The Effect of pH on the Binding Characteristics of Paracetamol to Bovine Serum Albumin

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Abstract- This research was done to study the effect of changes in pH on the binding characteristics of paracetamol to bovine serum albumin at 37^{o}C as a follow up to the work done previously by Okoro, et al (2015) on this title at 26^{0}C .The binding characteristics were investigated at a pH range of 6.0-8.0 using UV- Spectroscopy . The result showed that the binding of paracetamol to bovine serum albumin was impaired at almost neutral and near alkaline pH (6.8 -7.2) with the drug-protein binding constants (K_L) of $1.698X10^{2,}$ $0.330X10^{2}$ and $5.007X10^{2}$ respectively and was enhanced at higher alkaline pH of 8.0, with the drug-protein binding constant K. of $216{,}432x10^{2}$. This can be interpreted as conformation changes (neutral-base transition change that occurred at pH of 6.8 to 7.2. This confirmed that bovine serum albumin has two structures, one at the acidic pH and the other at the basic pH.

Index Terms- *Binding characteristics, Bovine serum Albumin, paracetamol ,pH*

I. INTRODUCTION

Drugs are chemical compounds that may be used on or administered to humans to help diagnose, treat, cure or prevent disease or other abnormal conditions. On administration, drugs undergo physiological movements in the body in four steps namely absorption, distribution, biotransformation (metabolism) and excretion, (Shargel et al , 2005).

Following absorption, drugs are rapidly distributed around the body by circulation. Some drugs are totally dissolved in the plasma water while others are transported and the rest bound to plasma proteins, particularly the albumins (Stockley, 1991).

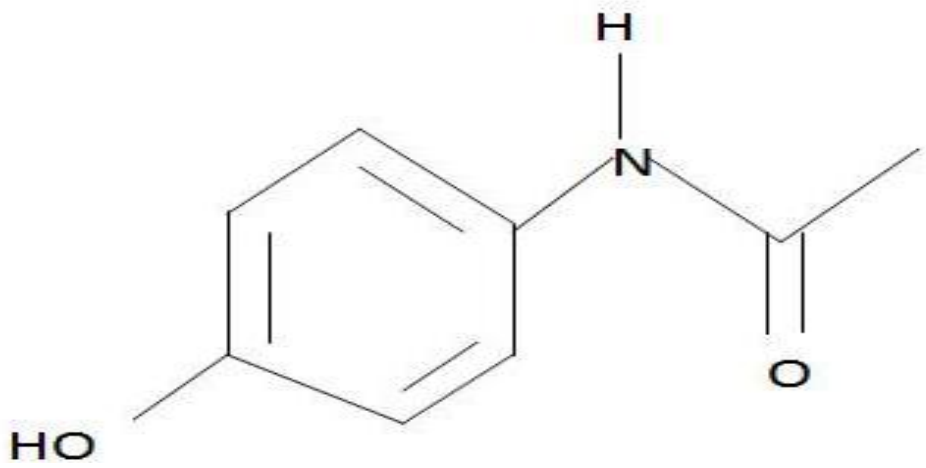
Serum albumin, commonly called “albumin” is the most abundant plasma protein in humans and other animals (Okoro et al, 2015). Serum albumin possesses a unique capacity to bind covalently or reversely to a great number of various endogenous and exogenous compounds. Several different transport proteins exist in blood plasma but only albumin is able to bind a wide diversity of ligands reversibly with high affinity (Stockley, 1991). The two major types of serum albumin used in pharmacological and biochemical investigations are human and bovine serum albumins. Humans’ serum albumin (HSA) is produced in the liver. It is soluble and monomeric. The molecular mass of HSA is 68,000.It contains a single polypeptide chain of about 580 amino acids (Wikipedia, 2015). Albumin has a low pH of 4.7 and represents about 25% of the protein produced in the liver. It has a serum half –life of approximately 20days. About half of the blood serum protein is albumin. HSA plays a key role in the transport of fatty acids, metabolites and drugs.

