II. Nonrespiratory (metabolic)		
1. Abnormal SID		
a. Water excess/deficit	$\downarrow$ SID, $\downarrow$ [Na$^+$]	$\uparrow$ SID, $\uparrow$ [Na$^+$]
b. Imbalance of strong anions		
i. Chloride excess/ deficit	$\downarrow$ SID, $\uparrow$ [Cl$^-$]	$\uparrow$ SID, $\downarrow$ [Cl$^-$]
ii. Unidentified anion excess	$\downarrow$ SID, $\uparrow$ [XA$^-$]	——
2.Nonvolatile weak acids		
a. Serum albumin	$\uparrow$ [Alb][&]	$\downarrow$ [Alb]
b. Inorganic phosphate	$\uparrow$ [Pi]	$\downarrow$ [Pi][‖]

Table 12: *Classification of acid-base disturbances according to Fencl, Jabor, Kazda and Figge* (17). *The acid-base disturbances are divided into three major groups, based on the three independent variables of the modern approach: pCO_2 SID and A_{tot}. SID can change due to sodium, chloride or unidentified anions. Changes in plasma weak acid (buffer) concentration are not called changes in A_{tot}, because these authors use linearized approximation of buffering (see next section for details). However, the concept clearly originates in Stewart's idea of A_{tot} causing acid-base disturbances.*

The link between electrolyte and acid-base physiology is emphasized in the modern approach. Hence, it is not surprising that the authors of modern approach contributed to the progress in identifying various factors that play role in the electroneutrality equation for plasma. Several improvements in the clinical assessment of so called unknown anions (X$^-$) (figure 30) were published. First, bed-side correction of the values of anion gap for abnormal values of albumin was published by Figge, Jabor, Kazda and Fencl (64). Later, even more precise formulas were published to get the value of [X$^-$] directly (17), (40). The value of [X$^-$] has also been called strong ion gap (SIG) (18), (9).

Weaknesses of the modern approach

This section is concerned with the weaknesses of the modern approach. Although it is always nicer to focus on the positive side of things, only weak points are covered in this section. My wish is to reunite the description of acid-base physiology; showing only the common features and links between the two approaches might not be enough for this purpose. Potential pitfalls and

shortcomings need to be highlighted as well. Strong points and progress in our understanding that has sprung from the modern approach are going to be discussed in the last chapter.

There are several weak points in the modern approach as it is commonly understood and implemented into the clinical practice. These weaknesses include inability to describe linear buffering of proteins, concept of independent and dependent variables as its implications, lack of compensation diagrams that would aid diagnosis and finally approach's problem with identifiability of model parameters. These weaknesses will now be considered in turn.

First weak point is connected to the fact that most non-bicarbonate buffers in plasma, whole blood and extracellular fluid are proteins. These proteins include albumin and globulins in plasma, hemoglobin in erythrocytes and various constitutional proteins of extracellular fluid. As has been shown for albumin (65), (40), globulins, whole plasma (66) and hemoglobin (28), when titration curve of these proteins is observed over pH range of interest (e.g. middle value ± 1), it is fairly close to straight line. This can also be expressed in terms of buffer being fairly constant over the range of interest. This fairly constant value of β generally cannot be approximated well by Stewart's single pK_A buffer, except when the pH range is very narrow (e.g. ±0.3). This is because β of single pK_A buffer varies substantially with changes of pH, as is apparent from figures 3 and 4 and table 3 in chapter 1. The only area where β is relatively constant (only -25% at each end) is in the pH interval (pK_A – 0.5) to (pK_A + 0.5). Still, the process of fitting Stewart's model parameters often leads to pK_A value estimates outside this range (table 11). The reason for this rather unsatisfactory parameter estimation is going to be looked at in more detail in the next section.

Linear or almost linear titration curves of protein buffers might seem surprising at first. This behaviour is connected to the fact that pK_A's of individual buffer residues are scattered around their average value by the influence of the surrounding protein moieties. This scattering essentially behaves as a random number generator, distributing pK_A's evenly in the pH range of interest. Titration curves of a random set of 10 buffer residues were modeled in chapter 2, section about linearization in describing titration curve of protein buffers, showing that linear approximation is generally much more suitable then single pK_A approximation.

It might be interesting to ask why the rather unsatisfactory behaviour of single pK_A buffer hasn't been addressed in literature so far. Some authors of the modern approach have probably become aware of it and used linear approximation instead of the single pK_A model, but without giving the issue much discussion (40), (17). For others, unsatisfactory behaviour of single pK_A model of plasma non-bicarbonate buffers became covered by dominant influence of bicarbonate. This deserves a bit of discussion: Carbon dioxide – bicarbonate buffer system is a single pK_A system and thus, its buffer

capacity changes significantly with pH, producing a bent titration curve. Furthermore, there is a huge pH dependent difference between buffer capacity of closed bicarbonate buffer system and open bicarbonate buffer system (one where pCO_2 is maintained at constant level). For instance, at pH = 7.4, buffer capacity of closed bicarbonate system can be calculated using equation (1.20), where normal concentration of CO_2 is calculated using its solubility 0.0306 mmol/l/mmHg. This gives us buffer value equal to 2.7 mmol/l. In case of open bicarbonate system, the buffer value has to be calculated using the definition equation of β (1.12). We substitute in C_B = [HCO_3^-], where [HCO_3^-] is expressed from the equation (2.2) and pCO_2 is taken as a constant (open buffer). When the derivative is taken, we get a following relationship

$$\beta_{Bic,open} = 2.303 * [HCO_3^-]$$ (3.17)

Given that normal concentration of bicarbonate is 24.4 mmol/l, buffer capacity of open bicarbonate buffer at pH = 7.4 is equal to 56 mmol/l. This is more than 20 times higher than the buffer capacity of closed bicarbonate buffer system. This value is also much higher than the buffer values of plasmatic non-bicarbonate buffers, which has been reported as 7.7 mmol/l (6), or the buffer value of albumin at physiological concentration 44 g/l, which has been reported as 5.4 (40).
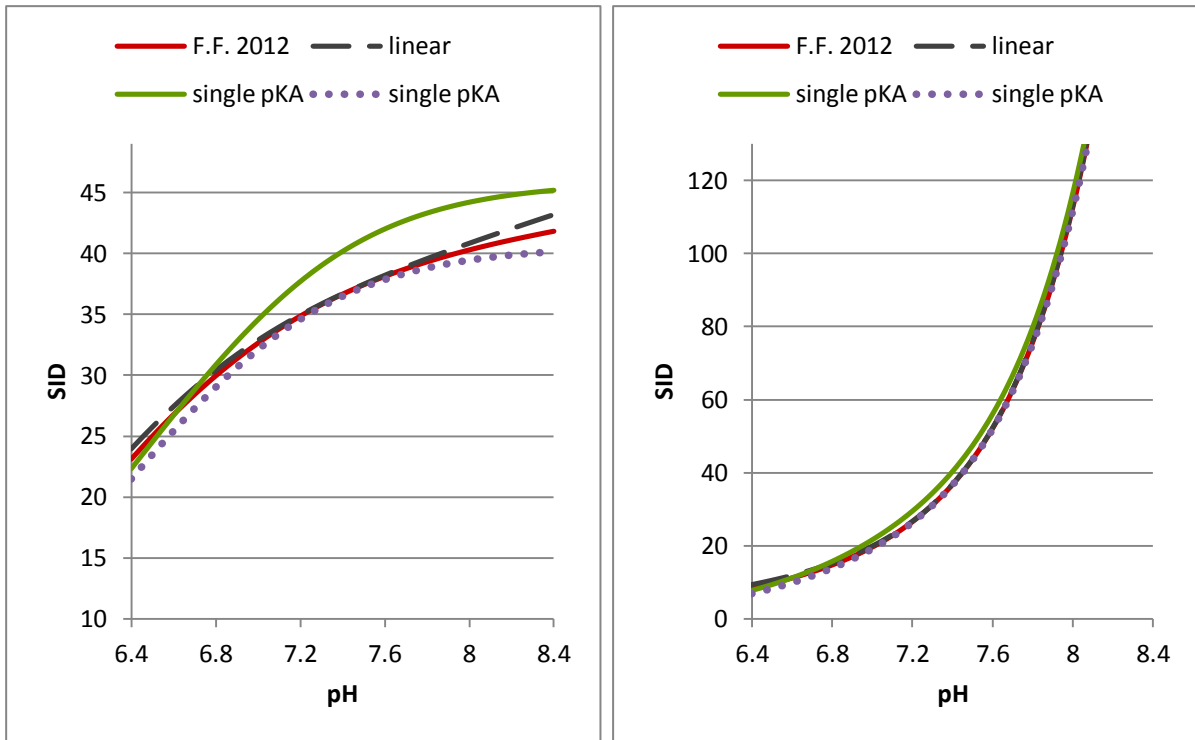
Figures 33 to 36 show titration curves of albumin either with or without bicarbonate buffer. Four models of albumin are presented. Figge-Fencl model of albumin charge and buffering, version 3.0 (43) (reference), linear approximation of the previous model suggested by Figge, Mydosh and Fencl ((Alb^-) = $C_{w,Alb}$*(0.123*pH-0.631)) (40), single K_A approach with values of K_A and A_{tot} determined by Constable (48), based on the data of Figge, Rossing and Fencl (65) and single K_A approach with values of K_A and A_{tot} determined by Matousek et al (9). Although the models were derived from a same data set, single pK_A model of Constable does not show very good fit, as is apparent when the titration curve of albumin is plotted alone (figures 33 and 34). This is due to the large uncertainty in indentifying the value of A_{tot} per gram of albumin and pK_A, as was reported by Constable. Constable reports the value of A_{tot} as 4.60*[albumin](g/dl), yet the 95% confidence interval for the value is 3.20 – 10.00. The value of K_A is determined as $1.40*10^{-7}$, with the confidence interval being $0.50 – 3.14 * 10^{-7}$. Also Constable's values were determined in a solution containing phosphate, which might add to the buffer capacity of albumin and SID was calculated from measured strong ion concentrations, which might cause disturbances, as discussed further. The values K_A and A_{tot} reported by Matousek et al were calculated from the known charge and buffer value of albumin at pH = 7.4 in such a way, as to assure the best possible fit around 7.4. These authors determined K_A= $1.65*10^{-7}$ and A_{tot} = 15.0 mmol/l (at albumin concentration 4.4 g/dl), which would give value of A_{tot} equal to 3.4 per g/dl of

albumin. It should be noted that calculating model parameters at other pH would give different values of K_A and A_{tot} per g/dl of albumin.

Figures 35 and 36 show how the differences in behaviour of different models become apparently smaller, when we have a mixture of albumin, strong ions, CO_2 and bicarbonate. In the case of closed bicarbonate buffer, the differences are still clear even though smaller than in case of pure albumin. In case of open bicarbonate buffer, the same differences become barely perceptible.
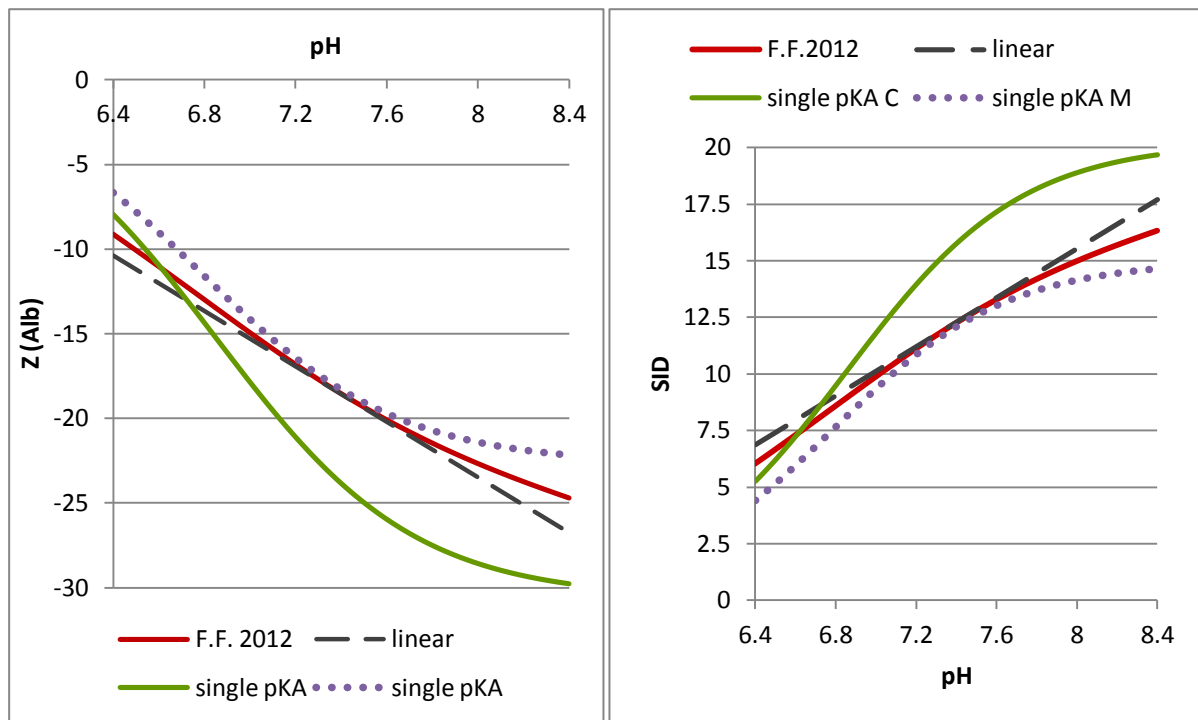
Figures 33 and 34: *Titration curves of four models of albumin plotted over the pH range of physiological interest. Red curve is the Figge-Fencl model version 3.0, which is considered reference model and has been fitted to data. The black dashed line is the linear approximation suggested by Figge, Mydosh and Fencl. Green curve is plotted according to the single pK_A version of the modern approach with parameters determined by Constable to give best possible fit for albumin filtrate and its measured SID. Purple dotted line is the single pK_A version of the modern approach with parameters determined by Matousek et al to give best possible fit to published albumin titration curve around pH = 7.4. For the relationship between average charge of albumin molecule Z (Alb) and SID due to charge of albumin, see equation (2.36). The right graph shows the SID due to albumin at physiological concentration of albumin 44 g/l.*

Figures 35 and 36: *Titration curves of albumin and bicarbonate buffer together, four models of albumin compared. The left graph shows the titration curves of albumin plus closed bicarbonate buffer, i.e. one where total concentration of CO_2 (tCO_2) is equal 25.6 mmol/l. The right graph shows the titration curves of albumin plus open bicarbonate buffer, i.e. one where pCO_2 is always equal to 40 mmHg (5.3 kPa). The colours and patterns of lines are same as in figures 33 and 34. Note that the differences between various titration curves appear much smaller when plotted together with open bicarbonate buffer. In each case, SID was calculated according to equation (2.36) as $-C_{Alb}*Z$ (Alb^-) + [HCO_3^-]. The values of SID of the left and of the right graphs are same at 7.4, because pCO_2 of the left graphs equals 40 mmHg at 7.4.*

This last graph is related to the situation when measured pH is compared to the pH calculated according to the modern approach given known pCO_2 and SID. The difference between the pH measured and pH calculated is analogical to the horizontal distance between the lines of different models (these lines are constant pCO_2 lines and horizontal intercept has constant SID). As mentioned, the distance is small, because the differences between various models of albumin (or between model and measurement) are overlaid by the dominant effect of good precision bicarbonate model.

Since the reference Figge-Fencl model version 3.0 appears to be in between the linearized relationship and the single pK_A model of Matousek et al on figures 33 and 34, the single pK_A might seem as not such a bad idea after all. For a narrow range of pH, single pK_A model can be surely used.
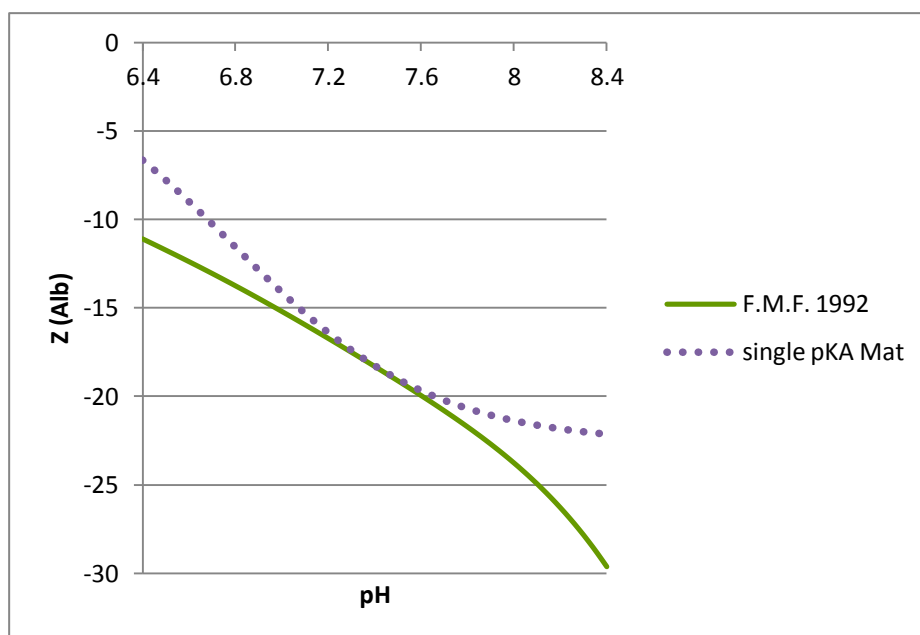
Figure 37: *Comparison of single pK_A model with parameters derived by Matousek et al. and Figge-Mydosh-Fencl model version 2.0. Z (Alb) is the average charge per molecule of albumin. Since F.M.F. model version 2.0 gives same charge and buffer value at pH=7.4 as model version 3.0, they both yield same single pK_A model parameters. However, unlike version 3.0, model version 2.0 is downwards curved, away from the single pK_A model. Here, linear approximation would be much closer to the reference model.*

However, the apparently same curvature of the two models is just a chance - the reference model could be curved the other way and it would still produce the same parameters of single pK_A model, because the parameters of single pK_A model depend only on the charge and its first derivative (i.e. buffer value) at the point of parameter determination (see appendix of reference (9) for details). This is shown at figure 37, where Figge-Mydosh-Fencl model version 2.0 (curved the other way, but with same charge and slope) is compared to the same single pK_A model.

Second weak point of the modern theory is comprised of some implications drawn from the concept of independent and dependent variables. The concept will be discussed on the set of Stewart's 6 original equations, but the simplified model with 4 equations could be discussed instead, leading to the same conclusions. There are 9 variables in the original model, 3 that were named independent ones by Stewart (SID, A_{tot}, pCO_2) and 6 that are called dependent ones ($[HCO_3^-]$, $[CO_3^{2-}]$, $[HA]$, $[A^-]$, $[OH^-]$ and $[H^+]$). Strictly mathematically speaking, any 3 of the 9 variables in the set of 6 equations could be considered inputs (or independent variables) and any remaining 6 variables could be considered functions of the inputs, or dependent variables. However, there is a fundamental difference between the two groups based on the underlying chemistry.

