Chemistry & Biology Interface, 2020, 10, 5, 121-127

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Buffer capacity of blood for interpretation on COVID-19 patients

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Received 27 July 2020, Accepted 1 September 2020

Abstract: Arterial blood gas (ABG) test is used to measure the acidity (pH), amounts of arterial gases namely oxygen (O_2) and carbon dioxide (CO_2) for critically ill patients. The test essentially gives information for lungs, heart and kidneys function. The acidity (pH), concentration of hydrogen carbonate (HCO_3^-) and partial pressure of CO_2 causes metabolic acidosis, metabolic alkalosis, respiratory acidosis and respiratory alkalosis. Till now one parameter is overlooked i.e. buffer capacity of the blood and above a particular value, it validates the above mention phenomena. Below which blood lost its buffer behaviour for proper functioning in human organs. Buffer capacity will not change the pH value of blood. Thus the symptoms found of a patient overwhelm doctor for treatment. This is happening for coronavirus patients. The ABG test for COVID-19 patients are analysed and medicines are proposed for their treatment on the new concept of buffer capacity. A completely new technique for injecting bicarbonate buffer has been proposed by changing preparation of bicarbonate buffer and modifying the medical device.

Keywords: pH, Buffer capacity, Arterial blood, Treatment, Covid-19.

Introduction:

The most important buffer systems in human body are bicarbonate buffer (in blood plasma, generally in the extracellular fluid), haemoglobin buffer (in erythrocytes), phosphate buffer, proteins, and ammonium buffer [1-10]. Bicarbonate buffer system mainly regulate cellular respiration i.e. carbon dioxide management in the blood and cells of different organs by converting bicarbonate ion to carbon dioxide or vice versa. Phosphate buffer regulates the internal fluids of all cells and protein buffer systems maintain acidity in and around the cells respectively. Haemoglobin is an example of protein buffer which binds small molecules in the blood [4,9]. Actually all these buffers maintain proper pH values within the body system so that all biochemical process can take place appropriately and smoothly. The

blood components buffer such as bicarbonate buffer, haemoglobin buffer, plasma protein buffer and phosphate buffer contribute 53% (plasma 35% and erythrocytes 18%), 35%, 7% and 5% respectively in total buffer system. All buffer systems are in equilibrium and any kind of concentration change of any component of any buffer influences pH and buffer capacity of the buffer systems. Buffer capacity is defined as the moles of an acid or base necessary for one litre of a buffer to change its pH by one unit. The buffer capacity depends on the amounts of the weak acid and its conjugated base present in the buffer. Higher is the buffer capacity best is the buffer. When the pH value of buffer and pKa value of weak acid are almost similar, it will be a very efficient buffer. The term buffer capacity for static buffer solution (in laboratory) is less important than dynamic buffer solution (the blood in human artery, vein and capillary). Higher the concentration of buffer components in the buffer, buffer capacity will be more or vice versa. If the buffer capacity of a dynamic buffer (blood buffer) solution is decreased by a certain limit then it will not act as buffer. The average highest and mean blood velocities are 66 and 11 cm/sec in the ascending aorta, 57 and 10 cm/sec in the pulmonary artery, 28 and 12 cm/sec in the superior vena cava, and 26 and 13 cm/sec in the inferior vena cava [11]. Bicarbonate buffer is the most important buffer system in blood plasma (mainly in extracellular fluid). If the buffer capacity of blood bicarbonate buffer is decreased (is due to decrease of sodium level in blood), its efficiency will be lost. Thus bicarbonate buffer will unable to maintain the acid and base balancing procedure by both normal and abnormal physiology, handling of carbon dioxide, the waste product of cellular respiration. As an effect human body shows several symptoms like fever, cough, shortness of breath or difficulty breathing, chills, repeated shaking with chills, muscle pain, headache, sore throat and new loss of taste or smell similar to the symptoms of coronavirus [12-17] infected person. Till now the terms mainly metabolic acidosis, metabolic alkalosis, respiratory acidosis and respiratory alkalosis are used to explain the ABG test reports. But there may arise certain ABG test report which cannot explain by conventional guideline. Thus we are introducing a factor, AG factor (Ashesh Garai factor by author name), which can perfectly explain of that ABG test report. It is completely a unique concept which not only explain COVID-19 patients ABG test reports but also useful for analysis of ABG test reports of critically ill ICU patients.