Serum albumin is of two major types namely Human Serum albumin (HSA) and Bovine serum albumin (BSA). Bovine serum albumin has similar structure to human serum albumin but is less dense than HSA

Its molecular mass is 66000. Bovine serum albumin has numerous biochemical applications.

For instance, BSA is used to determine the quantity of other proteins by comparing an unknown quantity of protein to known amounts of BSA; it is also used as a nutrient in cell and microbial culture etc.

Paracetamol is an analgesic (pain reliever) and antipyretic (fever reducer) widely used. Its analgesic properties commence at about 11 (eleven) minutes after oral administration of paracetamol. Its structural formula is as shown below:



IUPAC NAME: N-(4-hydroxyphenyl) acetamide.

Its molecular formula is $C_8H_9NO_2$. Acute over dose of paracetamol can cause potentially fatal liver damage; the risk is heightened by alcohol consumption (Okoro et al, 2015). A drug exists in two forms: bound and unbound. Depending on a specific drug's affinity for plasma proteins, a proportion of the drug may become bound to plasma proteins with the remainder being unbound. It is the unbound fraction that shows pharmacologic effects. It is also this unbound fractions that they may be metabolized and / or excreted.

The interaction of drugs with protein in solution is often studied with the use of spectroscopic techniques. This research work was done to investigate how pH affects the binding of paracetamol to bovine serum albumin under physiological conditions by UV-Visible spectroscopy.

II. MATERIALS AND METHODS

Paracetamol (powder) from China, Bovine serum Albumin and distilled water were purchased from lilichem laboratory, No. 1 Okigwe Road by over-rail Aba, Abia State. Disodium hydrogentetraoxophosphate (V) (Na_2HPO_4) and Sodium dihydrogentetraoxophosphate (V) (NaH2PO4) were purchased from Harrison Nigeria Ltd, No. 100 St Michael's Road, Aba, Abia State. All reagents used were of analytical grade and were used without further purification.

Phosphate buffers of various pH (6.0, 6.2, 6.4, 6.8⋯8.0) were prepared using distilled water, and a combination of sodium dihydrogentetraoxophosphate (V) and Disodium hydrogentetraoxophosphate(V) salts. $1.0x10^{-4}$M paracetamol solution was prepared by dissolving $1.5117x10^{-3}$g of the paracetamol powder in about $100cm^3$ of distilled water in a $100cm^3$ volumetric flask. Also, $1.0x10^{-6}$M Bovine Serum Albumin was prepared by dissolving $6.6x10^{-3}$g of BSA in about $100cm^3$ of distilled water.

APPARATUS: UV-Visible Spectrophotometer (Spectrumlab. 7525); pH Meter (Model PHS -3C.

2.1 DETERMINATION OF DRUG –PROTEIN BINDING CONSTANT

The paracetamol sample solution was prepared in $50cm^3$ volumetric flasks (seven in number), by adding a suitable aliquots ($0.1\text{-}0.5cm^3$) of the stock standard paracetamol solution to each of the first five flasks containing 2cm3 of the $1.0x\ 10^{-6}$M bovine serum albumin. The flasks were numbered 0.1, 2, 3, 4, 5 and "inf". The flask numbered "0" contained protein of known albumin concentration ($1.0x10^{-6}$M) and a suitable phosphate buffer only. The flask numbered "inf" contained the same concentration of albumin and excess of the paracetamol powder. The remaining five flocks also contained the same varying volume of the paracetamol solution being tested. Each flask was then made up to mark with the duffer of the appropriate pH being tested. The flasks were properly corked and vigorously shaken for a short while and incubation at 37^0C for three (3) hours. After incubation the absorption spectrums of the sample solutions were measured at 278nm against the reference buffer solution using a 1cm light pass cell (cuvette). This same procedure was used for each of the buffer solutions prepared.