Stewart originally defined each group as follows (13): "Independent variable values are imposed on a system from outside, and are not affected by the equations which govern the system, nor by changes in the system, nor by each other. Dependent variables are internal to a system; their values are determined by the system equations and by the values of the independent variables.... Only changes in the independent variables in a system can cause changes in the dependent ones."

"Two of the qualities involved, [SID] and [A_{tot}] are determined when we make up the solution, and are not affected in any way by the processes these equations represent. They are the independent variables for this system."...."CO_2 at an externally regulated partial pressure pCO_2" is the third independent variable.

Apparently, what Stewart meant by imposing the values of the independent variables from outside is that both SID and A_{tot} only change in the instant when we mix new constituents into the solution, or take some constituents out. That can be agreed on. Neither hydrogen ion redistributions nor any other acid-base reactions cause changes in concentration of strong ions, because the ions are inert to the acid-base reactions (limits of this notion are yet to be discussed); nor do these processes cause disappearance or appearance of complex buffer molecules like albumin that constitute the value of A_{tot}. In the moment that we pour something new in, so to say, SID and A_{tot} change "immediately" and discretely assume a new value, maintaining an interval of the change; otherwise, they maintain a constant value.

On the contrary, the dependent variables show different behaviour. When a new constituent is mixed in, one or more dependent variables also immediately assume a new concentration. However, in case of dependent variables, this generally leads to disequilibrium and part of the added substance reacts with the other buffers and water molecules until a new equilibrium is established for all substances. The independent variables act as *invariants* during the equilibrations, while the dependent variables gradually change their values from a state of disequilibrium into a new equilibrium. So, variables of each group behave differently when the system transits from an acid-base disequilibrium and reaches a new ballance.

Let's discuss this on an example: when HCO_3^- changes, for instance by tubular transport mechanism in kidneys, some other ion has to accompany it to keep electroneutrality, for instance positively charged Na^+ has to go across in the same direction. Let's consider the situation when the tubular transport increases the concentration of bicarbonate in the surrounding blood plasma by an amount of $[HCO_3^-]_{add}$; it would be tempting to think that the concentration of bicarbonate increases from the original level $[HCO_3^-]_{orig}$ to a new level $[HCO_3^-]_{orig}+[HCO_3^-]_{add}$. This is, however, not the case, because the newly added bicarbonate constitutes a new base added to the system and part of it serves as a

hydrogen ion acceptor; thus, hydrogen ion redistributes in the system (equation 1.34, together with equations 1.27 and 1.28)). Before this redistribution takes place (but after the bicarbonate has been added), the concentration of buffer base is equal $BB_{orig}+[HCO_3^-]_{Add}$; since the redistribution does not change the amount of buffer base (hydrogen ion just shuffles around), the sum stays same after the redistribution. It is also true that new SID equals $SID_{orig} + [Na^+]_{Add}$. Added sodium and the change in SID is definitely a handy measure that can quantify what has happened, but a more direct causative acid-base change is added bicarbonate and change in BB. Naturally, A_{tot} stays constant during the whole process. Notice, that we essentially have the following relationships

$$BB_{new} = BB_{orig} + [HCO_3^-]_{add}$$

$$SID_{new} = SID_{orig} + [Na^+]_{Add}$$

$$[HCO_3^-]_{Add} = [Na^+]_{Add}$$

(3.18)

Thus, we have shown for our particular case that

$$\Delta SID = \Delta BB = [HCO_3^-]_{Add} = [Na^+]_{Add}$$

(3.19)

This is true for all cases of buffering and hydrogen ion redistributions.

Similar analysis has been done in more detail for the C_H and addition of strong acid (equations 1.30 to 1.33 and the surrounding text), showing that C_H also behaves as an *invariant* during internal equilibrations and hydrogen ion redistributions. Thus, we have identified three variables that stay *invariant* during acid-base equilibrations: *SID*, C_H and C_B / BB.

Change in Na^+ changes SID and the solution of Stewart's equations – however, from the point of view of standard acid-base theory (67), (23) - it is a bystander ion, not participating in H^+ or OH^- exchange, thus not being a causative agent. A true cause of an acid-base effect is the original change in bicarbonate (that increases or decreases the amount of buffer base), although bicarbonate itself does not behave as *invariant* during equilibration. However, here, Stewart posits something different. He writes: "Changes in the dependent variables are necessarily correlated, as they all depend on changes in the independent ones, but the dependent variables are not causally related to each other, and do not determine each other." In other words, according to Stewart, a transport of bicarbonate, as happens for instance in kidney tubular transport, cannot be a causative agent of a acid-base change. This is totally against the universally accepted theory (in general chemistry) and all the evidence behind that standard theory.

Stewart also states: „To understand a change in [H^+], we must look for a change in one or more of the three independent variables which caused it. Nothing else is needed and nothing else will do!"... "In

particular, the so called "buffer equation" (equation 2, figure 32, basically eq. 1.10) can not, and must not, be interpreted to indicate how $[H^+]$ depends on, or is determined by, $[HA]$, $[A^-]$ or their ratio. $[H^+]$, $[HA]$ and $[A^-]$ are all *dependent* variables, so that *each* of them is separately determined by $[SID]$ and $[A_{tot}]$." ... ...The "dependent variables all change only with changes in the independent variables, and do *not* determine each other."

In other words, what Stewart wants us to do is to forget all the insight and everything we know about buffer behaviour and just concentrate on $[SID]$ and $[A_{tot}]$. Why would anybody want to do it? What positive effects can blinding ourselves to parts of well established knowledge bring? For instance, $[HA]$ and $[A^-]$ are not *invariant* during equilibration, but their ratio after equilibration does determine pH even according to Stewart (eq.2, figure 32). Or if we want to start telling the story on the other side, we could say that pH determines ratio of $[A^-]$ and $[HA]$ after equilibration. Obviously, neither statement is an expression of cause and effect going one way, because activities and concentrations of the species involved in a reaction determine equilibrium simultaneously. But why should using the meaning that the equation 2 (fig. 32) conveys be prohibited? I believe it is so apparently ridiculous that it needs no more argumentation.

Couple of other formulations of Stewart are dubious. Stewart states: Equations "say that the *only* way any of the four dependent variables in the system can change is as a result of a change in either $[SID]$ or $[A_{tot}]$, or both." I thing a more correct statement would be that the dependent variables can only change *simultaneously* (not necessarily as a result) with a change in $[SID]$ or $[A_{tot}]$ or both. Stewart states: "We can never change only one of the dependent variables in this system, because a change in either independent variable causes *all* the dependent variables to change at the same time." It is true that a change in one variable generally brings new equilibrium for all variables. But we can change two variables ($[A^-]$ and $[HA]$) in such a way that their ratio stays constant (this is where insight from the equation 1.10 comes handy). This causes no disequilibrium to the other buffer systems and all other dependent variables stay constant. On the other hand, both SID and Atot have to change during the process (68). Alteration of albumin levels can indeed lead to changes of two independent variables (SID and A_{tot}), as was discussed in this thesis in the parts dealing with *iSID* of albumin, and as was also previously pointed out by Constable (20).

The concept of independent variables being the principal ones to change and causing the change of the dependent ones has already been criticized in literature (69), (70). Kurtz et al. (70) published a thorough critique that includes historical perspective and Wooten pointed out (69) that Stewart's approach, "like any other method derived from considerations involving the calculation of interval change via the assessment of initial and final equilibrium states,... does not produce mechanistic

information" (i.e. the information about how the change has occurred)... "To believe otherwise risks falling prey to the *computo ergo est* (I calculate it, therefore it is) fallacy. What is thus required for mechanistic understanding is the collection of actual mechanistic data, perhaps obtainable through isotopic labeling and kinetic experiment." The only part of Wooten's notion I would slightly object is the actual need of kinetic experiments and isotopic labeling to disprove the concept of independent variables driving the change of the dependent ones. These experiments have long been performed for other types of reactions and even acid-base reactions themselves and standard theories are constructed to be in accordance with these experiments. These standard theories, having much wider area of validity then just acid—base physiology, tell us that the reaction equilibriums are primarily determined by concentration and activity of the species participating in the reaction and not bystander ions, albeit the latter might be needed to keep electroneutrality of the solution.

The third weak point of the modern approach is the lack of compensation diagrams similar to that given on figure 11 or empirical "Boston" rules (table 5) that would aid diagnosis. The ideas of respiratory compensation mitigating the pH derangement of metabolic disturbances and renal metabolic compensation mitigating the pH derangement of respiratory disturbances have well researched physiological basis. The mechanisms of these regulatory responses are described, published and almost universally accepted (71), (36). In case of respiratory compensation to a metabolic disturbance, they include pH sensing mechanisms of peripheral and central chemoreceptors that influence the rate of breathing. In case of metabolic compensation, they include regulatory mechanisms of kidney tubules, including varying rate of metabolism of glutamine, which serves to replenish serum bicarbonate levels, varying rate of excretion of organic acids, bicarbonate excretion (in case of alkalosis) and varying rate of bicarbonate synthesis in the reaction (2.1), where H^+ is excreted to the urine and HCO_3^- returned to plasma (36). The bicarbonate centered approach (table 5) and the traditional approach (figure 11) have algorithms to identify pH, pCO_2, $[HCO_3^-]$ and BE values typical for each compensated disturbance. This makes accurate diagnosis of the nature and causes of each disturbance more certain; for instance, mixed disorders caused by two different underlying mechanisms can be identified this way. However, the framework of the modern approach does not provide any equivalent to the Boston rules or compensation diagrams. This may lead to incorrect diagnosis as to which parameter has charged due to a pathological process and which has changed as a compensation of the pH regulatory system. Using only the numerical values can easily lead to one of the causes being missed especially in the case of mixed disturbances.

The fourth weak point of the modern approach lies in its inherent problems with identification of model parameters and normal values of independent variables used for the description of non-bicarbonate buffer system. The approach has been around for more than three decades, but the

value of the dissociation constant pK_A and normal values of SID and A_{tot} were never agreed on (table 11). The problem is not that there would be no values of pK_A, SID and A_{tot} giving reasonable fit to the measured buffer curves around pH = 7.4. The problem is that there are more possibilities than just one. The problems of identifiability for K_A and A_{tot} are going to be discussed in the next section, because the discussion needs mathematics used when comparing the modern and the traditional approach. Therefore, only *SID* is going to be covered here.

As seen in figure 11, various values have been identified when trying to find the normal value of SID in plasma. This has several reasons:

First: Some authors include minor inorganic ions like Mg^{++}, while others don't. Some authors include major organic ions, like lactate, while others don't. As discussed in the section dealing with plasma in chapter 2, both approaches have some logic into them. Anyway, minor organic ions are never completely covered.

Second: When concentrations of strong ions are measured, and *SID* calculated according to the formula (2.44) or analogical, the errors of each measurement add together, leading to a larger uncertainty in the determination of SID.

Third: When SID is calculated as BB (BB is called SID effective my authors of the modern approach), using the equation (3.14) there is a problem with the charge of globulins. Their charge is dependent not only of the globulin concentration, but also of the particular percentage of different proteins that comprise globulins (47). Thus globulins are not included in the formulation of the modern approach advocated by Figge, Fencl and their collaborators (65), (40), (17).

Fourth: The group of substances known as 'unknown anions' ($[X^-]$ or $[XA^-]$) is often impossible to measure as a whole by other means then measuring SID and subtracting BB. This is also known as strong ion gap. A question then arises, whether the measured SID or BB should be used as the 'true' SID.

Fifth: An effect known as chloride shift exists in full blood. When CO_2 diffuses into the blood from tissues, part of it is converted to bicarbonate in erythrocytes. This bicarbonate is then exchanged for chloride ion at erythrocyte membrane. Reverse process goes on in lungs. Due to this, SID of venous and arterial blood differs by several mmol/l. In fact, it should not be considered independent of pCO_2 due to this effect.

Sixth: When pH changes, buffer residues become charged or uncharged. This influences protein binding of various strong ions, including calcium, chloride and sodium. The effect has been well

documented for albumin (41). When activities of various ions are measured, only the free ionic concentration plays role. A question arises, whether the free or total concentration of each ion should be taken as part of *SID*. This has been already addressed by Staempfli and Constable (63). Also, this means that *SID* is not such a perfect *invariant* as it looks in the theory.

And seventh: Parts of the charge on proteins behaves as a fixed charge in the physiological range of pH, as was first pointed at by Watson (22) and also explored in this thesis. According to the pH-pK_A criterion, this charge should be considered part of strong-ion difference, but it is generally not, because it is on a protein. Again, question arises, which *SID* should be used. This issue has been, again, already addressed by Staempfli and Constable (63).

This overview shows that it is going to be very difficult to find a consensus as to which value should be considered a normal value of *SID*. Unfortunately, the value assigned to *SID* also influences the best choice for the value of K_A and normal value of the A_{tot}.

Transformation between the variables and equations of the two approaches

Acid-base disorders are characterized by 3 independent variables of the modern approach: A_{tot} or analogical measure of buffer concentration, SID and pCO_2. With these, several auxiliary parameters can be used to aid the diagnosis. These include $[Na^+]$, $[Cl^-]$, Anion gap (AG), Corrected AG and strong ion gap (SIG). Traditional approach characterized acid-base disorders by 2 major parameters, pCO_2 and base excess (BE). Base excess has been shown to be independent of acute changes in pCO_2. Several auxiliary parameters can also be used, including $[HCO_3^-]$, AG and corrected AG.

An interesting question arises about the nature of the relationship between the equations and variables of the traditional and the modern approach. Is there new important information included in the variables of the modern approach that has been missed by the traditional approach? Or possibly, is there a mapping between the equations and variables of the traditional and the modern approach that would transform the first into the latter and vise versa? And if such transformation exists, is it unique?

Answers to these questions only started to appear the last decade or so. From a clinical standpoint, common features, strengths and weaknesses of each approach and possibilities of using them in concert were researched by Schlichtig et al (19) and Kellum (8). First general description of the relationship between *SID* and *BE* was published by Wren Wooten (7), based on Guenther's formalism

of acid-base description (72). This formalism was also introduced in chapter 1 of this thesis. The disadvantage of Wooten's analysis is relatively complex mathematical notation used, which might be difficult to understand for a medical professional. The link was reexamined in the paper of Matousek et al (9), who used only the simplified models for comparison and discussed nature of some interpretation differences. Complete description of the specific transformation relationships is given in this thesis. Nature of the transformation coefficients and some important consequences of the existence of the transformation are discussed.

Let's first consider the relationship between the principal variables of each approach. Since pCO_2 is a variable in both approaches, this leaves us with the relationship between *SID*, A_{tot} and *BE*. Figure 38 summarizes the correspondences that exist between the equations of the two approaches, including representation of bicarbonate buffers, non-bicarbonate buffers and electroneutrality. Mathematical equations relating to the modern approach of Stewart are labeled 'St' and those of the traditional approach of Siggaard-Andersen are labeled 'SA'.

Mass action equations of the reactions for water dissociation and carbonate buffering are numbered St1 and St2. These equations have already been discussed in the section dealing with the modern approach, showing that their inclusion to the equation system does not bring any further precision to the values of the other dependent variables (2). These equations have no equivalent in the traditional approach. The reaction for bicarbonate buffering is represented by Stewart as equation St.3, the Henderson equation. For the traditional approach, this is the same equation in its logarithmic form, i.e. the Henderson-Hasselbalch equation (SA.1).

As was covered extensively in chapter 2, non-bicarbonate buffer system of common biological fluid, including blood plasma, is composed of multiple buffer residues and substances of various pK_A. Traditional approach collates all these contributions into simple linear approximation, characterized by its variable buffer capacity β, while the modern approach collates them into single pK_A buffer, characterized by its variable A_{tot} concentration. In the modern approach, the equations that describe non-bicarbonate system behaviour include mass action equation (St.4) linking the weak acid form ([HA]), and anions or buffer base ([A^-]), equation describing the conservation of mass of non-bicarbonate buffer in both forms (St.5) giving a total concentration A_{tot} and the electroneutrality equation that links non-bicarbonate and bicarbonate buffer systems to the value of SID (St.6). In the traditional approach, these reactions are represented by a single equation – van Slyke equation for plasma (SA.2a).
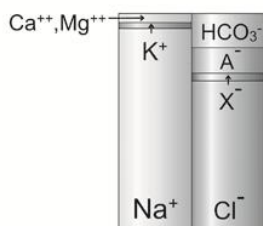
Chemical equilibria	Modern approach	Traditional approach
water: $H_2O \rightleftarrows H^+ + OH^-$	St.1 $[H^+] \cdot [OH^-] = K'_w$	} No equivalence
carbonate: $HCO_3^- \rightleftarrows H^+ + CO_3^{2-}$	St.2 $K_3 = \dfrac{[H^+] \cdot [CO_3^{2-}]}{[HCO_3^-]}$	
bicarbonate: $CO_2 + H_2O \rightleftarrows H^+ + HCO_3^-$	St.3 $K_C = \dfrac{[H^+] \cdot [HCO_3^-]}{pCO_2}$	$\leftrightarrow$ $pH = pK_C + \log \dfrac{[HCO_3^-]}{\alpha_{CO_2} \cdot pCO_2}$ SA.1
non-bicarbonate buffers: $HA \rightleftarrows H^+ + A^-$	St.4 $K_A = \dfrac{[H^+] \cdot [A^-]}{[HA]}$ St.5 $A_{tot} = [HA] + [A^-]$ St.6 $SID = [HCO_3^-] + [A^-]$	$BB = [HCO_3^-] + [A^-]$ SA.2 $\Delta BB = \Delta[HCO_3^-] + \text{ß} \cdot \Delta pH$ SA.2a If Δ is from pHn: $\Delta BB = BE$ $BE = ([HCO_3^-] - 24.4) + \text{ß}(f(Alb)) \cdot (pH - 7.4)$ SA.2b
electroneutrality 	St.7 $[Na^+] + [K^+] + [Mg^{++}] + [Ca^{++}] = [Cl^-] + [HCO_3^-] + [A^-] + [X^-]$ SA.3 St.8 $SID_A = [Na^+] + [K^+] - [Cl^-]$ St.9 $SID_E = [HCO_3^-] + [A^-]$ St.10 $SIG = SID_A - SID_E = [X^-]$	$AG = [Na^+] + [K^+] - [Cl^-] - [HCO_3^-]$ SA.4 $AG = [X^-] + [A^-]$ SA.5 $AG_{corr} = AG - [A^-]_n + [A^-]_n = [X^-] + [A^-]_n$ SA.6

Figure 38: *Relationship between the modern and classical approach to the acid-base chemistry of blood plasma. Left column contains the equations of chemical reactions and concept of electroneutrality. Central and right columns contain mathematical formulas of modern (Reactions labeled St.) and traditional approach to acid-base and shows the connection between the two. The meaning of some of the formulas: SID_A = apparent SID, SID calculated from the strong-ion side. SID_E = SID effective, which is another name of buffer base, sometimes used in the modern approach. AG – anion gap, SIG – strong ion gap, $AG_{torr.}$ – anion gap corrected to the concentration of albumin, α_{CO2} solubility of CO_2 (different notation to S of the equation 2.2), β – buffer capacity of non-bicarbonate buffers. Published in* (9).