Report and discussion: The Henderson-Hasselbalch equation [18,19] for the estimation of pH of a buffer solution (weak acid and its conjugate base) can be written as

$$pH = pK_a + log\frac{[conjugate\ base]}{[Acid]} \qquad \text{Equation (1)}$$

where K_a is the acid dissociation constant. This equation can explain the pH value of the buffer solution when either the concentration of acid or the concentration of conjugate base is changed. Let an acid buffer formed by HX (weak acid) and PX (X^- is the conjugate base and P is charge balancing cation). Then the equation will be

$$pH = pK_a + log\frac{[X^-]}{[HX]} \qquad \text{Equation (2)}$$

Then QX (Q is similar like P) is added to the buffer solution. Then the equation will be

$$pH = pK_a + log\frac{[X_P^- + X_Q^-]}{[HX]} \qquad \text{Equation (3)}$$

If a buffer solution prepared by mixing H_2CO_3 $(pK_a = 6.35)$ $NaHCO_3$ $(pK_a = 6.4)$ and $KHCO_3$ $(pK_a = 6.37)$ then the above equation will be

$$pH = pK_a + log\frac{[X_{Na}^- + X_K^-]}{[HX]} \qquad \text{Equation (4)}$$

Where X is HCO_3 i.e. X^- is HCO_3^- and X_{Na}^- is the concentration of HCO_3^- come from $NaHCO_3$

and X_K^- is the concentration of HCO_3^- come from $KHCO_3$. Now consider a macromolecule, $ZHCO_3$ dissociate into Z^+ and HCO_3^- in the solution, where Z is polypeptide or Protein (spike protein or membrane protein or any other protein) or RNA. The ionic state of Z (Z or Z^+) in solution depend on isoelectric point of polypeptide (indirectly pKa value of the amino acid) and pH of the solution. In blood, neither Stewart-Fencl strong ion difference (ΔSID = Na – Cl – 38) in the Van Slyke equation [The normal SID value in blood plasma is ~38 meq/L (25 meq/L bicarbonate, 12 meq/L albumin and 1 meq/L phosphate)] nor buffering in blood plasma [20] can explain this fact. For very fast kinetics (both forward and backward reaction) of $ZHCO_3 \leftrightarrow Z^+ + HCO_3^-$ reaction or any other reason $ZHCO_3$ may not share its HCO_3^- ion to other reaction but contribute for pH estimation. Then equation (4) will become

$$pH = pK_a + log\frac{[X_{Na}^- + X_K^- + X_Z^-]}{[HX]} \qquad \text{Equation (5)}$$

The buffer capacity value β [21] can be written as $\beta = -\frac{dA}{dpH}$ but this term may be able to explain the action of $ZHCO_3$. The X_Z^- will not take part any reaction i.e. it will not consume the proton when external acid will be added to the buffer. Then the effective pH of a buffer solution prepared by mixing H_2CO_3, $NaHCO_3$, $KHCO_3$ and $ZHCO_3$ will be

$$pH = pK_a + log\frac{[X_{Na}^- + X_K^-]}{[HX]} \qquad \text{Equation (6)}$$

though the solution contain X_Z^- ions. It means that the effective pH of the buffer solution will be less than the measured pH value. It will be apparent when buffer capacity will measured. Thus this type macromolecule though unable to change the pH value of buffer can able to change the buffer capacity of that buffer. Because when buffer capacity is measured by adding H^+, $X_{Na}^- + X_K^-$ will take part in the reaction and X_Z^- will remain attached with Z^+. For simplicity AG factor (α) is introduced which can be described as

$$\alpha_{HCO_3^-} = \frac{molar\ concentration\ of\ components\ active\ in\ the\ buffer\ solution}{molar\ concentration\ of\ components\ present\ in\ the\ buffer\ solution}$$

Thus (α) for buffer solution of H_2CO_3, $NaHCO_3$, $KHCO_3$ and $ZHCO_3$ will be

$\alpha_{HCO_3^-} = \frac{[X_{Na}^- + X_K^-]}{[X_{Na}^- + X_K^- + X_Z^-]}$ = fraction, <1 and effective pH will be

$$Effective\ pH = pK_a + log\frac{\frac{[X_{Na}^- + X_K^-]}{[X_{Na}^- + X_K^- + X_Z^-]}[X_{Na}^- + X_K^-]}{[HX]} = pK_a + log\frac{(\alpha)[X_{Na}^- + X_K^-]}{[HX]}$$

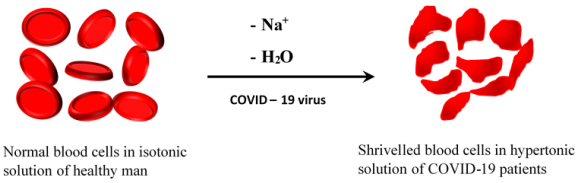
Equation (7)

It implies that the effective pH of the buffer solution will be less than the measured pH value. The value of AG factor is 1 for buffer made from small molecules and <1 for buffer made from macromolecules. Thus AG factor clearly explain the variation of percentage change of the individual buffer components. This is happening for ABG test reports of COVID-19 patients. Though the measure pH value for COVID-19 patient arterial blood is around 7.4 but the effective pH value of the individual buffer components will be less.

