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The Effects of Human Immunodeficiency Virus (HIV) Infection on the Absorbance Characteristics of Different Blood Components

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ABSTRACT: *The effects of human immunodeficiency virus infection on the absorbance characteristics of diverse blood components have been studied. The methodology involved taking blood samples from twenty HIV-infected persons and from twenty uninfected persons for absorbance measurement using Ultraviolet Visible Spectrophotometer (Ultrospec3100pro). From the absorbance data various variables (e.g. dielectric constant, etc) were derived. CD4 counts using the digital CD4 counter were also obtained. The decrease in the absorbance values of all the infected blood components (i.e. Red Blood Cells, White Blood Cells and the Plasma) is a clear indication of the role of the viral infection in variations observed in the absorbance levels of these components. This in effect suggests that infection has occurred thus confirming the role of this principle in HIV-blood interactions. A mathematical model for the HIV-blood interaction mechanism was developed from the principle of particle-particle interaction mechanism.*

IndexTerms: *Absorbance, Dielectric Constant, Hamaker Coeficient, Human Immunodeficiency Virus, Lymphocyte, van der Waal.*

I. INTRODUCTION

Over time diverse clinical approaches to the issue of HIV/AIDS have been employed to seek to proffer possible solutions to the threat occasioned by the viral infection. Progress in this regard has been slow and far in between but has given birth to some palliative measures which include the introduction of the Highly Active Anti-retroviral Therapy (HAART). However, the results have not actually shown an easy and comprehensive solution due to the rapid mutative genetic nature of the virus [1].

Much research has been and is still on, on this subject with a cure not yet in view. The choice to approach it via the vehicle of surface thermodynamics against the conventional clinical methods is a novel one. The optimism stems from the great successes recorded with this approach in related areas of biology and medicine. The role of surface properties in various biological processes is now well established. In particular, interfacial tensions have been shown to play an important, if not crucial role in phenomena as diverse as the critical closing and opening of vessels in the microcirculation, cell adhesion, protein adsorption, antigen-antibody interactions, and phagocytosis [2].

The HIV is assumed to be a particle which is dispersed in a liquid (the serum) and attacks another particle (the white blood cell). The virus attaches itself on the surface of the blood cell before penetrating it to attack the RNA. If the surface of the blood cell is such that it will repel the virus, access to the virus into the interior of the cell would have been denied. Thus, the initial actions take place on the surfaces of the cell and of the virus (assumed to be particles). This interaction which involves two surfaces coming together in the first instance can be viewed as a surface effect.

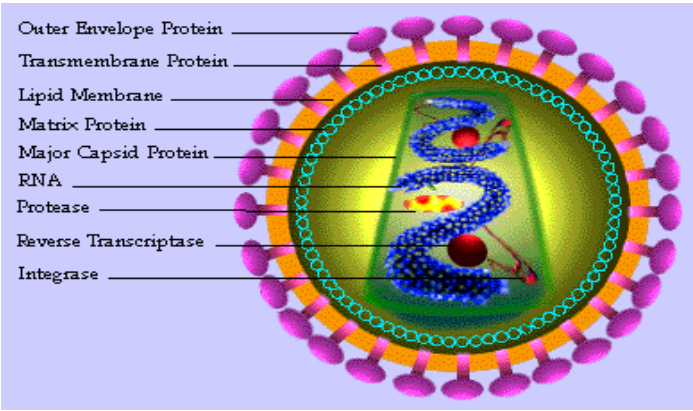

Fig. 1: Human Immunodeficiency Virus (HIV) Anatomy [3]

It therefore stands to reason that, if it is possible to determine the surface properties of the interacting particles, then one can predict the mechanisms of their interactions. When two particles make contact, they establish a common area of contact. Some original area of each particle has been displaced, and the work done to displace a unit area of the surface is referred to as the surface free energy. The actions therefore that take place on the surfaces are termed surface thermodynamic effects. These actions are assumed to occur slowly so that thermodynamic equilibrium is assured. This concept will be employed in this research work to characterize the HIV-blood interactions with the serum as the intervening medium. The clinicians have analyzed the surfaces of blood cells on which the virus binds. There are receptors and coreceptors that aid these interactions.