The link that exists between these approximations is now going to be explored. We are going to start from the Stewart's formulation, which collates all non-bicarbonate buffer bases under the term A$^-$. However, we are also going to explore the link in a more general view. For the purpose of derivation, we are now going to make a distinction between buffer base *BB* and total titratable base C_B. *BB* is going to be understood as the negative charge on buffer substances (including proteins), regardless what type of residues bear this charge. This is common in acid-base physiology. C_B is going to represent the true concentration of the base forms of protein buffer residues, in agreement with the general theory.

The sum of negative charges on plasmatic non-bicarbonate buffers and bicarbonate is, when we want to keep things simple, equal to *SID* (St.6) and *BB.*

$$SID = BB = [A^-] + [HCO_3^-]$$
(3.20)

However, as was discussed in the connection with the equation (2.46) and (3.14), things get more complex, when we take a more detailed look. For instance, the negative charge of albumin can be divided into the fixed charge of the residues that behave like the strong ions and the charge of buffer residues. The charge of buffer residues is further equal to the base form concentration added with the maximum charge of buffer residues times albumin concentration (equation 2.39 and table 10)

$$[Alb^-] = -iSID_{Alb} - C_{Alb} * \bar{z}_{\max (Alb)} + C_{B(Alb)}$$
(3.21)

The negative sign of the *iSID* and $C_{Alb}*\bar{z}_{\max (Alb)}$ terms is due to the fact that [Alb⁻] represents negative charge, while these two terms represent positive charge. This relationship can be generalized for the whole negative charge of non-bicarbonate buffers that is represented by A⁻ term in our notation

$$A^- = \sum_i \left(-iSID_{(i)} - C_i * \bar{z}_{\max (i)} + C_{B(i)}\right)$$
(3.22)

The correspondence between the two approaches will be considered in two phases, first in the simplified thinking of the equation (3.20) that is relevant for the original formulation of the modern approach and then in the more complex thinking represented in the equation (3.22).

Equation St.6 can be written in its general form (3.20) and for normal conditions, meaning that all the variables constituting *SID* and *BB* are in their average normal concentration. These average normal values are going to be denoted by the subscript *N*.

$$SID_N = BB_N = [A^-]_N + [HCO_3^-]_N$$
(3.23)

When first parts of the equation (3.20) and eq. (3.23) are combined and subtracted, we get the differences of each parameter from average normal values

$$SID - SID_N = BB - BB_N$$
(3.24)

The right hand side can also be written as

$$BB - BB_N = (BB - BB_{pHn}) - (BB_N - BB_{pHn})$$
(3.25)

The first two terms on the right constitute the definition of base excess from the equation (3.1). BB_{pHn} is the normal buffers base, as it is understood in the traditional approach, i.e. buffer base of the

given blood plasma (with any particular total concentration of non-bicarbonate buffers), when it has been titrated back to pH = 7.4 (at pCO_2 = 40 mmHg).

The last two terms of the equation (3.25) are both values of buffer base at pH = 7.4 and pCO_2 equal 40 mmHg (normal acid-base conditions). The only difference is that BB_N is the value of negative charge on buffers at normal acid-base conditions and normal average concentration plasmatic buffers. BB_{pHn} is the value of negative charge on buffers at normal acid-base conditions and any concentration of plasmatic non-bicarbonate buffers ([HCO_3^-] is still fixed at its normal average value by Henderson-Hasselbalch equation and normal acid-base conditions)

$$BB_N = [HCO_3^-]_N + [A^-]_N \tag{3.26}$$

and

$$BB_{pHn} = [HCO_3^-]_N + [A^-]_{pHn} \tag{3.27}$$

Obviously, the terms [HCO_3^-]$_N$ cancel out when we subtract eq. (3.27) from (3.26). Now, substituting for [HA] term in the equation St.4 by using equation St.5 gives

$$K_A = \frac{[H^+] * [A^-]}{A_{tot} - [A^-]} \tag{3.28}$$

Rearranging to isolate [A$^-$], and expressing H$^+$ in terms of pH yields

$$[A^-] = A_{tot} * \frac{K_A}{[H^+] + K_A} = A_{tot} * \frac{K_A}{10^{-pH} + K_A} \tag{3.29}$$

When the equation (3.29) is applied to the normal acid-base conditions, the last two terms of the equation (3.25) can be expressed as

$$BB_N - BB_{pHn} = [A^-]_N - [A^-]_{pHn} = \{(A_{tot})_N - A_{tot}\} * \frac{K_A}{10^{-pHn} + K_A} \tag{3.30}$$

Combining equation (3.24), (3.25), (3.1) and (3.30) finally yields the first relationship between the variables of the modern and the traditional approach

$$SID - SID_N = BE + \{A_{tot} - (A_{tot})_N\} * \frac{K_A}{10^{-pHn} + K_A} \tag{3.31}$$

Written more concisely with the Δ notation signifying the difference from the average normal values

$$\Delta SID = BE + \Delta A_{tot} * \frac{K_A}{10^{-7.4} + K_A} \tag{3.32}$$

This relationship has already been described in words by Siggaard-Andersen and Fogh-Andersen (6) in their paper criticizing modern approach. This is however, the first time that the relationship is

formally derived and written down as a formula, at least to my knowledge. The last term of the equation (3.32) can be represented graphically as a vertical line ΔA^-_{pHn} of figure 39, i.e. change in the charge of non-bicarbonate plasma buffers at pH = 7.4, when their concentration is varied.

Next part of the link describes how BE can be derived from the variables of the modern approach. According to its definition, BE is equal to

$$BE \stackrel{\text{def}}{=} BB - BB_{pHn} = [HCO_3^-] + [A^-] - [HCO_3^-]_N - [A^-]_{pHn} \tag{3.33}$$

In this derivation, we have again used the fact of bicarbonate concentration being fully determined by normal pH and pCO$_2$, i.e. that $[HCO_3^-]_N = [HCO_3^-]_{pHn}$. Substitution to the Henderson Hasselbalch equation (2.2) gives normal value of bicarbonate 24.4 mmol/l.
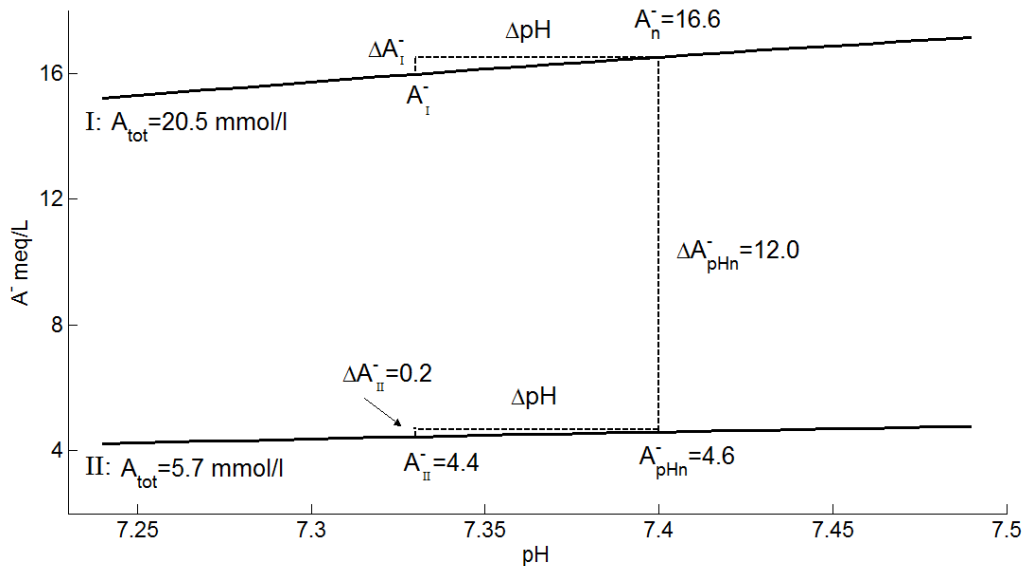

Figure 39: *Titration curve and charge of non-bicarbonate buffers [A⁻] in plasma according to simplified SID model, showing the different perspective of the traditional and the modern approach. Only the values of ΔA⁻$_{II}$ (or ΔA⁻$_I$) are included in the base excess, because it only takes as relevant changes from [A⁻]$_{pHn}$. The [A⁻] vs. pH relationship is illustrated for: I – a normal value of plasma buffers (mainly albumin) and hence total non-bicarbonate buffer (Atot); and II - a reduced value of plasma buffers (mainly) albumin and hence total non-bicarbonate buffer (Atot). ΔA⁻$_I$ and ΔA⁻$_{II}$ represent the change in A⁻ when pH changes from 7.4 to 7.6. ΔA⁻$_{pHn}$ represents the change in A⁻ on variation of A$_{tot}$ at normal pH, i.e. the term ΔA$_{tot}$*(K$_A$/(K$_A$+10$^{-7.4}$)) of the equation (3.32). The figure was first published in the article of Matousek et al (9).The numbers are taken from the clinical example presented in (9).*

$$BE = ([HCO_3^-] - 24.4) + ([A^-] - [A^-]_{\text{pHn}}) \tag{3.34}$$

The last two terms represent change of charge of non-bicarbonate buffers with titration starting (or finishing) at pH = 7.4. As has been shown in this thesis, Stewart's approximation with single K_A system produces curved titration curve, while the traditional approach uses linear approximation, which is generally closer to the real titration curves. Link from the (3.34) to the linear approximation uses the following substitution, which is based on first two terms of Taylor series, where the constant terms cancel out (equation (3.5))

$$[A^-] - [A^-]_{\text{pHn}} = \beta * \Delta pH \tag{3.35}$$

This link is only valid when the rate of [A⁻] change with respect to the pH (i.e. slope on the left) actually equals the buffer value the of non-bicarbonate buffers (β of the right hand side). This can't be assured everywhere, because the line and the curve are slightly different, but it makes sense to make best possible fit at least around normal pH = 7.4. Mathematically speaking, the same slope is assured, when the derivative of the left hand side equals β. The term $[A^-]_{\text{pHn}}$ is constant during changes of pH, thus we only need to consider [A⁻] term, being expressed by the equation (3.29), when taking the derivative of the left hand side

$$\frac{d([A^-] - [A^-]_{\text{pHn}})}{d\text{pH}} = \frac{d[A^-]}{d\text{pH}} = A_{tot} * \frac{2.303 * K_A * 10^{-7.4}}{(10^{-7.4} + K_A)^2} \tag{3.36}$$

As already said, this expression has to equal β

$$\beta = A_{tot} * \frac{2.303 * K_A * 10^{-7.4}}{(10^{-7.4} + K_A)^2} \tag{3.37}$$

This is basically the same relationship as was derived for buffer capacity in the chapter 1 (equation (1.19)). With beta calculated according to the equation (3.37), we can substitute the equation (3.35) into (3.34), getting

$$BE = ([HCO_3^-] - 24.4) + \beta * \Delta pH \tag{3.38}$$

This is the well known van Slyke equation for plasma of the traditional approach, obtained by using and combining the equations of the modern approach. The value of the second term is equal (as a linear approximation) to the value of ΔA_I^- and ΔA_{II}^- of figure 39, which is the only difference of non-bicarbonate buffer charge that is considered relevant in the traditional approach.

It is interesting to note that the equation (3.37) contains only two variables, A_{tot} and β. Values of these variables are directly proportional. Each one of them can be considered a measure of total concentration of plasmatic buffers. For instance, Matousek et al determined following coefficients relating their value to the measured concentration of albumin, globulins and phosphates

$$\beta = 0.123 * C_{W,Alb} + 0.049 * C_{W,Glb} + 0.309 * C_P \tag{3.39}$$

$$A_{tot} = 0.34 * C_{W,Alb} + 0.14 * C_{W,Glb} + 1.0 * C_P \tag{3.40}$$

Normal average value of concentration of albumin, $C_{W,Alb}$ is 44 g/l, normal average value of concentration of glubulins, $C_{W,Glb}$ is 33 g/l and normal average value of phosphates, C_P is 1.2 mmol/l.

Albumin is by far the most important plasmatic buffer and frequently measured in clinical practice. It might be a clinically useful simplification to lump the influences of the other buffers to the albumin by considering their plasmatic concentration to change in proportion to albumin

$$A_{tot} = 0.47 * C_{W,Alb} \qquad\qquad \beta = 0.17 * C_{W,Alb} \tag{3.41}$$

Interestingly, the left coefficient is very close to the value 4.6 ((mmol/l)/(g/dl)), i.e. 0.46 ((mmol/l)/(g/l)), determined for albumin filtrands by Constable (48). These relationships necessarily mean one thing – there are two equivalent measures of non-bicarbonate buffer concentration in plasma: A_{tot} for the modern approach and β for the traditional approach. Neither one changes with buffering nor the changes that buffering brings to *SID* or *BE.* Neither one is dependent of pCO$_2$. In other words, β can be considered an independent variable of the traditional approach, analogical to A_{tot} in the modern approach.

This gives us three independent variables of the modern approach, pCO$_2$, SID and A_{tot} and three "independent" variables of the traditional approach pCO$_2$, BE and β. As was explained in the previous section, the word *invariants* might be a better term for what they really are. It is not burdened with the misleading deductions attributed to their role by the modern approach, yet it shows that they really have a special role, because neither one of them changes during buffering and hydrogen ion redistributions. pCO$_2$ is same in both approaches and it is actually only an *invariant*, when we consider blood plasma an open system with externally regulated pCO$_2$. *SID* and *BE* are both measures of buffering, they both change by same amount during buffering, as was proven by Wooten (7). A_{tot} and β are both measures of non-bicarbonate buffer concentration.

Given these two threesomes of *invariants*, it might be interesting to derive the exact transformation relationships. When the right hand side of the equation (3.23) is substituted for the SID_N term of the equation (3.31), we get

$$SID - \{[HCO_3^-]_N + [A^-]_N\} = BE + \{A_{tot} - (A_{tot})_N\} * \frac{K_A}{10^{-pHn} + K_A} \tag{3.42}$$

Looking at the equation (3.29), we can see that the last term is equal to $[A^-]_N$

$$(A_{tot})_N * \frac{K_A}{10^{-pHn} + K_A} = [A^-]_N \tag{3.43}$$

Substituting (3.43) into (3.42) and rearranging, we get

$$SID - [HCO_3^-]_N - [A^-]_N = BE + A_{tot} * \frac{K_A}{10^{-pHn} + K_A} - [A^-]_N \tag{3.44}$$

Obviously, the terms $[A^-]_N$ cancel out, which, after little rearrangement gives us the first transformation relationship

$$BE = SID - [HCO_3^-]_N - A_{tot} * \frac{K_A}{10^{-pHn} + K_A} \tag{3.45}$$

Recall that pHn = 7.4 and $[HCO_3^-]_N$ equals 24.4 (equation 2.2). The second transformation relationship from the variables of the modern approach to the variables of the traditional approach is the equation (3.37)

$$\beta = A_{tot} * \frac{2.303 * K_A * 10^{-pHn}}{(10^{-pHn} + K_A)^2} \tag{3.46}$$

Simple rearrangements give us the reverse relationships, i.e. ones from the variables of the traditional approach to the variables of the modern approach

$$SID = BE + [HCO_3^-]_N + \beta * \frac{K_A + 10^{-pHn}}{2.303 * 10^{-pHn}} \tag{3.47}$$

$$A_{tot} = \beta * \frac{(K_A + 10^{-pHn})^2}{2.303 * K_A * 10^{-pHn}} \tag{3.48}$$

Equations (3.45) to (3.48) are general relationships that hold true for any version of the modern approach. These formulas can be used with the normal value of A_{tot} and the value of K_A derived by Matousek et al (9) from the albumin data, getting the following number representations

$$BE = SID - 24.4 - 0.8\,A_{tot} \tag{3.49}$$

$$\beta = 0.36\,A_{tot} \tag{3.50}$$

$$SID = BE + 24.4 + 2.22\,\beta \tag{3.51}$$

$$A_{tot} = 2.77\,\beta \tag{3.52}$$

Remember that *BE* in these formulas is the base excess for plasma, not the one for full blood or extracellular fluid.

This completes the phase 1 of link from the modern approach to the traditional approach, i.e. the link when using the simplified thinking of the equation (3.20). The key elements of the link are the

transformation equations (3.45) to (3.52), equation (3.32) that relates ΔSID, ΔA$_{tot}$ and BE and equations (3.37) and (3.38) that relates the van Slyke equation for plasma to the modern approach.

Let's now explore what the link between the approaches would look like if we divide the charge on the [A$^-$] into the terms of the equation (3.22). After substituting in (3.22), equation (3.20) becomes

$$SID + \sum_i iSID_{(i)} = [HCO_3^-] + \sum_i C_{B(i)} - \sum_i C_i * \bar{z}_{\max(i)}$$
(3.53)

Recall that $iSID_{(i)}$ is defined for a single protein i in the equation (2.41), in accordance with the notions of Staempfli and Constable (63). Let's now define total $iSID$ of plasmatic proteins (or other substances that include buffer residues) and total titratable base of plasmatic proteins as

$$iSID_{nonB} = \sum_i iSID_{(i)} \qquad C_{B(nonB)} = \sum_i C_{B(i)}$$
(3.54)

Equation (3.53) can now be written in the familiar form derived in chapter 2 (equation 2.39)

$$SID + iSID_{nonB} = C_{B(nonB)} + [HCO_3^-] - \sum_i C_i * \bar{z}_{\max(i)}$$
(3.55)

In this formula, only three terms vary with pH (i.e. titrations by strong acids or bases) – SID, $C_{B(nonB)}$ and [HCO_3^-]. The non-bicarbonate buffers are represented by the $C_{B(nonB)}$ term. In the simplification of the modern approach, this term can again be approximated by a single K$_A$, A$_{tot}$ system. Only this time, the charge of the base form of non-bicarbonate buffers (A$^-$) does not have to be -1. It can be essentially any number; its value is characterized by the value of the $C_i * \bar{z}_{\max(i)}$ term (the charge of base forms can be considered -1 only when $C_i * \bar{z}_{\max(i)}$ term equals zero). Furthermore, $iSID_{nonB}$, the fixed charge of protein residues that do not function as buffers is now excluded by pH-pK$_A$ criterion and added to SID. This model structure is much closer to reality then the common representation. As said, the term $C_{B(nonB)}$ can be approximated by a single K$_A$, A$_{tot}$ system, which gives us expression similar to (1.46)

$$C_{B(nonB)} = A_{tot2} * \frac{K_{A2}}{K_{A2} + 10^{-pH}}$$
(3.56)

But this time, the values of A_{tot} and K_A are going to be different, which is why they received extra subscript 2. The issues concerned with their identification are going to be covered shortly. Now, we are still interested with the relationship of these variables and the equation (3.55) to the traditional approach. Equation (3.55) can be rearranged, yielding

$$SID + iSID_{nonB} + \sum_i C_i * \bar{z}_{\max(i)} = C_{B(nonB)} + [HCO_3^-]$$
(3.57)

Now, because the term $C_{B(nonB)}$ is expressed as eq. (3.56), analogically to the expression for the [A⁻] in the equation (3.29) and because all the terms on the left represent charge that is indifferent to buffering, behaving similarly to the strong ion difference, we have an equation analogical to the equation (3.20)

$$SID + iSID_{nonB} + \sum_i C_i * \bar{z}_{\max(i)} = C_B = C_{B(nonB)} + [HCO_3^-] \qquad (3.58)$$

Informally (and bit incorrectly), we could even group all the terms on the left under umbrella variable "super SID", to see the analogy with the equation (3.20) even more clearly

$$"supSID" \overset{\text{def}}{=} SID + iSID_{nonB} + \sum_i C_i * \bar{z}_{\max(i)} \qquad (3.59)$$

With this informal terminology, equation (3.58) becomes

$$"supSID" = C_B = C_{B(nonB)} + [HCO_3^-] \qquad (3.60)$$

Equation (3.60) is completely analogical to the equation (3.20). We could now perform same analysis that compared the two approaches step by step, starting from the equation (3.20) only this time, we would start from the analogical equation (3.60). Only difference is that in the first analysis, we started from a simplified assumption that SID equals BB and now we would be using a more precise thinking. The analysis would also lead to analogical results, these being especially

$$\Delta\left(SID + iSID + \sum_i C_i * \bar{z}_{\max(i)}\right) = BE + \Delta A_{tot2} * \frac{K_{A2}}{10^{-7.4} + K_{A2}} \qquad (3.61)$$

This is analogical to the equation (3.32). Interestingly, the two extra terms on the left are a function of albumin, globulin and phosphate concentration, similarly to A_{tot}.