In addition of fever, COVID-19 patients shows symptom of an increase in urinary frequency with urosepsis in the differential diagnosis of both in ambulatory care and in emergency rooms [22]. With frequent peeing kidneys release more salt (sodium) from the body (including blood) into the urine. It is found in ABG test reports of COVID-19 patients that reported Na^+ values (110 mmol/L) is less than the normal average value (142 mmol/L) keeping the bicarbonate concentration almost similar within the range of normal value. It indicates coronavirus replace Na^+ ions in blood and kidneys release it.

$$coronavirus^+ + NaHCO_3 = coronavirus - HCO_3 + Na^+$$

To understand the probable mechanism it is important to understand the structure of coronavirus. The structure of coronavirus mainly contains RNA, Nucleocapsid Protein (N), Envelope Protein (E), Membrane Protein (M) and Spike Protein (S) [23]. The function of these proteins likely are essential for coronavirus entry and viral replication into host cell [24]. The spike protein (S) binds to the human cell receptor and the viral membrane (M) glycoproteins (helping the immune, digestive, and reproductive systems) fuses into the human cell membrane, allowing the genome of the coronavirus to enter human cells and begin infection. The spike protein has amino acids in the range of 1160 for avian infectious bronchitis virus (IBV) and up to 1400 for feline coronavirus (FCoV). The isoelectric point of Lysine, histidine and arginine are 9.74, 7.59 and 10.76 respectively and the human blood pH value is in the range of 7.35-7.45. These three amino acids will remain as acidic form in the blood. Thus it may indicate that coronavirus have these type of amino acids in its proteins of structural unit. As the pK_a value, isoelectric point of histidine fall in the blood pH range so giving (oral or injection) pure histidine to COVID-19 patients may improve their condition. As the basic unit of histidine is imidazole so the drug have imidazole moiety (example: tinidazole) may be more effective than other nitrogen base moiety drug. The kinetics implies that higher dose may be more favourable. Blood plasma contribute 10%, protein contribute 30% and chemical (bicarbonate and others) contribute 60% in total respiration from tissue to blood and blood to alveoli. If coronavirus (behave like ZA in buffer) present in blood the respiration percentage contribution will be decreased and it is seen on ABG test reports of COVID-19 patients. It is hard to predict which parameter decrease how much for CO_2 breathing. Each haemoglobin molecule can bind with four oxygen molecules for which the haemoglobin molecule changes its shape or conformation. The distal histidine from the haemoglobin molecule further stabilizes the O_2 molecule packing by hydrogen-bonding interactions. The proximal histidine is pulled the O_2 molecule along with the iron ion. May be coronavirus consumes histidine from blood for its replica preparation thus O_2 binding capacity of haemoglobin decreases. That is why ABG test reports of COVID-19 patients show less pO_2 value. As buffer capacity of blood is our primary discussion so we are not going in details in protein chemistry. As a chemist viewpoint the osmotic pressure of blood have vital role for COVID-19 patients. The decrease of sodium ion from blood as seen on ABG reports made the blood hypertonic and that is why blood cell clotted which has seen for COVID-19 patients. The normal blood cells and COVID-19 patients' blood cells are presented in scheme 1.



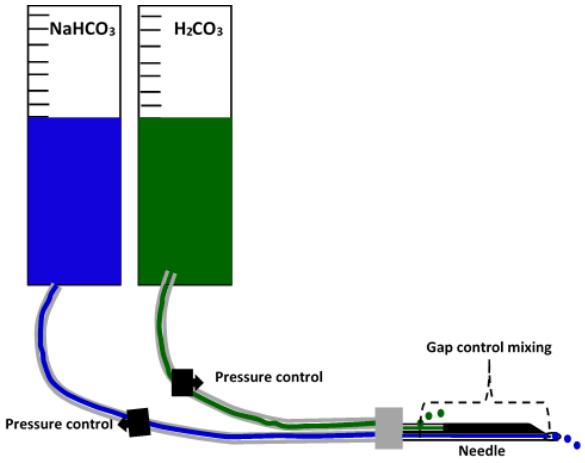
Scheme 1: Schematic presentation of normal blood cells and COVID-19 patients' blood cells.