2.2 TREATMENT OF DATA

The values used in plotting the graph were obtained by using the following formula:

$$L_b = \frac{(D_o - D_t)}{(D_o - D_\infty)} \times [P_T]$$ where

L_b = Concentration of the bound drug

D_o = Optical density (absorbance) of the protein solution only at any pH between 6 and 9.

D_∞ = Optical density of the protein solution in the presence of the excess drug at the same pH

D_t = Optical density of the protein solution in the presence of drug at a low drug to protein ration.

Pt = Total protein concentration.

$L_f = L_t - L_b$ where

L_b = Concentration of the bound drug

L_t = Total drug concentration.

$$K_{obs} = \frac{(D_o - D_t)}{(D_t - D_\infty)(L_t - L_b)}$$

But K_{obs} was found to be apparent and does not represent the true drug-protein binding constant for proteins that undergo ionization within the physiological pH region (Ejele, 2004). To obtain the true biding constant, a correction is made to account for the extent of protein ionization using the equation:

$$K_{obs} = \frac{1+Ka}{[H^t]}$$

Where

K_L = True drug-protein binding constant

K_a = Ionization constant of the drug and

$[H_t]$ = pH of the phosphate buffer used.

III. RESULTS AND DISCUSSION

The effect of pH on the binding characteristics of paracetamol to bovine serum albumin has been investigated using the UV-Spectroscopy method. The results of the drug-protein binding constant ($K_{L)}$ is presented in table 1and represented graphically figure 1. The graph shows that increasing the pH of the medium increases the drug-protein binding constant.

TABLE 1: Variation of K_{obs} and K_L with pH for the Binding of paracetamol with Bovine serum Albumin (BSA).

pH	K_{obs} x 10^{-12}	K_L x 10^2
6.0	0.0754	0.0130
6.2	1.3406	O.3270
6.4	2.9611	1.1460
6.6	2.8424	1.7440
6.8	1.7422	1.6980
7.0	0.2145	0.3300
7.2	2.0521	5.0070
7.4	4.0142	15.8520
7.6	1.1722	7.2210
7.8	2.2574	23.1840
8.0	14.054	216.4320