Transformation relationships between the variables of the traditional and the modern approach (analogical to the equations 3.45 -3.48) become

$$BE = SID + iSID + \sum_i C_i * \bar{z}_{\max(i)} - [HCO_3^-]_N - A_{tot2} * \frac{K_{A2}}{10^{-pHn} + K_{A2}} \qquad (3.62)$$

$$\beta = A_{tot2} * \frac{2.303 * K_{A2} * 10^{-pHn}}{(10^{-pHn} + K_{A2})^2} \qquad (3.63)$$

$$SID + iSID = BE + [HCO_3^-]_N + \beta * \frac{K_{A2} + 10^{-pHn}}{2.303 * 10^{-pHn}} - \sum_i C_i * \bar{z}_{\max(i)} \qquad (3.64)$$

$$A_{tot2} = \beta * \frac{(K_{A2} + 10^{-pHn})^2}{2.303 * K_{A2} * 10^{-pHn}} \qquad (3.65)$$

Equation (3.63) is same as (3.37), except for the subscript 2 at A_{tot} and K_A. Same form has also the van Slyke equation for plasma, i.e. equation (3.38), being exactly same as (3.66)

$$BE = ([HCO_3^-] - 24.4) + \beta * \Delta pH \tag{3.66}$$

The reason why van Slyke equation is the same is this: Although the values of A_{tot2} and K_{A2} are different then values A_{tot} and K_A of phase one of comparison, they have to give same value of β around pH = 7.4. This is because β is a measured parameter that each model has to be fitted to.

This brings us to the last point in comparing modern and traditional approach: Issues connected to the identification of modern approach parameter K_A and normal value of A_{tot}. Neither A_{tot} nor K_A can be directly measured. A_{tot} is dependent of the plasma buffer concentration (like the concentration of albumin), but the principal question is how much of A_{tot} should be assigned to a gram of albumin. On the contrary, what can be directly measured are the values of plasma buffer capacity, which is equal to the slope of the titration curve, β. Also charge can be measured, albeit with some difficulties. In case of a single protein, isoelectric point can be measured, i.e. a point where the total charge of the protein is zero, resulting in zero movement in electric field. From this point, protein can be titrated back to the pH of interest, the amount of monovalent acid or base used during titration has to equal the amount of free (negligible around pH = 7) and bound charge. This way, charge of albumin has been determined. However, to determine charge of the whole plasma is more difficult, because there are many proteins involved, often in negligible amounts. Most commonly, the total charge of plasmatic buffers, denoted [A⁻] has been estimated from the equation St.6 (figure 38).

$$[A^-] = SID - [HCO_3^-] \tag{3.67}$$

Once the value of non-plasmatic buffer charge [A⁻] is known at least for one value of pH (typically 7.4), it and value of β can be used to find the value of parameters of the modern approach. Recall that the equations St.4 and St.5 can be combined to yield the equation (3.29), which is given here one more time

$$[A^-] = A_{tot} * \frac{K_A}{10^{-pH} + K_A} \tag{3.68}$$

As already discussed, this equation can be differentiated with respect to pH, yielding the expression for β

$$\beta = A_{tot} * \frac{2.303 * K_A * 10^{-7.4}}{(10^{-7.4} + K_A)^2} \tag{3.69}$$

These equations seem handy for calculating [A⁻], eventually β, knowing A_{tot} and K_A. But now, things need to be reversed. [A⁻] and β are known from measurement and A_{tot} and K_A are to be determined. This can be done by solving this system of two equations for the two unknowns:

$$A_{tot} = \frac{2.303 * [A^-]^2}{2.303[A^-] - \beta}$$ (3.70)

$$K_A = \frac{10^{-pH} * (2.303[A^-] - \beta)}{\beta}$$ (3.71)

The last two equations were used by Matousek et al to determine A_{tot} and K_A of albumin. As seen on the figures 33 to 36, this yields excellent fit of the titration curves around pH = 7.4 for both albumin alone and in mixture with bicarbonate. However, the determination of [A⁻] in the case of whole plasma is problematic when the equation (3.67) has to be used. One of the factors in determining [A⁻] this way is SID. However, SID cannot be easily and uniquely determined, as was discussed at the end of the previous section (weaknesses of the modern approach). Since the normal value of SID cannot be uniquely determined, the normal values of A_{tot} and the value of K_A cannot be uniquely determined as well (equations 3.67, 3.70 and 3.71). In fact, the equivalence equations in the detailed thinking (phase 2) can be looked at as just another value of "*SID*" (which is the "super SID"), for which there are just another values of A_{tot} and K_A. The difficulty in identifying model parameters is one of the major drawbacks of the modern approach.

According to the equation (3.69), there are many values of K_A and A_{tot} that can result in same measured value of β at 7.4. The titration curves determined using these different combinations of K_A and A_{tot} only differ in the second derivative, i.e. in how the pH vs. SID line is curved. This could theoretically allow for better fitting, where the values of K_A and A_{tot} are chosen to approximate even the curving of the true (experimentally determined) titration line. However, the true experimentally determined curving of the titration line is not taken into consideration when determining the values of K_A and A_{tot}. These values are only determined to be in agreement with the charge (determined from the value of SID, which contains an arbitrary component) and the rate of change in charge with pH (β, first derivative). The curving (second derivative) of the true titration line is not taken into account. This can be seen on figure 37, where the curving of the reference titration line and the one produced by the modern approach go in opposite directions.

The fact that there are many solutions that give similar titration curves around pH = 7.4 has one more important consequence. When the parameters of the modern approach are successfully determined, they are nothing more than model parameters, straying from the original meaning as it was explained by Stewart. This is especially true for the value of A_{tot}. Should it really be total amount

of weak acid (i.e. buffer), its definition would be something in the line of the equation (2.42). The two-sided iSID model based of Figge-Fencl model version 3.0 has 27 buffer residues per molecule. Thus, its A_{tot} as determined by the equation (2.42) at pH=7.4 and physiological concentration of albumin (44 g/l) should be 17.9, while the value determined by solving the equations (3.70) and (3.71) is 15.0. This is almost 20% difference, which is actually surprisingly little, given that the A_{tot} was determined to account also for the fixed charge of the strong ion residues, which should theoretically be excluded.

In the comparison the traditional and the modern approach that me and my co-authors have published (9), we have derived A_{tot} and K_A of plasma from the known charge and buffer capacity of albumin, using the equations (3.70) and (3.71). Then, we have used the normal buffer capacity ratio of albumin, globulin and phosphates determined by Siggaard-Andersen et al (6) to determine their contribution to A_{tot} (according to the equation 3.46, β and A_{tot} are directly proportional). Using the K_A determined for albumin in case of globulin is a simplification. It effectively means that we assign globulins same ratio between average charge of molecule at pH 7.4 and the buffer value. However, according to Figge et al (40), the average charge of globulins should be around zero at pH = 7.4 (this does not mean that their buffer capacity should be zero). When using single K_A approach, one has to give up either the precision in charge or buffer capacity. We have given up the charge, considering it less important for the acid-base physiology. Still, trying to fit all the plasmatic buffers into single K_A system is a bit like trying to tune a bad guitar.

Last thing that needs to be discussed when comparing the two approaches is the relationship between the auxiliary parameters that aid diagnosis of metabolic acidosis. These parameters aim at indirectly determining the elevated level of unmeasured anions (such as lactate and keto-anions) or other disturbances of less common plasmatic ions. They include anion gap (AG), corrected AG ($AG_{corr.}$) and strong ion gap (SIG). Out of these parameters SIG is the most precise, although it requires most measurements and calculations to be accurately determined. It is calculated as *SID* (also called SID_A) minus *BB* (also called SID_E) (Equation St.10). *BB* is determined using linear approximation and *SIG* calculated by measuring concentrations of all significant plasmatic ions. Historically, calculation of AG was used to estimate the difference in [X$^-$] from normal. However, in situations where protein concentrations are significantly modified AG (SA.5, fig. 1) does not reliably represent [X$^-$]. This criticism of the traditional approach led to formulation of AG corrected (AG_{corr}) (57), changes in which approximate those in SIG as described by Matousek et al (9).

Summary

The modern approach as it is commonly understood contains major weaknesses and flaws, which was one of the topics elucidated in this chapter. These weaknesses include its inability to describe almost linear buffering of proteins over wider range of pH, concept of independent and dependent variables as posited by Stewart, lack of compensation diagrams and approach's inherent problems with measurement and identification of key parameters, these being K_A and normal values of SID and A_{tot}. As was also shown in detail, there is a mathematical transformation or a mapping between the key variables of each approach. Existence of this transformation has important implication: Each approach contains equivalent amount of information, otherwise this transformation would not be possible. However, this information is structured differently and its usefulness for direct clinical interpretation is not necessarily same.

As was shown in the paper of Matousek et al (9), the BE effectively separates the change in buffer ions (equal to change in SID due to electroneutrality) due to buffering from the one due to change in concentration of buffers (see figure 39). The inability to provide this separation in the modern approach has been previously criticized by Siggard-Andersen and Fogh-Andersen (6). Theoretically, one can obtain this separation using modern approach as well, but extra calculation is required, where the effects of abnormal levels of A_{tot} on BB and SID are considered (see clinical example in (9)).

The analysis contained in this chapter can be used in construction of educational simulators. It can also be used to draw a picture of reunified acid-base physiology and chemistry of blood plasma. Obviously, due to the problems of the modern approach, reunified description should be based more on the description of the traditional approach. However, not all features of the modern approach should be discarded and not every feature of the traditional approach should be taken for granted.

Chapter 4

Use of the insight gained at comparing traditional and modern approach

Chapter three described and compared two most popular approaches to the mathematical description of acid-base chemistry of plasma and blood; traditional and modern. First, both approaches are introduced, including historical perspectives of their development. Then, four major weak points of the modern approach are covered: Its inability to describe linear buffering of proteins, concept of independent and dependent variables as posited by Stewart, lack of compensation diagrams and approach's inherent problems with identification of key parameters. These problems are major and affect the very foundations of the modern approach. However, they still make it possible to find a detailed transformation between the variables and equations of the modern and the traditional approach, as is described at the end of the previous chapter. The transformation shows, that both approaches can be considered three-dimensional description of reality: Modern approach with variables pCO_2, SID and A_{tot} and traditional approach with the variables pCO_2, BE and β.

Chapter four is the final chapter of this thesis. It shows how the insight gained in the previous chapters can be used. First use is the one for which the work was originally started: Design of educational acid-base simulators. These simulators can be an efficient tool in teaching a detailed understanding to the demanding concepts of acid-base physiology and chemistry, including the understanding of the link between the modern and the traditional approach. Gained insight can also be used in trying to draw a picture of what should the reunified description of acid-base physiology look like, given the findings of the previous chapters. Finally, contributions of this work in the context of current and past development of acid-base theory are discussed, as well as its limitations and future perspectives of the field.

Design of educational acid-base simulators

The original purpose of this work was to aid the design of educational acid-base simulators. This includes formulation of the mathematical structure of the underlying model, detailed description of simulator functionality based on understanding the teaching goals and designing graphic user

interphase (GUI). I have been a part of the model design in our group, including acid-base simulators, and some of the results are shown in this section. There are other, more technical aspects of successfully building computer simulators, such as a well-thought of choice of the modelling environment and connection between the simulator layers. These aspects are more in scope of my technically oriented colleagues.

Formulation of the structure of underlying simulation model is a part of computer modelling in medicine. Generally speaking, underlying models can be static, containing only algebraic system of equations (in the area of acid-base these models often describe the equilibrium), or dynamic, containing also a set of differential equations with respect to time. The size of the model can also vary, depending on the purpose of the underlying model. There are three main uses of the underlying models. These include medical education, clinical domain and understanding underlying physiology. The work group that I am part of in Prague is focused more on the use of models in medical education and understanding underlying physiology. Substantial parts of this thesis were also conceived in Aalborg, Denmark. The center for model-based medical decision support at Aalborg University is focused on using computer models directly in clinical practice, which requires a slightly different perspective. Having these different perspectives of modelling acid-base chemistry and physiology has helped me to maintain broader scope of my work.

My research group was originally focused on building large scale models of physiological regulation similar to that by A. C. Guyton et al in 1972 (73) (described in more detail in 1973 and 1975 (74), (75)) Ikeda et al (76) or Amosov et al (77). Some of these large scale models got more focused on education, gradually becoming learning simulators. First of this type was the simulator Human of T Coleman (78), collaborator of Guyton. In the late 1990's, Kofranek et al have built a large scale simulator Golem, designed as an open source (79) (80). The simulator was focused on the internal environment disorders and included detailed description of acid-base physiology and its regulation in the context of blood gas transport and ionic and water homeostasis.

 The simulator Golem has been used at several medical faculties. It proved itself especially in demonstrating interconnections of individual physiological subsystems and influence of these connections on pathogenesis of complex disorders of internal environment. When using the simulator in education, the possibility of disconnecting and reconnecting individual regulatory bonds was often used. Disconnection limits the simulation to an individual physiological subsystem and its behaviour can be studied independent of the complex regulatory relationships within the whole body. This was a key feature that contributed to better understanding of the physiological relationships among students. The simulator proved to have disadvantages as well – namely its

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complexity of control and visualization. Experience, both ours and of others, showed that complicated simulators require much longer period of user adaptation to the use, structure of various inputs and outputs, etc. External guidance was generally needed in this period (81), (82), (83).

This brought us to the concept of using simpler aggregated models (giving the user option to observe only several variables) as a suitable educational tool. The models can be arranged as single interactive screens, where one can proceed from simple to more complex concepts. Lumped, simplified structure of these models is often similar to the models used in clinical decision support system, where models need to have only few parameters so be well identifiable to individual patient data. Acid base concepts described in this thesis can be readily used in these models.
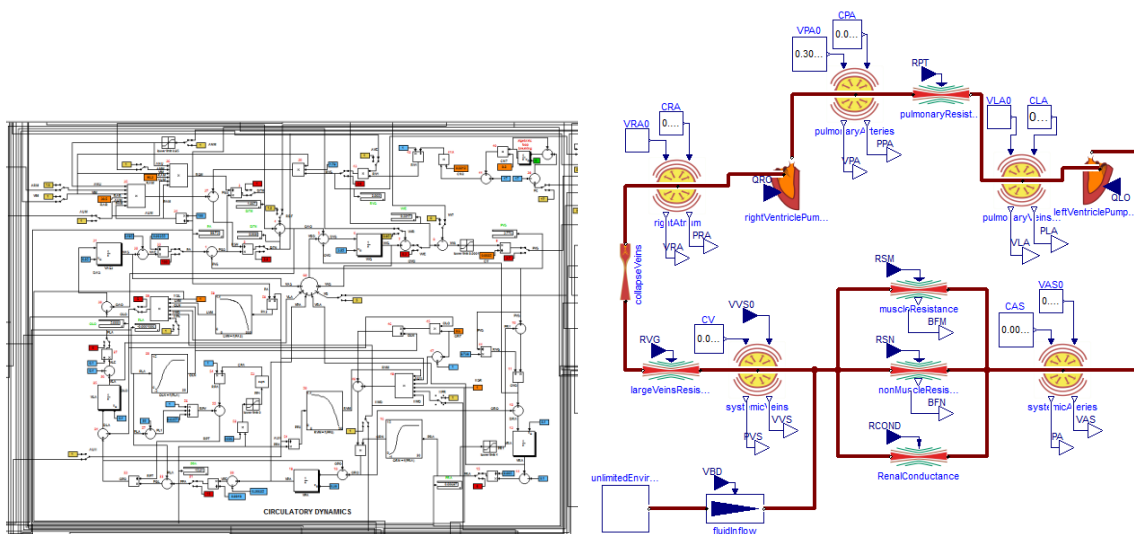

Figure 40: *Circulatory dynamics: Central part of the Guyton's original model* (84)*, implemented in Simulink (left) and Modelica (right). The structure on the left is very similar to the original diagram, i.e. the form in which the model was published. The complex web of lines and symbols require some toil if one wants to understand the model structure. Same model is implemented in Modelica on the right. The model structure is same as that of circulatory system itself. Two red quarter-circles or triangles in the upper left and right represent right and left ventricle and their pumping properties. The upmost part represents pulmonary circulation, while the three parallel branches at the bottom represent branches of systemic circulation. Circles with yellow lumen represent elasticity of blood vessels, blue and red elongated symbols in the branches of systemic circulation (and elsewhere) represent resistance of venules and capillaries. Each of these components is regulated by other parts of the model.*
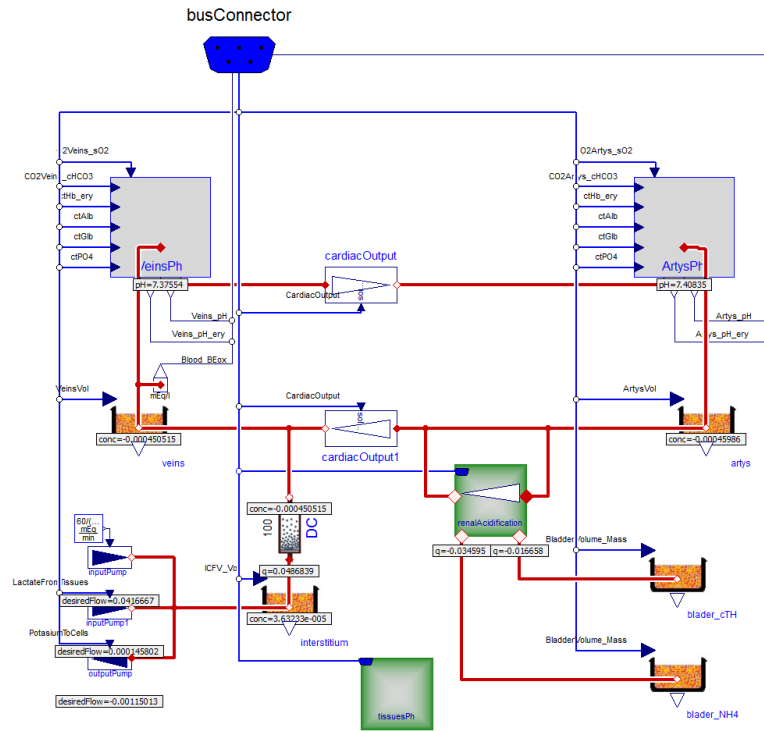
Figure 41: *Modelling the buffering properties of blood, including the effects of metabolic production of strong acids and renal excretion of the same, top layer. This compartment is part of HumMod, Golem edition. The gray boxes represent arterial and venous blood and contain equations (such as van Slyke equation) calculating values of so called dependent variables (such as bicarbonate) from invariants of Siggaard-Andersen.*

An example of a complex acid-base simulator, where I had a role in debugging the acid-base section is a Modelica/Dymola implementation of probably the most complex model of human physiology so far, HumMod of T Coleman and coauthors (85). The model was implemented by my colleague M Matejak. The Modelica implementation allows substantial simplification of model structure, because all the unnecessary symbols and code representing computing algorithms rather than underlying model logic disappear, an example of which is given in figure 40. The implementation gives the model more clarity, making it easier to understand for those interested.