For average arterial true plasma, HCO_3^- = 24.0 mM, H_2CO_3 = 1.2 mM, Total CO_2 = 25.2 mM, pH = 7.40, and pCO_2 =40 mm Hg [20,25]. To keep the pH value of blood 7.4, the ratio of [HCO_3^-/H_2CO_3] should be 20:1. If the concentration of HCO_3^- in blood changed to 12.0 mM and the concentration of H_2CO_3 changed to 0.6 mM, the blood also shows the pH value 7.4. But buffer capacity will be changed and blood cannot function appropriately. As the blood pH value is 7.4 thus it cannot predict from ABG report whether it is acidosis or alkalosis. So before refereeing acidosis or alkalosis, it is necessary to check the buffer capacity value of blood. In human body several organ are pH dependent i.e. lungs, kidneys, stomach, blood, muscle, tongue (saliva) etc. need a particular pH for

its functioning. The pH value at which lungs, kidneys, stomach, blood, muscle (living) and tongue (saliva) work are 7.38-7.42, 7.4, 1.5-3.5, 7.35-7.45, 7.1and 7.4 respectively. Symptom arise when the above mention pH value differ from organ's working pH value and the symptom become mild to severe depending on the amount of pH change. Thus any basic compounds give an impact for the treatment of COVID-19 patients by increasing basicity of different organs.

Hydroxychloroquine (HCQ) is used for the treatment of COVID-19 patients [26] and it partially works. The mechanism of hydroxychloroquine action is that it increase pH within intracellular vacuoles and interference of lysosomal activity and autophagy. As it is a basic compound and increase intracellular vacuoles pH value hence act as drug for treatment COVID-19 patients. But its lysosomal activity and autophagy may be restrict for further application. Famotidine also a promising drug for treatment COVID-19 patients. As famotidine is a competitive inhibitor of histamine H2-receptors, it inhibit gastric juice secretion, reduces the acid and pepsin content and stimulate gastric secretion. This medicine change the stomach pH value and thus it work. But it cannot directly increase the pH value of blood and other organs. Hence famotidine has limitation for treatment COVID-19 patients. Recently convalescent plasma therapy [27] is added to standard treatment for COVID-19 with severe or life-threatening condition. But the success rate is low and mechanism is still unknown. The success may be due to antibody developed in blood or buffer capacity changed (200 ml blood plasma added which contain sodium and other buffer components). Before giving blood plasma lots of parameter have to be matched and donor is also limited. Again Bicarbonate therapy though remain controversial [28] is commonly use for the treatment of acute acidemia and chronic buffer depletion. Adverse effects of bicarbonate therapy are also include hypokalemia [29], hypocalcemia [30], hypercapnia [31], hypernatremia and fluid overload [32]. Buffer therapy mitigating acidemia, increased binding of O_2 in haemoglobin and reduced delivery to tissues, potentially contributing to an increase in tissue hypoxia.

Instead of giving blood plasma adequate amount of bicarbonate buffer (not only bicarbonate) may be injected. It will not only correct blood buffer capacity but also help to decrease the acidic nature of organs to function properly. Till now there are no report for injecting bicarbonate buffer in human blood because of its preparation and injecting technique. In normal condition it is impossible to prepare desire concentration of bicarbonate buffer in a container. It can be solved by preparing the bicarbonate buffer in two different containers i.e. in one container $NaHCO_3$ solution and in other container H_2CO_3 solution. The concentration and amount of bicarbonate buffer injection will be vary from patient to patient. The calculation will depend on blood ABG report of the patient. It is not simple like normal saline (0.9% NaCl) injection. We can only say a mother solutions (relatively higher concentration) of those two solutions should be diluted before injecting considering the osmotic pressure. The needle for injecting these two solutions needs modification. A schematic presentation for injecting bicarbonate buffer is presented in Scheme 2. The bicarbonate buffer solution not only help COVID-19 patients but also help other patients admitted in ICU for acidemia or alkalemia and other blood related symptoms. The term fluidic overload can be eliminated by injecting 200 ml bicarbonate buffer into the blood and taking out 200 ml blood from blood stream.



Scheme 2: Schematic presentation of the device for injecting bicarbonate buffer (osmotic pressure should be consider).

Conclusion: Till now Henderson-Hasselbalch equation is used for the estimation of pH of a buffer solution. But there may arise a situation when its component/s will not contribute for appropriate buffer function. Blood is such type of buffer and bicarbonate is the component. Using AG factor effective pH value of the buffer can be calculated. The effective pH value has an imperative significance for explaining blood function. A unique equipment has been engineered for injecting bicarbonate buffer into the blood. Bicarbonate buffer therapy has better perfection than bicarbonate therapy. Bicarbonate buffer therapy can mitigate hypokalemia, hypocalcemia, hypercapnia, hypernatremia and fluid overload. Coronavirus is using amino acid, protein and inorganic chemicals for its survival and replica construction in human blood and other organs. Blood inorganic chemical are related to blood pH and blood buffer capacity. Thus maintaining blood buffer capacity can help the organs function well to decrease severity and mortality of coronavirus infection.

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