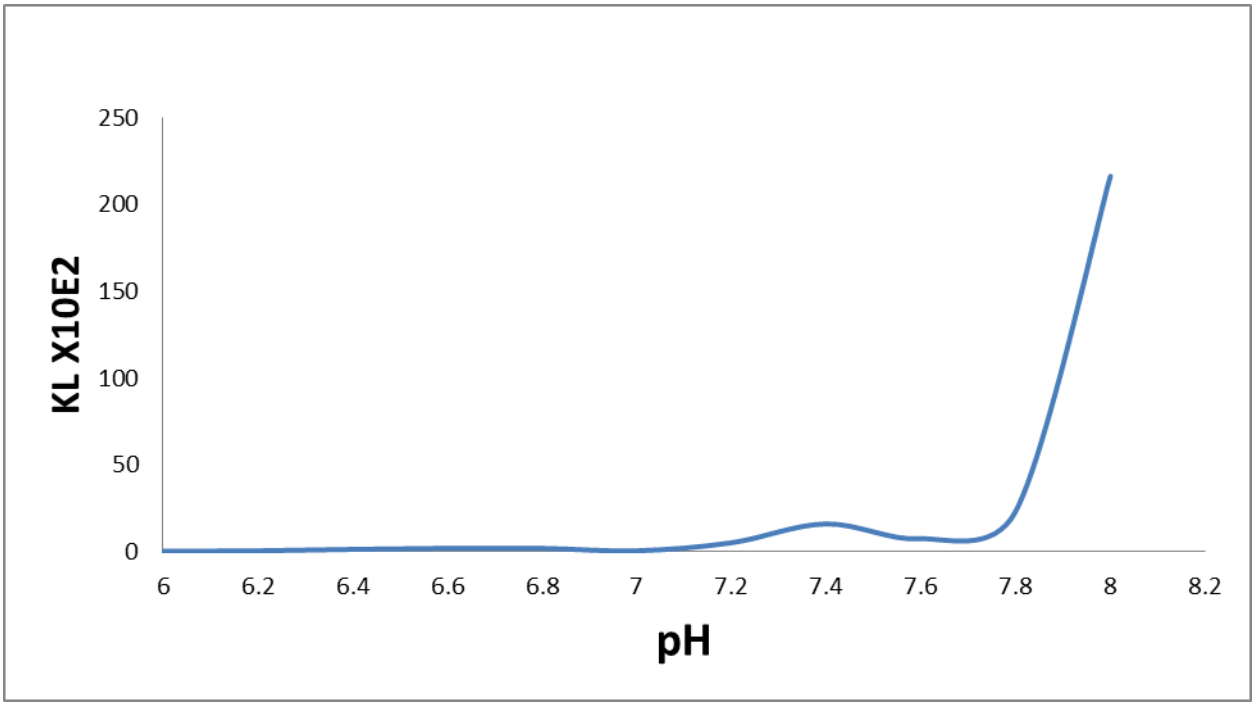


Figure 1:The true drug-protein binding constants Vs pH for the binding with paracetamol

Table 1 showed that the drug –protein binding constant increased with increasing pH from $0.013x10^2$ at pH 6.0 to $261.432x10^2$ at pH 8.0. Figure 1 showed that the drug-protein binding constant was low at low and neutral pH between pH6.0-7.0 but increased with pH from pH 7.2 to 8.0 with marked increase between the pH of 7.8 and 8.0. This result agrees with earlier work done by Okoro et al (2015) on the effect of pH on the binding characteristics of paracetamol to bovine serum albumin (at 26^oC). This shows that there is no pronounced difference between the effect of the binding of this drug to serum albumin at 26^oC and at 37^OC in which this work was done. The pH at which the drug-protein binding constant showed the change was taken as the characteristic pH (or pHch) and is dependent on the nature and type of drug. This result was explained in terms of the conformational change called neutral – base conformation which occurs in the protein within the physiological pH region.

Earlier reports have shown that serum albumin can exist in two main forms within the pH range 6-9; the neutral (N-) form exists predominantly at pH 6.0 while the basic (B-) form occurs almost exclusively at pH 9 (labro and Jansen, 1986). The result of this work showed that the binding affinity increases rather gradually with pH within the neutral pH region 6-7. A more rapid increase was observed in the slightly alkaline pH region of 7.2 - 8.0 particularly from 7.8-8.0 suggesting that the basic (B-) form of the protein (BSA) has a higher affinity for the paracetamol than the neutral (N-) form. Several reports have shown that the drug-albumin binding constants increase with pH [O'Reilly and Kowitz (1967); Caossolo et al (1978); Ejele and Anusiem (2001). By varying the pH from 6 to 10 O' Reilly noted that the binding

constant increased with pH. This observation eliminates electrostatic bonding because increasing the pH increases the net negative charge on the albumin molecule which is expected to reduce the binding affinity of anions if an electrostatic mechanism controlled the interaction. [O'Reilly and Kowit (1967) and Mcmenamy (1965). Therefore an increase in the binding constant of the drug-albumin interaction with increase in pH is not consistent with an electrostatic mechanism [O'Reilly (1967), Fujita (1972), Caossolo et al (1978), Labro and Janssen (1986), Ejele and Anusiem (2001) and Mcmenamy (1965)]. Thus, the result of this work confirms these earlier reports that the binding constants of drug-protein interactions increase with increase in pH.

IV. CONCLUSION

The interaction of Paracetamol with bovine serum albumin at various pH shows the importance of drug binding to serum albumin and the conformational changes it can cause in the serum. The results obtained showed that the binding of paracetamol was impaired at neutral and near alkaline pH of 7.0-7.2 which agrees with earlier works reported by Witting, et al (1980); Lambert, et al (1985) and Okoro, et al (2015).

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