The acid-base part of the original HumMod model is done according to the modern approach. Renal regulation is calculated from SID, where level of each strong ion is regulated in an independent fashion. This is exactly according to the original postulate of the modern approach, including the omission of the so called dependent variables and concentration on the so called independent variables. The result is an inability of the whole model to maintain stable plasmatic pH, which is

obviously not in agreement with the real behaviour and shows that important part of renal regulation was omitted. The tendency of the modern approach to encourage blindness to parts of reality was already discussed in previous chapter, in the section weaknesses of the modern approach. We have reworked this section of the model. Acid base chemistry is characterized by *invariants* of Siggaart-Andersen, similarly to Golem. The variables that change during buffering are calculated in both arterial and venous blood. Renal regulation is done using modified relationships of Ikeda (ref). These are based on known (measured) patterns of hydrogen ion excretion. Unfortunately, interdependence of bicarbonate and strong ions excretion is not included in the model, which is its current weakness. Top layer of this acid-base model is seen in figure 41.
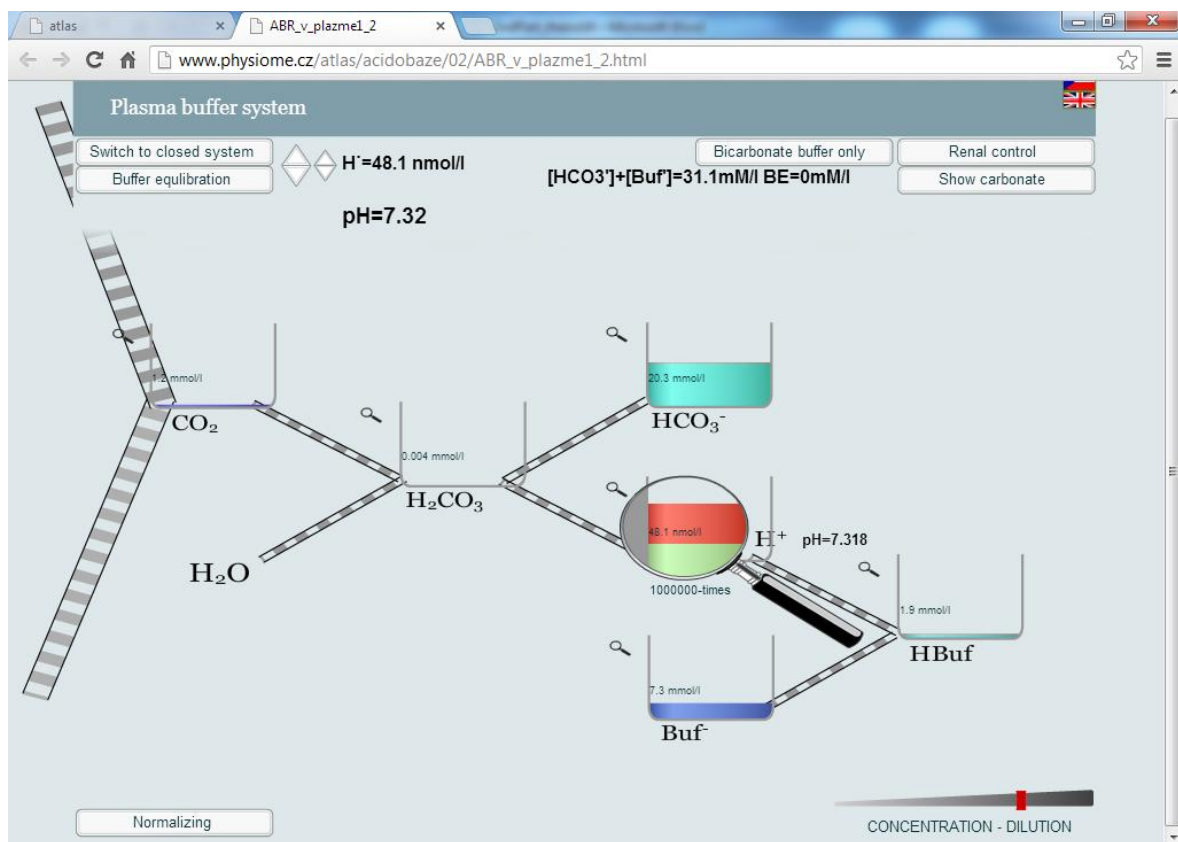

Figure 42: *Simple acid-base simulator based on a static model of equilibrium after each user intervention (equations of modern approach (22)). However, unlike a "typical approach to the modern approach", this simulator actually shows the equilibria of different forms of buffers, i.e. values of so called dependent variables. As discussed in the previous chapter, this gives much better insight than using the values of independent variables only. Pathogenesis of dilution acidosis is shown here. In dilution acidosis, concentrations of all buffers get diluted except for CO_2, which is constantly produced and set to a constant value by respiratory regulator. Less base and same amount of acid (CO_2) lead to acidosis.*

The user interface resembles cartoon-like pictures of the printed Atlas of Physiology (86) or Atlas of Pathophysiology (87). However, unlike printed illustrations, the pictures of the atlas are "alive" and often interactive – changes of variables of the simulation model are reflected in a change of the picture. There was a substantial technical development behind the Atlas, as described by Kofranek et al (88). I had a substantial role in the coordination of the whole project, as well as scenario writing.

Acid-base chemistry and physiology is included in two models of the Atlas. Large model of blood gas transport and a simple acid-base model based largely on the equations of the modern approach. However, unlike the other "approaches to the modern approach" the model actually shows the buffer equilibria that are described in the mathematical formulation of the approach. The model can also illustrate the disorders of dilution acidosis and contraction alkalosis. These disorders are often omitted as a possible pathogenetic basis of acid-base disorders, especially within the framework of the traditional approach.

Recently, we have substantially improved our simulator building technology. We now have a know-how and technological tools to convert simpler models built in Modelica environment into full, browser based simulators with multimedia animations and rich functionality. I and dr. Kofranek are designing a new series of aggregated acid-base simulators, starting with simple acid-base concepts, such as definition of pH and dynamic relationship between H^+ and OH^- ions and explanation of simple buffer behaviour and continuing to explain more challenging concepts, as those covered in this thesis (as is the relationship between variables of the modern and the traditional approach). These simulators are being gradually implemented by our collegues Filip Jezek, Martin Tribula and graphic designer Veronika Sykorova. Technical solution was developed mainly by Pavol Privitzer. Modelling support is given by Marek Matejak. Underlying models of these simulators are dynamic, where the equilibrium is achieved in time according to the chemical theory of reaction velocities. This allows user to see what is actually happening when the system transits from disequilibrium into a new equilibrium.

Figure 43 shows the structure of a single buffer model, implemented in Modelica. Again, model structure in Modelica represents real structure of the chemical equations. There are three reactions implemented in this model (white boxes *a, b* and *w*), each having its own there and back velocity. For a system of two products and two reactants reacting in 1:1 ratio, each velocity is dependent on the reactant concentration according to the formula

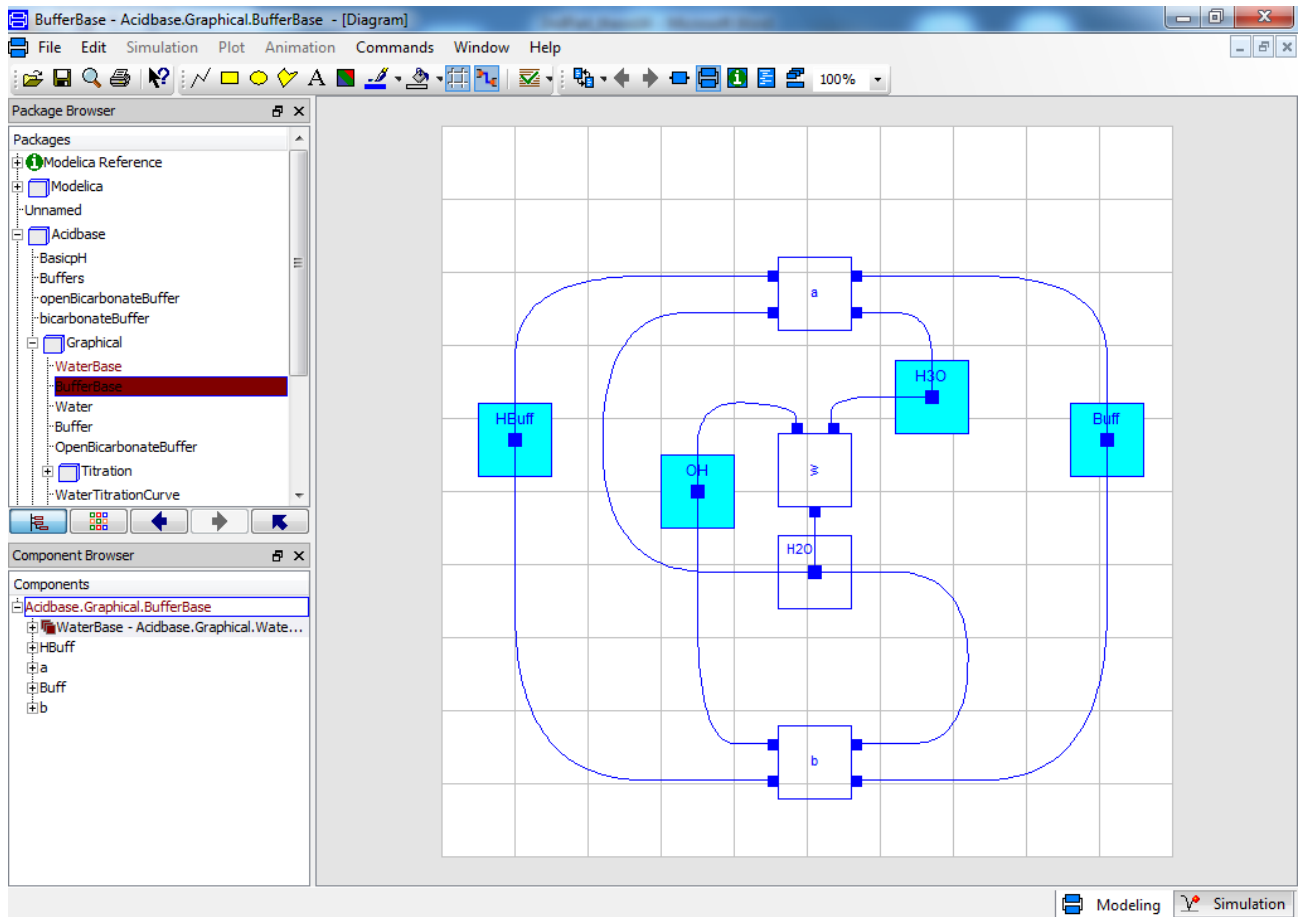$$\vec{v}_r = \vec{k} * [A] * [B] \tag{4.1}$$

Figure 43: Dynamic model of a single buffer acid-base system. This is a dynamic model of a system of five substances and three chemical reactions, as described in the text. The resulting simulation gives concentration of each substance as a function of time. This is the underlying model to the browser located acid-base simulator seen at figure 45.

$\vec{v}_r$ is the reaction velocity to the right, $\vec{k}$ is the reaction coefficient and [A] and [B] are the concentrations of reactants. The law for the reverse reaction velocity is same, except that original products now function as reactants

$$\overleftarrow{v}_r = \overleftarrow{k} * [Y] * [Z] \tag{4.2}$$

The velocity coefficients to the left and to the right and bound by the equilibrium constant of each reaction

$$K_A = \frac{\vec{k}}{\overleftarrow{k}} = \frac{[Y] * [Z]}{[A] * [B]} \tag{4.3}$$

The rate of change of each substance depends on reaction velocities. For instance, we have (for reactant A)
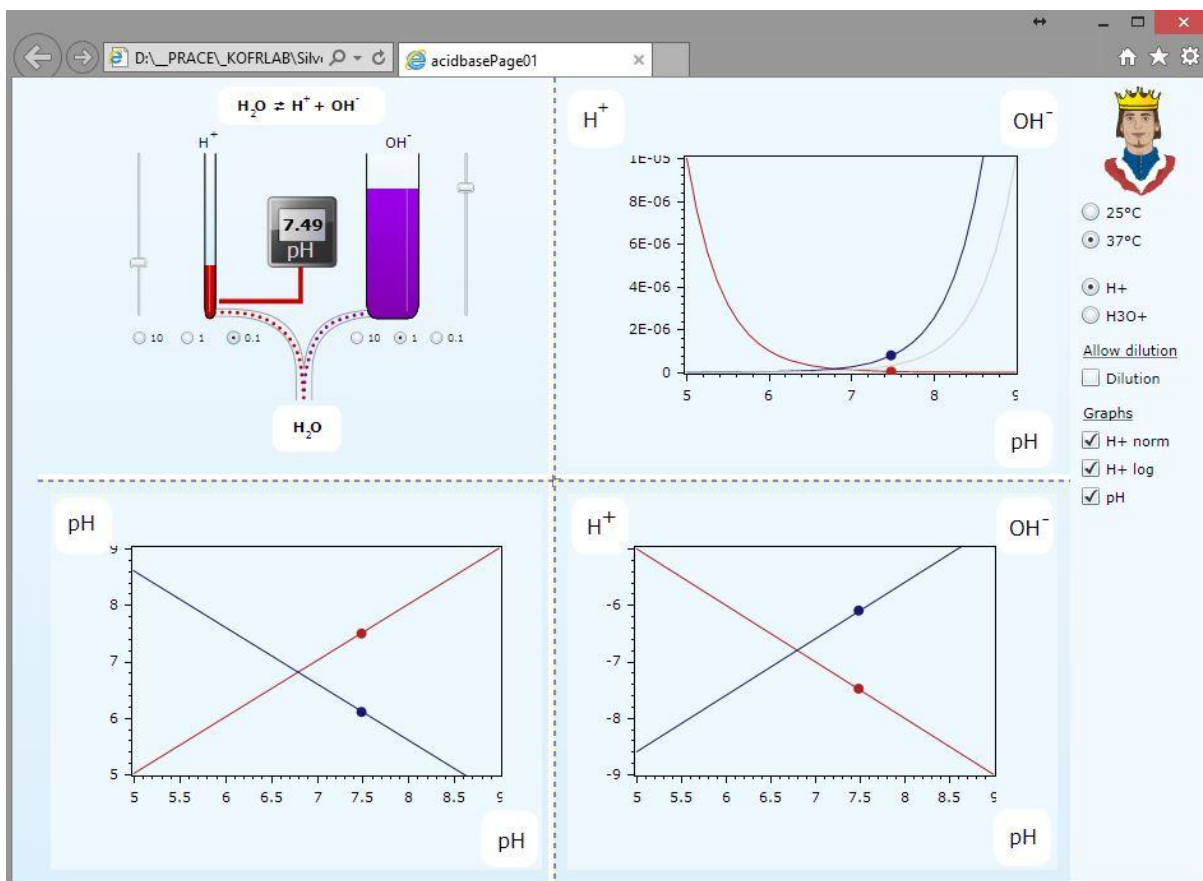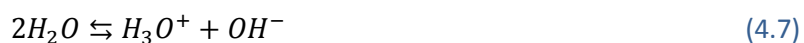
Figure 44: *First screen of the series of aggregated acid-base simulators. This first simulator focuses on the basic equilibrium between H⁺ and OH⁻ ions. User can add either H⁺ or OH⁻ ions by dragging the sliders in the upper left corner. This causes disequilibrium – points in the graphs and the levels in the test-tubes then gradually reach the new equilibrium. This screen is also used to explain well the relationship between concentration of hydrogen ions and pH. Tasks and questions and explicatory text can be rolled out from the left.*

$$\frac{d[A]}{dt} = \overleftarrow{v}_r - \vec{v}_r = \overleftarrow{k} * [Y] * [Z] - \vec{k} * [A] * [B] \tag{4.4}$$

and analogically for the other reacting substances.

There are three chemical reactions in the model seen at figure 43. These reactions are represented by the boxes named *a*, *b* and *w*. Each box represents following reaction, respectively

$$HBuf + H_2O \leftrightarrows Buf^- + H_3O^+ \tag{4.5}$$

$$HBuf + OH^- \leftrightarrows Buf^- + H_2O \tag{4.6}$$

$$2H_2O \leftrightarrows H_3O^+ + OH^- \tag{4.7}$$

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Thus, the model could be mathematically represented by five differential equations, one for each substance, where the rate of change of each substance is a sum of contributions from the chemical reactions in which the substance appears. Each reaction contribution is expressed by equation (4.4) or analogical.

So, even behind a relatively simple looking model, there is a more complex structure of differential equations. Resulting on-line simulator is seen in figure 44, which shows an OH^- H^+ relationship in pure water. Figure 45 shows a simulator of single buffer behaviour based on the mathematical model just discussed. This series of acid-base simulators is probably going to be part of the internet Atlas of Physiology and Pathophysiology. We plan to use the acid-base theory described in this work as a basis for the design of the more advanced screens (models) of the simulator, for instance the screen explaining the relationship between buffers and strong ions or the screen comparing the variables of the modern and the traditional approach.
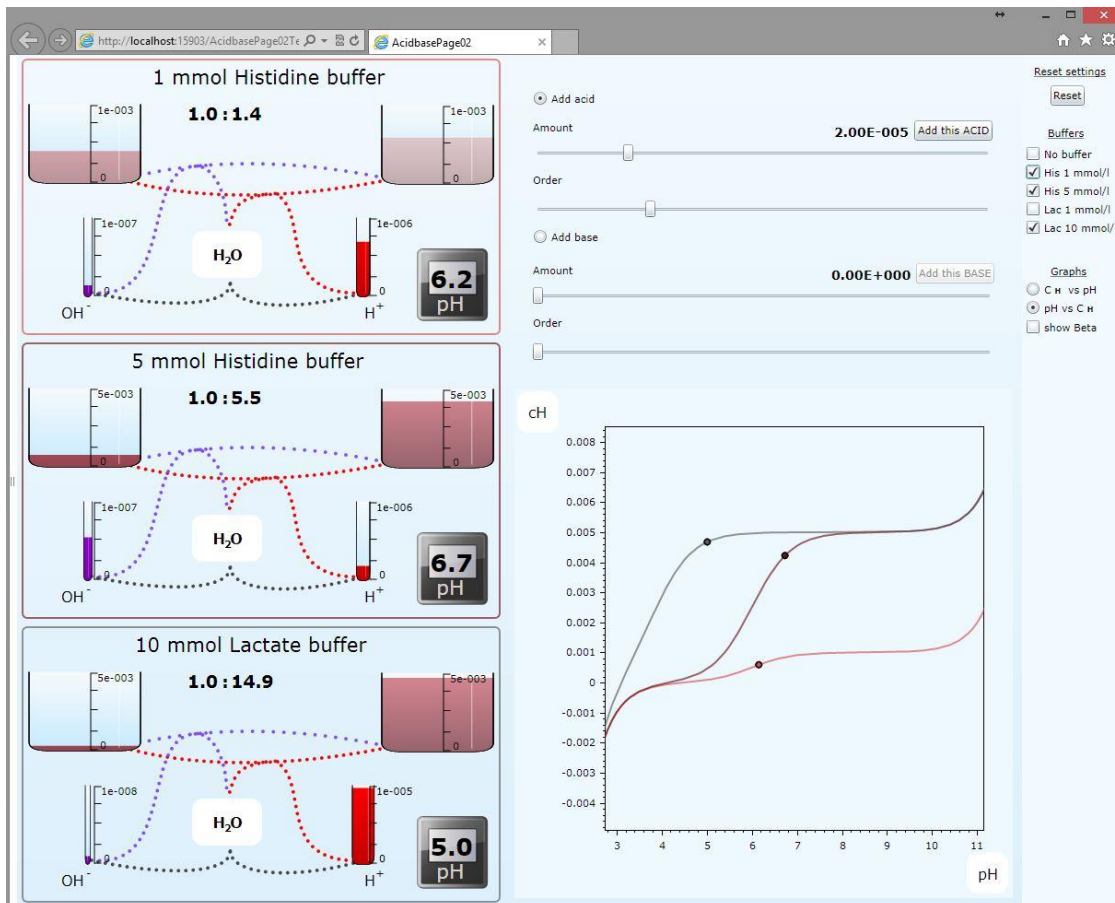

Figure 45: One of the series of the acid-base simulators. This simulator is focused on understanding basic properties of buffers. User can choose from four different buffers, fifth one being pure water (for comparison). The simulator is based on the dynamic model of buffering implemented in Modelica, as is depicted in the figure 43.

Reunified description of acid-base physiology and chemistry of blood plasma

Current clinical picture of the acid-base status description is not at all encouraging. A specialist in intensive care medicine, nephrology or pneumology is presented with two theories and two sets of parameters, while the underlying physiology and chemistry obviously has to be only one. Practicing clinicians generally have neither time nor training to make a detailed comparison such as the one in this thesis. Thus, they end up choosing their approach according to the local tradition, their psychological preference and the ability of each approach to "sell" the key ideas.

The concepts covered in the previous chapters can form a solid basis in the effort to reunify the clinical description of the acid-base status. Suggestions as to which parts of each approach seem reasonable and which ones less so and how can the two approaches be eventually used together are presented in this section. The reasons behind each choice are going to be discussed as well, so eventually, reader can make their own mind.

As discussed extensively in the previous three chapters, there are several possible buffering *invariants,* i.e. variables that do not change during buffering of added acid or base or hydrogen ion redistributions (they change by a step when the acid or base is added, which is why they can also be called interval variables). These include C_H, C_B/BB, *SID*, *BE* (derived from C_B/BB) and lately also the inverse of *BE*, called titratable concentration of hydrogen ion, ctH^+ (derived also from C_B/BB). The usefulness of each of them for clinical practice is going to be discussed in turn. There are also two possible choices measuring concentration of non-bicarbonate buffers, A_{tot} and β. Total concentration of non-bicarbonate buffers also does not change during hydrogen ion redistributions. Hence, these two quantities can be considered buffering *invariants* as well. Theoretically, any parameter of the first group can be used as a measure of acidosis or alkalosis, and any parameter of the second group can be used as a measure of total buffer concentration.

Before we start discussing parameters that stay *invariant* during redistributions and equilibrations, we should recall that there are also variables whose value changes during these processes. These variables are called dependent variables in the modern approach. The most important dependent variable is the concentration of bicarbonate $[HCO_3^-]$, concentration of hydrogen ion $[H^+]$ and its logarithmic measure pH. According to Stewart, values of dependent variables should not be considered in acid-base discussions. However, as was covered in detail in chapter 3, section weaknesses of the modern approach, this notion of dependent variables is flawed. There is no theoretical reason for not using the values of these parameters in clinical thinking and diagnostics, for instance when using so called Boston compensation rules. Their only limitation one should be

aware of is that they are not *invariants*. So, for instance, [HCO$_3^-$] changes with pCO$_2$. In case of bicarbonate, the dependence on pCO$_2$ can be gotten rid of by considering so called standard bicarbonate, i.e. [HCO$_3^-$] of the same blood or plasma moved to pCO$_2$ = 40 mmHg. Given the relationship between average BE and bicarbonate concentration discussed at figure 11, bicarbonate and especially standard bicarbonate can be considered satisfactory measures of metabolic acid-base disturbances for those, who want to use it.

For those who want to use the interval variables as measures of acid-base disturbances, there seem to be more options. First three include C_H, C_B/*BB* and *SID*. None of these parameters is completely anchored to a specific pH (chapter 1 and 2); therefore, their values can vary. Values of total titratable acid C_H and total titratable base C_B depend on where the line between titratable and strong acids and ions is drawn. Value of plasmatic *SID* is commonly taken as equal to *BB*. However, in more precise thinking, it can be different, measuring the charge of strong ions, whether free or bound to plasma proteins. In the latter case, its value depends on where the line between strong ion charge and buffer charge is drawn.

In the general chemistry, C_H would probably be the most used of these parameters, because it is a measure of total (bound and free) hydrogen ion concentration, being complementary to the pH as a measure of free hydrogen ion concentration. However, in acid-base physiology, C_H cannot be used as a good measure of metabolic disturbances, because CO$_2$ behaves as an acid and its turnover is much higher than that of any other acid or base (figure 9). This results in co-dependence between C_H and pCO$_2$ as can be seen in figure 46. Figure 46 also shows that C_H also changes during buffering of acid (by different amount than the amount of the acid added), when the bicarbonate buffer is open.

Both *SID* and *BB* can be used as measures of metabolic disturbances, as can be seen on figure 46. The two should be equal according to the simplified thinking of the equation (3.20), which is normally the level of detail used in clinical practice. It should be mentioned that the value assigned to the two is often neither *SID*, as was rigorously defined in the eq. (2.32), because parts of the strong ion charge are missed, nor is it *BB*, because it includes both *iSID* of the protein molecules and $Ci * \bar{z}_{max}$ term. There are so many possibilities as to which values to assign to these parameters (discussed extensively in chapter 3) that the effort in trying to find an agreement seems almost futile. However: Whatever value is assigned to these parameters, when there is a change, as an addition of X mmol/l of acid or base, these parameters change by an interval, which is exactly equal to the amount of added acid or base.
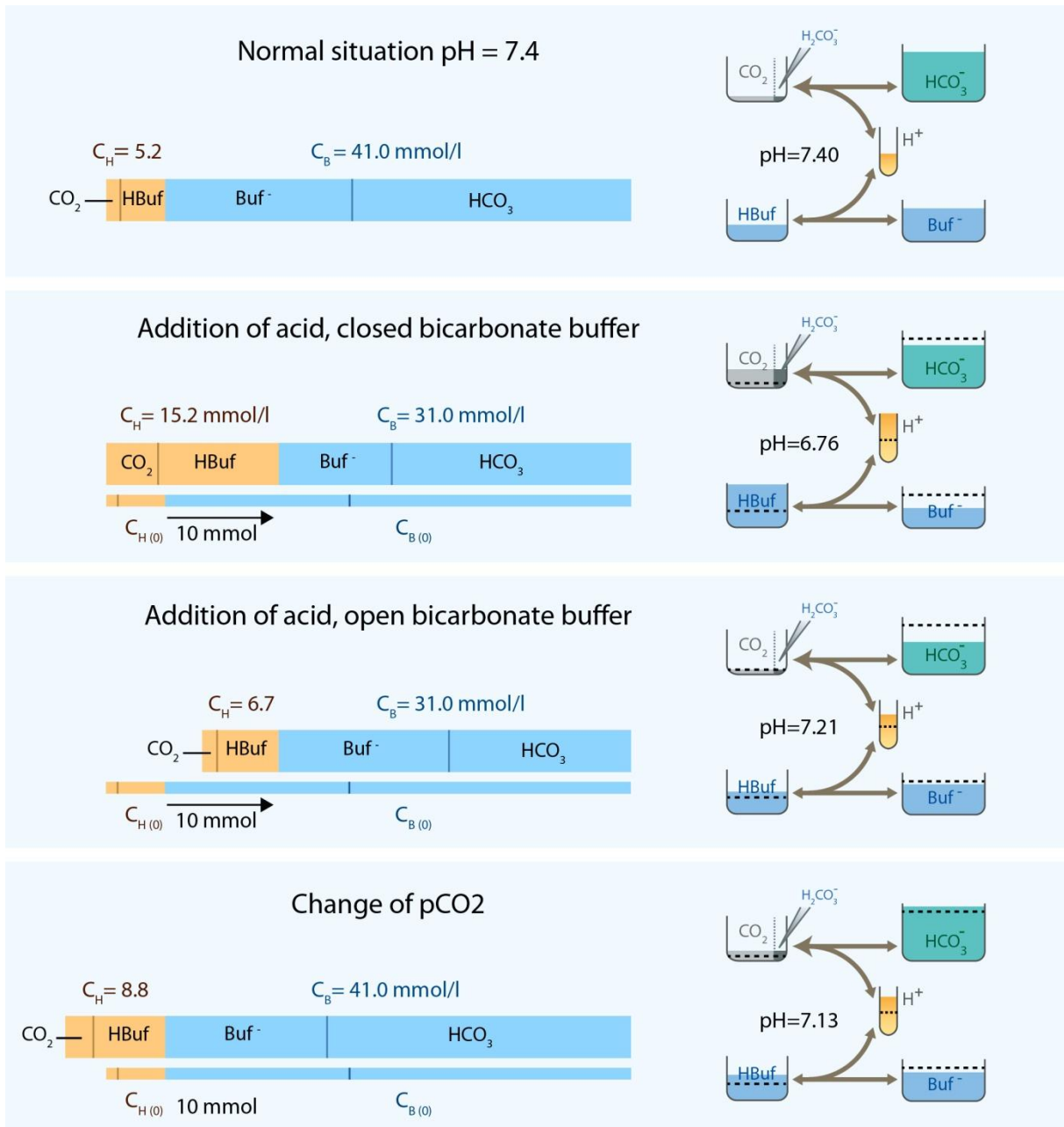
Figure 46: *Usefulness of total titratable acid C_H and total titratable base C_B as measures of metabolic acid-base disturbances in physiology. a) First part shows normal concentration of the bicarbonate and non-bicarbonate buffer bases and buffer acids, according to the values given in (9). Other values can be used, as is discussed in the text. This does not change the logic of the situations that follow. b) When 10 mmol of acid are added per liter of plasma with closed bicarbonate buffer (no CO_2 allowed to leave), pH changes substantially (to 6.76). At the same time C_B decreases by 10 mmol/l and C_H increases by 10 mmol/l. c) When 10 mmol of acid are added per liter of plasma with open bicarbonate buffer (pCO$_2$ allowed to equilibrate back to 40 mmHg), pH changes much less (to 7.21). At the same time, C_B still decreases by 10 mmol/l, while C_H changes only by 1.5 mmol/l. This is because CO_2 created during buffering is allowed to leave the system. Obviously C_H is not a good measure of*

metabolic change in this case. d) When pCO_2 changes from 40 mmHg to 80 mmHg, pH changes as well (to 7.13). Note that even though there is redistribution between HCO_3^- and Buf^-, total C_B behaves as an invariant with respect to changes of pCO_2. This is contrary to the behaviour of C_H, which increases during the process (both CO_2 and HBuf concentrations increase).

Various practical problems encountered when trying to use *SID* as a measure of acid-base disturbances are summarized in chapter 3, section weaknesses of the modern approach. From the clinical point of view, the principal ones are relatively large errors when determining *SID* as difference of major ion concentration, binding of strong ions to albumin and other proteins with changes of pH, chloride shift and varying presence of various minor anions (so called unmeasured anions). This is why even the proponents of the modern approach (17) do not advocate its direct use in clinical practice and use the value of *BB* instead, calling it strong ion difference effective (SID_E).

When *BB* (or SID_E) is used as a parameter in diagnostics of metabolic disturbances, its total value depends both on pH and total concentration of buffers. In this situation, it is crucial to know the total concentration of non-bicarbonate buffers, because otherwise, these two factors cannot be distinguished. This is described in the equation (3.32) for SID; it is same for the *BB* (equation 3.24) and seen also on figure 39. Theoretically, there is no difference between using A_{tot} or β to quantify the total concentration of non-bicarbonate buffers (eq. 3.39 – 3.41, 3.46 and 3.48).

BE was designed to provide effective separation between the buffer charge due to buffering (from pH 7.4) and due to change in concentration of buffers. This is done by means of its definition, where a difference of actual BB from the BB at pH 7.4 (called BB_{pHn} here) is taken (equation 3.1 and 3.2). BE has been criticized by the authors of the modern approach because it does not account for full difference in buffer charge from the norm. However, it has actually been constructed as to exclude the buffer base changes due to abnormal total buffer concentration and to focus only on the changes due to buffering (figure 39).

When we want to use the modern approach in clinical diagnostics, it actually means considering ΔBB = ΔSID_E from the norm (equation 3.23). The separation between the effect of buffering and the effect of total buffer concentration is usually done by also knowing the value of A_{tot}. The thinking in this separation is similar to the equation (3.32). For instance ΔA_{tot} is negative (there is a decrease in non-bicarbonate buffer concentration), this is considered to cause alkalosis. This alkalosis is exactly offset by the effects of concurrent negative change in ΔBB causing acidosis, as is covered in detail by Matousek et al (9). This is what was mentioned as a less practical way by which the information is structured in the modern approach, although the existence of the transformation between the two approaches means that one can always arrive to the same conclusion, if sufficient effort is made.

So, the problem of the modern approach is not that it doesn't describe the reality well (at least around pH = 7.4), but that it describes reality in unnecessarily complicated manner, where one has to consider interplay of two principal parameters (SID, A_{tot}) instead of one principal (BE) and one minor parameter (β). Furthermore, the possible choice of values for SID_E and A_{tot} is not unique, as was discussed in chapter three. SID_E is a parameter whose value is not well anchored and the values of A_{tot} and K_A depend on what value is assigned to SID_E. In fact, these two (A_{tot} and K_A) are just a different expression for the value of experimentally determined β and the value (partially) assigned to *SID* (equations 3.70 and 3.71). This is why I think BE should be used as a preferred *invariant* measure of metabolic disturbances in clinical practice; it should be given more forefront positions in acid-base considerations then the invariant *BB = SID_E*. The remaining two invariants (*SID* and C_H) cannot be used in clinical practice and physiology. *SID* cannot be used due to problems in measurement of all the strong ions and C_H cannot be used because it varies with pCO_2.

In the last years, Siggaard-Andersen has switched from advocating the use of *BE* into promoting the use of reverse parameter, so called concentration of titratable hydrogen ion, abbreviated *ctH⁺*. It is defined as "defined as the concentration of hydrogen ion determined by titration with strong acid or base to an end point pH of 7.40, at a *p*CO2 = 5.33 kPa, at 37 °C" (89). This definition means same as

$$ctH^+ = -BE \qquad (4.8)$$

Siggaard-Andersen argues that hydrogen ion, both free and bound is a more principal parameter than the concentration of the mirror parameter, hydrogen ion binding group. However, I consider this change unfortunate. By concentrating on the acid side, it gives an impression that the parameter is somehow related to ΔC_H, while in reality, it is only equal to $-\Delta C_B$. ΔC_H cannot be used as a measure of metabolic disturbances, because of the presence of open bicarbonate buffer (figure 47). Also, use of this parameter somehow suggests, that the principal mechanisms by which *ctH⁺* (or *BE*) changes in physiology are titrations by strong acids or bases. However, there are other mechanisms, and equally important, as is still going to be discussed at last. And finally, the change in principal known parameter is a further hindrance to the use of the traditional approach, where a clinician interested in acid-base looses ground as to how things are in relation to the previously used concepts (BE) and the modern approach.

This might be a good place to discuss the issues of reader accessibility of the relevant literature. Here, the modern approach seems to target the clinical audience much better; its theory is structured gradually from simpler to more complex and explained in well-known chemistry concepts and simple mathematical apparatus. For instance, titration curves of complex buffers are approximated with a curve of single buffer, which is a more straight-forward concept then

linearization, albeit less precise (as shown in the chapter 2, section concept of linearization of protein buffers). On the contrary, the articles of the traditional approach and the monograph of Siggaard-Andersen (28) seem to target more at chemists and clinical biochemists. Reading them requires proficiency in calculus and getting used to the complex notation. A clinician generally has neither time to get used to the difficult writing style nor good knowledge of advanced mathematics, which is generally not a focus in medical training. The authors of the traditional approach have never tried to explain the key concepts of their approach in simpler language to this wider, but important audience of users. For instance, there seems to be a lack of "everyday language" literature explaining the reasoning behind the general formulation of the van Slyke equation - literature that would explain why it looks the way it looks and what it tells us. Similarly, there was never a discussion around the change from BE to ctH^+. Father of the BE, O Siggaard-Andersen simply does his things and leaves the clinicians to believe him or not – take it or leave it. Given the complexities and challenges of acid-base physiology, this might not be a very wise strategy.

I believe the lack of relevant literature aimed at explaining the traditional approach to the important clinician audience is the key psychological reason why the modern approach has attracted so much attention, even though its main concepts are often oversimplified and sometimes even flawed (as is the concept of dependent and independent variables). In this context, it might be actually surprising to learn that the modern approach offers more complicated description of reality (two principal parameters) than the traditional approach (one key parameter and one secondary), without offering additional information.

As already mentioned, there is no difference in precision between the use of A_{tot} or β to quantify the total concentration of non-bicarbonate buffers (eq. 3.39 – 3.41, 3.46 and 3.48) – both are directly proportional to the concentration of key buffers and each other. The problems with determination of A_{tot} and K_A have actually led some of the authors associated with the modern approach to advocating the use of β instead, albeit rather discretely (17). An interesting question, already partially discussed, is whether the changes in A_{tot} or β cause alkalosis or acidosis. In the modern approach, the changes of A_{tot} have been shown to cause substantial changes of pH (5), (4), called hyperproteinenic acidosis and hypoproteinemic alkalosis. These pH changes have never been reported with regard to β in the traditional approach, which seems contrary to the interchangeable nature of these two parameters.
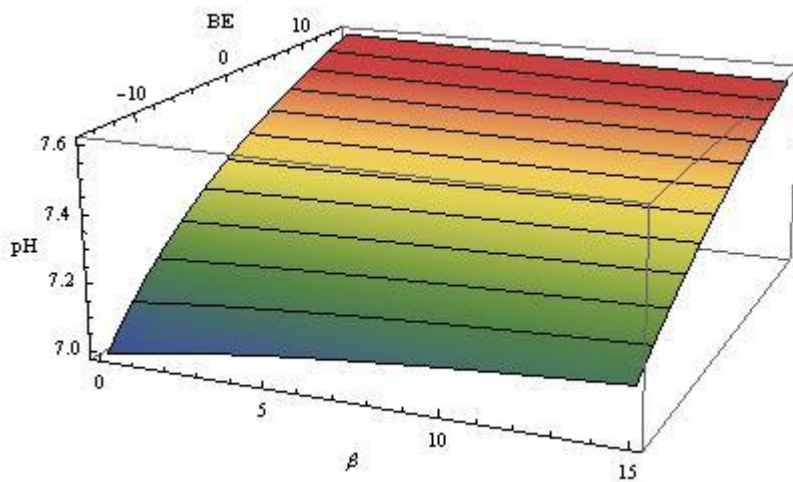
Figure 47: *3D plot illustrating a relationship between β, BE and pH. pCO₂ is kept constant, equal to 40 mmHg. As can be seen, pH varies mainly with BE. For very acidic pH (negative BE) and to a smaller degree for very alkalotic pH (positive BE), it also varies with β. However, around BE = 0, no or very small relationship between pH and β is seen.*
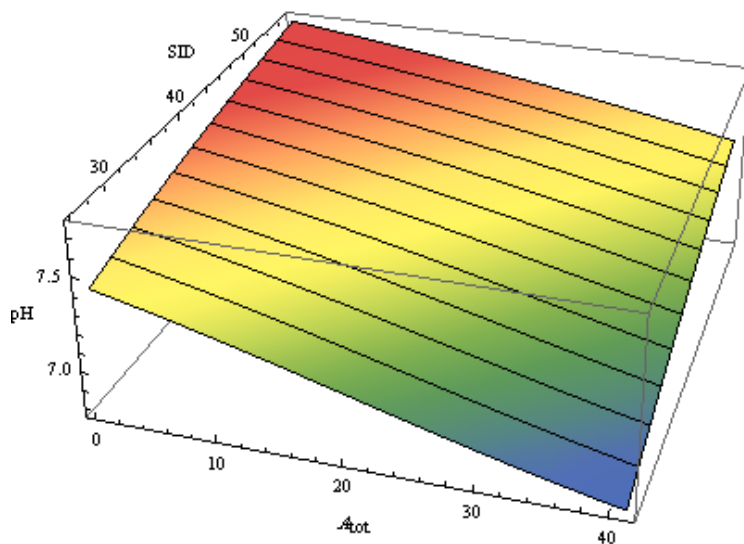
Figure 48: *3D plot illustrating a relationship between A_{tot}, SID/BB and pH. pCO₂ is kept constant, equal to 40 mmHg. As can be seen pH varies almost equally much with SID/BB and A_{tot}. The dependence on A_{tot} is named hyperproteinemic acidosis (high A_{tot}) and hypoproteinemic alkalosis (low A_{tot}). There is a direct proportionality between A_{tot} and β; same relationship would be seen if pH was plotted as a function of SID/BB and β. What is a key determinant of the shape of the graph is that A_{tot} is varied while SID/BB (as it is commonly understood, i.e. not including iSID) is kept constant.*

The answer to this apparent problem might seem surprising at first. For the phenomena of hyperproteinenic acidosis and hypoproteinemic alkalosis to appear, it is actually not important which parameter we choose in quantifying the total concentration of buffers (i.e. A_{tot} or β). It is only

important which other *invariant* of the other group (quantifying buffering) stays fixed during the change of A_{tot} or β. When *SID = BB* stays fixed during the process of changing plasma protein concentration, substantial changes of pH are seen during the process and described as hyperproteinenic acidosis and hypoproteinemic alkalosis. When BE is fixed during the process of changing plasma protein concentration, the observed changes of pH are much smaller (and even zero at 7.4). The relationships are plotted in 3D graphs on figures 47 and 48. Put simply, these interesting relationships can be explained by the acid form of non-bicarbonate buffer changing concentration when SID is kept constant, while both acid and base forms change almost in proportion when BE is kept constant. Also recall, that *SID* as it is commonly used is only a simplification of the situation and the value determined can vary due to different reasons. For instance, in a more complex thinking, it should also include *iSID*, i.e. strong ion charge on plasmatic proteins themselves. Astonishingly, the relationship between albumin concentration and pH would look different for each of these different determinations of *SID!* This is because each determination includes slightly different strong ions, and their charge is kept constant while concentration of albumin is varied. This means that each variation has to happen in different proportion of acid and base forms of albumin buffer residues, resulting in different changes of pH.

Given the direct proportionality between A_{tot} and β as possible measures of non-bicarbonate buffer concentration, it seems that the choice as which one to use is arbitrary. However, remember that A_{tot} is a part of a single K_A buffer representation; titration curve that this representation produces is not as good a fit to true (measured or theoretically approximated) titration curves as a linear approximation. Furthermore, A_{tot} is a parameter, whose value cannot be directly measured, of course its value depends on concentration of albumin and other buffers, but the problem is what should be its normal value at normal concentration of albumin. That, however, depends on the normal value assigned to SID, which is partially arbitrary (chapter 3, relationship between modern and traditional approach). On the contrary, β can be directly measured and can be considered well observable. So, again, we have a correspondence, yet choosing the variable of the traditional approach, i.e. β, seems more substantiated.

So, what is left of the modern approach? Among the possible *invariant* measures of metabolic disturbances, BE appears to be the best option. Among the possible *invariant* measures of non-bicarbonate buffer concentration, β appears to be the best option. Both of these are parameters of the traditional approach. Besides this, the modern approach was shown to contain substantial weaknesses (chapter 3). In fact, is there anything left at all after all this scrutiny? Surprisingly, yes. First of all, there is the improvement that the modern approach has brought to the use of anion gap

(AG), a parameter used mostly for estimation of the concentration of unmeasured anions. Their increased concentration can be an important parameter in diagnosing a type of metabolic acidosis. However, without the correction proposed by the authors associated with the modern approach, presence of unmeasured anions could be missed in the presence of hypoalbuminemia (64), (9). Even more precise determination of the concentration of unmeasured anions offers so called strong ion gap (SIG) (18). In fact, SIG equals the concentration of unmeasured anions. Recently, this parameter has been shown to predict trauma patient mortality better then pH, $[HCO_3^-]$ or lactate measurements (90). The only drawback to its use is its slightly more complex calculation. Unlike AG, to compute SIG requires at least hand calculator.

These improvements wouldn't be possible without a detailed model of albumin charge as a function of pH, which is associated with the same (or overlapping) group of authors (65), (40), (43). And finally, the modern approach has put more emphasis on the link between the acid-base and electrolyte physiology. Although original Stewart's notion of the relationship between dependent and independent variables is flawed (70),(chapter 3), meaning that also his notion about the strong ions concentrations govern the concentration of buffer ions is also flawed, electroneutrality and the interplay between strong ions and buffer ions is important. This is especially true for the renal regulation of acid-base and electrolyte homeostasis. Each inorganic ion is regulated separately, yet in concert with other ions. This is also true for bicarbonate and the hydrogen ion activity (pH). The regulation is complex, because several parameters that have to be regulated as well are linked to the excretion of individual ions (e.g osmolarity or pH). Furthermore, electroneutrality of both plasma and primary urine has to be kept in all moments, which can both limit or fasten the transport of cations and anions.

 It is now a well established knowledge that a primary disturbance in renal transport of strong ions can lead to an acid-base disorders as well. An example includes Bartter's syndrome, congenital disorder characterized by hypokalaemic alkalosis, low blood pressure and severe volume depletion, where the primary disturbance is a mutation of renal Na^+-K^+-$2\,Cl^-$ co-transporter (91) or paradoxical aciduria in hypochloremia (92), (93), (36). These examples show the strong link between electrolyte and acid-base physiology. Therefore, when assessing the acid-base status, the concentrations of the main inorganic ions should be taken into account, perhaps including the value of inorganic SID, i.e. one calculated from the concentrations main inorganic ions. Also, I believe that there should be further research that would clarify the behaviour of renal acid-base regulator in the conditions of abnormal ionic concentrations, abnormal concentration of albumin and abnormal inorganic SID. This

research might even show that SID should be, after all, given some role in acid-base quantification, albeit different than originally envisioned by Peter Stewart.

However, this connection between strong ions and buffer ion should not be perceived as if the strong ions per se had properties of acids or basis, which they had according to surpassed Arrhenius theory. As Christensen (94) has noted already in 1950's, long before the strong ion was conceived: "The only reason the neutrality can be described by the relationship between the fixed anion and the cation levels is that this relationship can give us indirectly the concentration of the *buffer anions.* These, together with the H^+ which they tend to bind, are the real actors in the drama. . . . As long as we treat the fixed-ion levels as a *reflection* of the hydrogen ion distribution rather than the *cause,* we have gained a valuable ancillary approach."

The reunited description should take into account possible physiological mechanisms leading to the acid-base disturbances (95). Figure 49, which is equal to the figure 9, provides good starting point to the discussion of possible causes. These include:

1) Change in the elimination of CO_2 or a change in the rate of production of CO_2. Because significant changes in the production of CO_2 are considered physiological (exercise), only the changes on the elimination side are among pathologies. Most important cause of disturbance is lung disease, which can first manifest during increased demand of elimination (exercise). Key diagnostic parameter is pCO_2.

2) A change in the inflow of hydrogen ion, most commonly its significant increase. The increased inflow can be from the metabolism, or from ingested toxic substances. The hydrogen ion reacts with both non-bicarbonate base and bicarbonate, decreasing its concentration. When the inflow exceeds regulatory capacity of kidneys, change in BE, $[HCO_3^-]$ and SID follows. The dissociated anion generally causes an increase in AG corrected and SIG. Analogical, but reverse situation occurs during increased inflow of base.

3) Loss or increased inflow of bicarbonate. This leads to the change in BE, $[HCO_3^-]$ and SID by other means than buffering acid/base. AG corrected and SIG are generally unchanged. Loss or increase in concentration can typically occur in gastrointestinal tract or kidneys. Concentrations of other ions are also an important player in these processes.
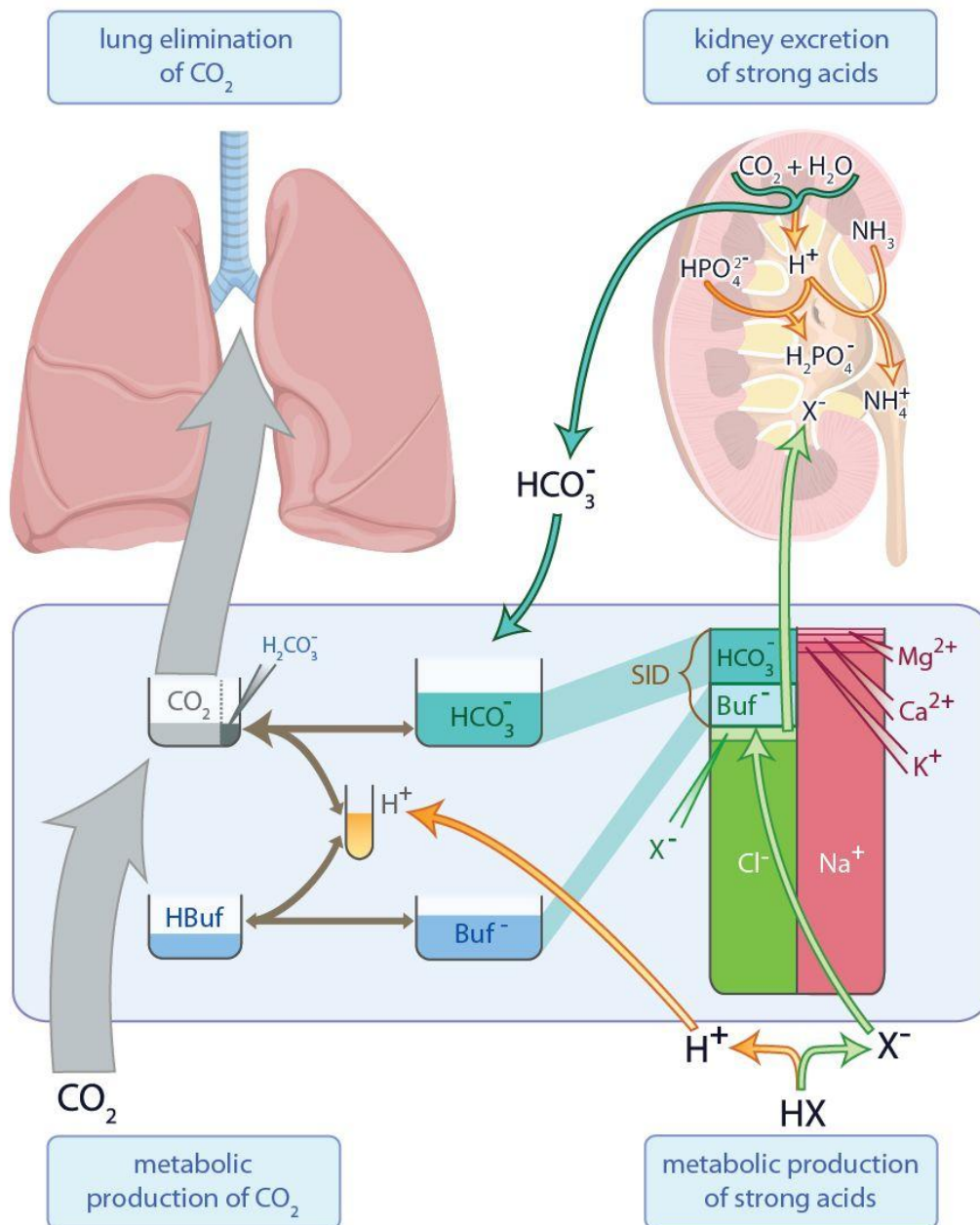
Figure 49: *Plasma buffer and electrolyte system in the context of physiological regulation. pH is determined by the equilibrium of all buffer systems that are coupled by concentration of hydrogen ion. The charge of buffers is in equilibrium with the charge of other ion, maintaining electroneutrality.*

4) Change of concentration of non-bicarbonate buffers. Loss can happen anywhere, increase of concentration happens mostly in liver. This can be quantified by β (or possibly A_{tot}). The resulting pH change depends on which other *invariant* or variable is kept constant i.e. under which conditions do we describe the process. These changes, if isolated, probably do not tend to cause significant acid-base disturbances in vivo (i.e. BE stays constant during the change rather than SID).

5) Dilution or concentration of the whole buffering system. These changes are caused by dilution with crystalloid solution (containing inorganic ions only) or loss of such solution. All buffers change their concentration, except CO_2, which is maintained constant by the respiratory regulator (50); dilution results in acidosis, concentration in alkalosis. There is no single parameter to indicate this possibility, so it should be thought of based on the administered infusions, concentration of electrolytes, change in weight, etc. If the disturbance is caused by extra water or missing water, return of the volume back to normal should treat the disturbance (in dilution acidosis, parameters like BE or *ctH+* indicate loss of base or an increase of titratable acid, but this has not been lost or infused, system has just been diluted).

Summary

This thesis addresses an important problem of biomedical acid-base theory, where there are two apparently contradictory descriptions, while the underlying physiology and chemistry obviously has to be only one. These two descriptions are called the traditional approach, based mainly on the work of Siggaard-Andersen (96), (28), (55) and the so called modern approach, based on the work of Peter Stewart and his followers (54), (13), (40) , (17), (18), (47). Modern approach is based on the use of three principal, so called independent variables (pCO_2, SID, A_{tot}), while the traditional approach generally uses only two principal variables (pCO_2, BE). This leaves every medical professional who needs to understand and use acid-base concepts in their work (intensivist, nephrologist and others) before an uneasy choice as to which description should be used. A researcher, who wants to build computer models of acid-base chemistry, small or large, helping in clinical decision support or serving as educational simulators, is put in front of a same choice. Unraveling the relationship between the two descriptions might even be the most difficult task in modelling already complex field of acid-base physiology and chemistry.

An elegant formalism to the description of acid-base phenomena in solutions of complex composition was developed by Guether two decades ago (23). This work starts by thoroughly explaining the formalism, so that its universal characteristics can be appreciated. Well established basic notions of acid-base chemistry and physiology are also introduced. This serves as a basis for the deductive work that covers some more advanced topics of acid-base theory, including titration curves of proteins, relationship between bicarbonate and protein buffers, strong ion charge and

protein buffer charge and fuzzy division between strong ions and buffer ions. This is amended by titration curves constructed using simple models built in Microsoft Excel, while other parts of this thesis also use simpler models built in Wolfram Mathematica and Modelica / Dymola. The modeling work then proceeds to comparing various models of albumin, principal protein buffer of blood plasma. Original theoretical concepts of this work, such as pH-pK_A criterion are validated by the behaviour of these models.

The two approaches to the description of acid-base chemistry of blood plasma are then introduced in the historical perspective of their development. This also gives an opportunity to link them to the general theory presented in the beginning, as both approaches are older than this general formalism and to my knowledge, thorough comparison has not been done yet. The general theory also provides a necessary apparatus to elucidate several fundamental weaknesses of the modern approach. Detailed mathematical comparison of the two approaches is carried out subsequently, showing that there is a unique transformation and neither that approach contains extra information to the other one. Out of this comparison, reunited description is suggested, combining the strengths of both approaches and eliminating the weak points. More is taken from the traditional approach, where several key concepts appear more rooted either in clinical needs or in experiment. Finally, the use of the gained insight for building educational acid-base simulators is outlined, returning to the point where it has originally started.

While the use of the united theory in computer modeling was the primary focus, the process also gave results that can be directly used in clinical diagnostics and clinical practice. To my knowledge, several accomplishments of this work can be considered original contributions to the field of biomedical acid-base theory. First of all, this appears to be the most detailed and comprehensive comparison of the two approaches published so far, both between themselves with regard to the general formalism of Guenther. In comparing them, this work is also the first one to publish explicit transformation relationships between the variables and equations of both approaches, including the transformation between *SID*, A_{tot}, *BE* and β.

By generating buffering curves of random pK_A proteins, the work also demonstrates that there is a theoretical reason, why the linear approximation to protein buffering curve generally performs better than single pK_A approximation. This is one of the weaknesses of the modern approach, which hasn't been given much discussion so far. Finally, the problem of identifiability of the modern approach parameters seems to be approached in a novel way.

Original contribution is also in the structure and design of the presented simulators.

The development in the biomedical acid-base theory has been astonishing in the past two decades. First, it seemed that the traditional approach has been done with. New articles were often strongly promoting the modern approach, steaming with titles and strong statements like: "Acid-base physiology in the post-Copernican era" (97), "strong ion model offers a unique insight into the pathophysiology of acid-base derangements" (2). "A revolution is underway in the clinical assessment of acid-base status" (20) and "another nail in the coffin of traditional acid–base quantification" (1). However, at the same time, the opposition against the modern approach was also growing (50), (70), (6).

Gradually, articles showing common links also appeared. Several reviews have been published, comparing the two approaches to acid-base chemistry. Schlichtig et al were the first one to compare the clinical usefulness of both approaches, as well as showing parts of the mathematical link between them (19), (10). Kellum authored a qualitative clinical review (8), pointing out many common links and advising the clinicians to use both approaches in concert rather than contradiction. Some of the features of the relationship between the two approaches, covered here in formal mathematical notation (including the key relationship (3.32)), were also described in words by Siggaard-Andersen (6). Siggaard-Andersen was also the first one to point out that the concepts of hyperproteinemic acidosis and hypoproteinemic alkalosis depend on the choice of other independent variables (figures 48 and 49), although again only in words. Maybe due to the critical tone of the father of the traditional approach, his explanation never seemed to get much appreciation. Major breakthrough came, when Wooten published a theoretical analysis (7) that links both approaches together mathematically and placed them in the context of elegant Guenther's formalism (23). Wooten was the one to introduce Guenther's ideas to clinical biochemistry and acid-base physiology. However, due to the limited space of a common physiology article, Wooten's review contains surpassingly condensed mathematical expressions (such as the equation 1.70) with only minimum commentary, possibly yielding it inaccessible to most of interested readers.

This thesis builds on the work of Guenther, Wooten and Siggaard-Andersen. Larger format of the text makes it possible to properly introduce Guenther's ideas, which was done on a physiologically important phosphate/ phosphoric acid buffer system. While linking Guenther's ideas to the common linear approximation of protein buffering, this thesis shows that linearization makes more sense than single K_A approximation. Same ideas are illustrated while comparing different models of albumin. The thesis also builds on the article of me and my co-authors, which compared both approaches both mathematically and clinically (9). The article describes how the parameters of the modern approach can be identified given the known charge and slope of buffering curve. This thesis brings this further by showing that the values of A_{tot} (per gram of albumin) and K_A critically depend on the normal value

of *SID* (i.e. also the buffer charge), which is partially arbitrary. The lack of consensus over the value of *SID* means that there is not a unique way of identifying the values of A_{tot} and K_A.

The presented analysis shows that reunified description is not only possible, but also that it should, using pure rationality, contain much larger portions of the traditional approach than expected from some late opinions.

This thesis was originally envisioned to include description of full blood as well. Indeed, the traditional approach is commonly used for the description of full blood or even extracellular fluid. The modern approach has typically been used just for description of blood plasma, even though Wooten (58) and Rees et al (60) have broadened it to the whole blood as well. Since the major disagreement among the two approaches is in the acid-base description of blood plasma, limiting comparison to the blood plasma only has shown to contain enough substance. This approach has helped to cover the topic in a level of detail that would otherwise not have been possible. In doing so, the thesis has focused on the mathematical aspects of describing the buffer systems, both in detail and in an overview. Physiological regulatory systems of acid-base were used to frame the theory. Again, the structure of these regulators could have been dealt with in much more detail, has this interesting area of mathematical formalization been focused on.

There are several possible directions of future development of the field covered in this thesis. Hopefully, this work might help to stimulate a renewed interest in the possibilities of diagnostics of acid-base disorders among clinicians. When assessing clinical usefulness of various parameters, it is not only important to correlate various parameters with various outcomes, but also to understand the relationships between them. SIG, for instance, measures different thing then BE, and they should only be compared with this knowledge. Or, to give another example, A_{tot} and SID can be combined to give similar information as BE.

Development of educational simulators that would depict the concepts of this thesis in a more graphical way (rather than writing them down as formulas) can greatly enhance the intuitive understanding of these parameters and of coupled acid-base equilibria. Be it with the simulators from us or from others, I believe that guided simulation is the future of teaching acid-base.

In terms of modelling research, there might not be much more to do in terms of plasma other than getting ever more detailed description of individual buffer moieties on individual plasma proteins, which seems like rather tedious job. Much more interesting area lies in developing new models of transport and equilibrium phenomena on the red blood cell membrane. These models should ideally be based on the first principles, as currently used formulas are most commonly semi-empirical.

Modelling the hemoglobin and intracellular buffering properties and possibly combining these models with the models of membrane transport and plasma is another promising area. Finally, interesting area, where the reunified description of acid-base chemistry might find it use is in modelling acid-base regulation of the kidney. The complicated interplay between buffer ions, bicarbonate, hydrogen ion and strong ions is fascinating; thanks to electroneutrality, both buffer and strong ions are important and neither part should be neglected (which happens with the buffer ions in the HumMod model (85) or with the strong ions in the model of Ikeda (76)). Indeed, renal regulation of the internal environment is an area integrative physiology, where combining insight and variables of both approaches might bring new surprising results in the future.

Conclusion

Widespread use of computers and the development of formalized description of acid-base equilibria even in complex solutions give us new possibilities to understanding acid-base physiology and chemistry of physiological fluids. The two most used approaches to the description of acid-base status of plasma were compared with this knowledge, leading to the following conclusions: 1) Modern approach of Peter Stewart contains major weaknesses. 2) There is a mathematical transformation between the equations and variables of the traditional and the modern approach.

The existence of transformation does not mean that the information in each approach is structured equally well in sense of its direct clinical usefulness. However, it gives a framework for combining the strong parts of each approach and using them in concert. A rational choice for diagnostics of acid-base disturbances seems to be the combination of parameters pH, pCO_2, $[HCO_3^-]$, BE, β, SIG or AG corrected, $[Na^+]$ and $[Cl^-]$.

The field is complex, but not beyond grasp. Interactive computer models can be an effective teaching tool to understanding the interplay between various parameters. Indeed, the future of teaching acid-base physiology seems to be in the use of educational simulators based on these models.

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List of abbreviations

α_j — Ratio (percentage) of substance in the form (species) of j hydrogen ions bound.

A^- — Base form of non-bicarbonate buffers, weak (buffer) base, especially in the modern approach; alternatively base form that is negatively charged as opposed to electroneutral base form *B* (only in this thesis)

$[A^-]$ — Concentration of non-bicarbonate buffer base, in meq/l

$[A^-]_N$ — Normal concentration of non-bicarbonate buffer base, i.e. concentration at pH = 7.4, pCO2 = 40 mmHg and normal average concentration of all buffers (in this last condition, it differs from NBB_{NonB}). Notation from the modern approach.

AG — Anion gap, in meq/L, generally computed as $[Na^+] + [K^+]-[Cl^-]-[HCO_3^-]$

AG_{corr} — Corrected AG, in meq/L, AG corrected for abnormal concentrations of albumin. Correction formula: $AG_{corr} = AG + 0.25*(44 - C_{W,Alb})$. $C_{W,Alb}$ (concentration of albumin in g/l).

$[Alb^-]$ — Concentration of the negative charge on albumin, which is generally viewed as equal to albumin buffer base. However, this is not true on a close inspection, as the charge is composed of the residues that behave like strong ions and both negatively and positively charged buffer residues, in meq/l.

$[A^-]_{pHn}$ — Charge of plasma buffer base at pH=7.4 and pCO_2 = 40 mmHg, directly proportional to A_{tot}. It depends linearly on the concentration of albumins, globulins and phosphates. It should be equal to NBB_{NonB}. Term from the modern approach.

A_{tot} — Apparent non-bicarbonate buffer concentratition of the modern approach, i.e. sum of the concentration of the acid and base form of non-bicarbonate buffers. According to the definition, this should be approximately equal to the concentration of the buffer residues as determined according to the pH - pK_A criterion. However, in practice, this ends up being only a model parameter, whose value depends on measured charge and buffer capacity, without much relationship to the true number of buffer residues.

A_{tot2} Value of A_{tot} determined in a more complex thinking that takes into account internal structure of buffer proteins, as opposed to the value determined by common (simplified) thinking that equals negative charge and concentration of buffer base.

$(A_{tot})_N$ Normal average concentration of non-bicarbonate buffers (originally referred to as weak acid by Stewart); in plasma, it includes albumin, (globulins) and phosphates.

β   Buffer capacity or buffer value (these terms are used interchangeably in this work), amount of strong base needed to increase pH by 1, in meq/L. In physiological literature, this term generally refers to non-bicarbonate buffer capacity, i.e. $β_{NonB}$

$β_i$ Internal buffer capacity or buffer value of substance i. It depends on concentration (C_i) and pH. In mmol/l.

$β_{M (Alb)}$ Molar buffer capacity (value) of albumin, given as 8.0 by Siggaard-Andersen

$β_{M(Pi)}$ Molar buffer capacity (value) of phosphates, determined at 7.4. Given as 0.309 by Siggaard-Andersen.

$β_{W (Alb)}$ Mass buffer value of albumin, i.e. buffer value of 1 g/l of albumin. The coefficient is given as 0.123 by Figge et al and 0.120 by Siggaard-Andersen (recalculation).

$β_{W (Glb)}$ Average mass buffer value of globulins, i.e. buffer value of 1 g/l of plasma globulins. Given as 0.075 mmol/g by Siggaard-Andersen; authors of the modern consider this value unimportant due to the nearly zero charge of globulins. This conclusion is, however, not substantiated by general theory.

$β_{M(i)}$ Molar buffer capacity of substance (i)

$β_{Max(i)}$ Maximum buffer capacity or buffer value of substance i. It is achieved when pH = pK_A.

$β_{NonB}$ Buffer capacity of non-bicarbonate buffers, i.e. of all buffers other than bicarbonate. In physiological literature, this is often simply referred to as β

$β_{i\,Relative}$ Relative buffer capacity or buffer value of substance i. Ratio of $β_i$ and $β_{Max(i)}$

B base form (species) of a substance that is electroneutral as opposed to negatively charged base form A^- (only in this thesis)

B^- Base form (species) of a substance reacting in acid-base reactions

$[B^-]$ Concentration of the base form of a substance reacting in acid-base reactions

BB	Buffer base. Term introduced to physiology by Singer and Hastings. Theoretically, it could be taken equal to C_B, total titratable base. In practice, it is often taken as equal to the total negative charge of buffer molecules of plasma, i.e. of albumin, globulins and phosphates. These two notions are not equal, they are referred to as more complex and simplified thinking, respectively, in this work, in meq/l.
BB_N	Normal average buffer base of plasma, i.e. that of pH = 7.4, pCO2 = 40 mmHg and normal average concentration of all buffers.
BB_{pHn}	Buffer base concentration at normal pH = 7.4 and pCO_2 40 mmHg. It is equal to sum of bicarbonate and NBB_{NonB}
BE	Base excess; amount of strong acid, needed to titrate 1 liter of solution (blood, plasma, extracellular fluid) back to pH = 7.4, when pCO_2 is moved back to the normal value 40 mmHg, in mmol/l or meq/l. Also defined as $BB - BB_{pHn}$
C_{Alb}	Plasmatic concentration of albumin in mmol/l. Normal average value is 0.66 mmol/l.
C_B	total titratable base; it includes the concentration of free hydrogen binding sites, plus concentration of hydroxide ion (minus [H^+]), conservative interval variable, *invariant* during hydrogen ion redistribution, , in mmol/l
$C_{B(Alb)}$	Total titratable base of albumin molecule; it is equal to the concentration of the base forms of those amino acid residues that can function as buffers, regardless of their charge. pH-pK_A criterion can be taken to distinguish between buffer residues and strong ion residues.
$C_{B(i)}$	Total titratable base of protein (or any substance) *i*
$C_{B(nonB)}$	Total titratable base total plasma substances that include buffer as well as strong ion residues (known as non-bicarbonate buffers). The term *BB* commonly takes the negative charge of plasma proteins as equal to *BB,* which is a simplification. The term *$C_{B(nonB)}$* differentiates between the charge of strong ion residues and buffer residues.
C_H	total titratable acid, it includes the total concentration of hydrogen ions, both free and bound, conservative interval variable, *invariant* during hydrogen ion redistributions, in mmol/l
C_i	Concentration of substance *i*. It is equal to the sum of all forms, in case of monovalent substance C_i = [HB] + [B⁻]; in mmol/l

C_P Total concentration of phosphate/phosphoric acid in all four possible species (forms), plasmatic concentration is 0.85 − 1.45 mmol/l in adults and 1.1 − 2.0 in children. Average value is taken as 1.2 mmol/l.

$C_{W,Glb}$ Mass (weight) concentration of globulins in g/l, norm being 26 − 46 g/l.

$C_{W,Alb}$ Mass concentration of albumin in g/l, norm being 35 − 52 g/l, average value 44 g/l.

ΔA_I^- Change in charge of non-bicarbonate buffer base due to buffering, at normal albumin (plasma protein and phosphate) concentration

ΔA_{II}^- Change in charge of non-bicarbonate buffer base due to buffering, at any albumin (plasma protein and phosphate) concentration

ΔA_{pHn}^- Change in charge of non-bicarbonate buffer base due to change in albumin (plasma protein and phosphate) concentration at normal pH = 7.4

$\bar{e}_i$ Average number of free hydrogen ion binding sites per molecule of substance i. It is equal to $[B^-]/C_i$ for monovalent substances

$\bar{e}_A, \bar{e}_B, \bar{e}_Z$ $\bar{e}_i$ of proteins A,B and Z, i.e average number of free hydrogen ion binding sites per molecule of A,B and Z; alternatively $\bar{e}_i$ of amino acid residues type A and type B per molecule of protein i (see $\bar{z}_A$ and $\bar{z}_B$ for details)

$\bar{e}_{N(i)}$ is the value of $\bar{e}_i(pH)$ at the normal pH (pH_n)

$[Glb^-]$ Concentration of the negative charge on albumin, which is generally viewed as equal to albumin buffer base. However, this is not true on a close inspection (see albumin for details), in meq/l.

$[H^+]$ often referred to as concentration of free hydrogen ion; in physiological literature, this commonly denotes activity of hydrogen ion without explicitly mentioning this.

HA   Acid form of non-bicarbonate buffers, weak acid, especially in the modern approach; alternatively acid form that is electroneutral as opposed to positively charged acid form HB^+ (only in this thesis)

[HA]   Concentration of HA

HB   Acid form of a substance reacting in acid-base reactions, in meq/l

HB^+ Acid form of a buffer that is positively charged, as opposed to electroneutral form HA

[HB]	Concentration HB or HB$^+$, respectively
HLac, Lac$^-$	Lactic acid, lactate
$[I_k^{z_k}]$	Concentration of the strong ion k
K_A	Equilibrium constant of the mass action equation; in case of acid-base theory, this reaction concerned is the dissociation of hydrogen ion from conjugate acid; alternatively, lumped dissociation constant of non-bicarbonate buffers of the modern approach, in meq/l
K_{A2}	Lumped dissociation constant of non-bicarbonate buffers of the modern approach, determined when the inner structure of protein buffers is taken into consideration (as opposed to the different value determined when the negative charge of protein buffers is taken as equal to the non-bicarbonate buffer base).
K_W	Ionic product of water, dissociation constant for auto-ionization of water.
$K_{j(i)}$	Dissociation constant of j-th buffer residue of protein i.
K_3	Equilibrium constant of second dissociation of carbonic acid, i.e. of the reaction bicarbonate - carbonate
NBB_{NonB}	Normal buffer base of non-bicarbonate buffers, i.e. buffer base concentration at pH = pHn, regardless of the total buffer concentration of the particular plasma/blood considered.
$N_{Buf\,(i)}$	Number of buffer residues per molecule of protein i.
$\bar{n}_i$	Average number of bound hydrogen ions per molecule of i. It is equal to [HB]/C$_i$ for monovalent substances
$\bar{n}_A, \bar{n}_B$	$\bar{n}_i$ of amino acid residues type A and type B per molecule of protein i (see $\bar{z}_A$ and $\bar{z}_B$ for details)
$\bar{n}_{\max\,(i)}$	Maximum number of bound hydrogen ions per molecule of i.
[P]	Short notation for $[PO_4{}^{3-}]$, in mmol/l

[P_i^-]	Concentration of the negative charge on phosphates, which is generally viewed as buffer base of phosphate/ phosphoric acid system. However, as shown, part of this charge can be treated as strong ion charge in physiology.
pCO_2	Partial pressure of carbon dioxide (generally measured in arterial blood), in mmHg
pH	negative decadic logarithm of [H^+]
pHn	physiologically normal "neutral" pH = 7.4. Alternatively, any other point taken as a reference point for linearization
pK_A	negative decadic logarithm of K_A , logaritmic form of the dissociation constant of the mass action equation
pK_C	dissociation constant of carbon dioxide – bicarbonate buffer
S	solubility of CO_2, i.e. the coefficient between the molar concentration of dissolved CO_2 and its partial pressure; sometimes also denoted as α_{CO2}.
SID	Strong ion difference, sum of strong cations minus sum of strong anions, also referred to as strong ion difference apparent (SID_A) in literature, in meq/L
SID_A	Apparent SID, i.e. SID calculated from the measured concentrations of major inorganic ions. It does not include minor ions that are difficult to measure as a whole (so called unmeasured anions). These ions are generally calculated as SIG.
SID_E	Effective SID, name that the authors of the modern approach give to buffer base, i.e. sum of bicarbonate and non-bicarbonate buffer bases, in meq/L.
SID_N	Normal strong ion difference, i.e. one when each strong ion is in its normal average concentration.
$iSID_{(i)}$	Internal strong ion difference of a protein i. Difference between the concentration of residues that behave like strong cations according to pH-pK_A criterion and those that behave like strong anions.
$iSID_{Alb}$	Internal strong ion difference of albumin. It is equal to $Z_{Fix}* C_{Alb}$. According to the 2 sided $iSID$ model based on Figge-Fencl model 3.0, Z_{Fix} is equal -25.

$iSID_{NonB}$ total internal strong ion difference of those substances that include buffer as well as strong ion residues in plasma. Because these substances include buffer residues, they are often called non-bicarbonate buffers of plasma.

"supSID" "super SID", informal name given to the sum of parameters that behave similarly to SID, staying constant during titration with strong acid and base. However, unlike *SID*, it also includes maximum charge of buffer residues.

SIG Strong ion gap, equal to measured (apparent) SID minus BB (=SID_E) , in meq/l

X So called unmeasured anions, generally measured as SIG, in meq/l

$\bar{z}_i$ Average charge per mole or molecule of substance (protein) *i.*

$\bar{z}_A$ Average charge of amino acid residues of type A (acid form electro neutral, base form negatively charged), per molecule of substance (protein) *i*

$\bar{z}_B$ Average charge of amino acid residues of type B (acid form positively charged, base form electroneutral), per molecule of substance (protein) i,

$Z_{Fix(i)}$ Fixed charge on the protein i, i.e. sum of the charge of all amino acid residues that behave like strong ions according to pH - pK_A criterion.

Z_k Charge of a strong ion k (per molecule).

$\bar{z}_{max\,(i)}$ Maximum charge per mole or molecule of substance (protein) i,

$\bar{z}_{min(i)}$ Minimum charge per mole or molecule of the substance (protein) i.