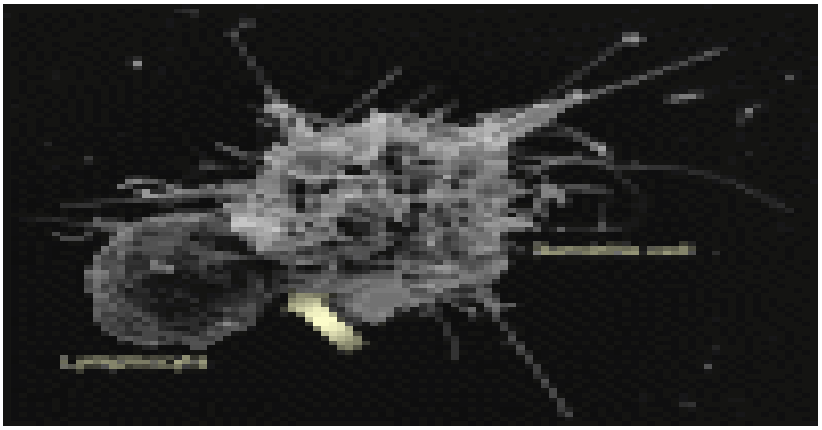

Fig. 2: Interaction of a Dendritic Cell (right) having HIV bound to its surface (arrow) with a Lymphocyte (left) [4]

The discovery and application of highly active anti-retroviral therapy (HAART) to suppress HIV has revolutionized the clinical management of HIV/AIDS cases. The HIV however, has the capacity to develop resistance to the antiretroviral drugs and this phenomenon has turned out to be a significant cause of failure of HAART. HIV, being an RNA-based rapidly mutating virus, (unlike the DNA-based counterparts) lacks the ability to check for and correct genetic mutations that can occur during replication. In chronic HIV cases, about ten billion new viral species can be generated daily. This rapid genetic variation has made it rather very difficult to proffer a clinical solution to the problem [1] and the worldwide picture is one of increasing rates of infection [5].

It is against this backdrop that this study explores a novel and rare approach using surface thermodynamics to seek a way forward in the research on the topic of HIV-blood interactions. The successes recorded in the use of this approach in finding solutions that have brought about many scientific applications cannot be overemphasized [2].

II. RESEARCH METHODOLOGY

A. Major Considerations

This research work is aimed at employing the concept of surface thermodynamics to study the interaction effects of the virus on the blood cells. The following tasks therefore, were undertaken;

- Determine the mechanism of interaction of HIV with white blood cells.
- Seek a thermodynamic interpretation of such interactions and their effects on other blood components.
- Quantify such interactions through actual measurements (absorbance measurements).

B. Sample Collection

This research work involved collection of blood samples from twenty HIV infected and twenty uninfected persons. The collected blood samples were screened to determine the infection status thus giving a total of forty blood samples from different individuals. The blood samples were collected from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria. Anticoagulant test tubes were used to ensure the freshness of the collected samples and to avoid the samples becoming lysed (spoilt). Good storage and refrigeration facilities were also used to ensure that the samples were healthy enough so as to obtain reliable results.

C. Sample Preparation

The collected samples were loaded into a centrifugal separator and the blood components separated. This was done at Peace Diagnostic Laboratory Awka. This helped to obtain such components as Red Blood Cells (RBC), White Blood Cells (WBC) also called the Lymphocytes or the Buffy Coat and the Plasma or

Serum. The glass slides were prepared and smeared with the samples for absorbance measurements. The slide preparations and sample smearing were done at the same laboratory.

D. Measurements

The CD4+ counts of the blood samples were obtained using a digital CD4+ Counter. This in a sense is an indicator of the level and progression of the infection process in the subjects. Absorbance measurements were done on all the different components of all forty samples (both HIV infected and uninfected samples). A digital Ultraviolet Visible Spectrophotometer (Ultrospec3100pro) was used in the measurements. The absorbance values of the samples were measured over a range of wavelength spanning between 230 and 890 Hertz. The data obtained were used to obtain the plots as presented in this work.

III. THEORETICAL CONSIDERATIONS

A. The Thermodynamic Approach to Particle-Particle Interaction:

The thermodynamic free energy of adhesion of a particle P on a solid S in a liquid L at a separation d_0 [6], is given by;

$$\Delta F_{pls}^{adh}(d_o) = \gamma_{ps} - \gamma_{pl} - \gamma_{sl} \quad (1)$$

Where ΔF^{adh} is the free energy of adhesion, integrated from infinity to the equilibrium separation distance d_o; γ_{ps} is the interfacial free energy between P and S; γ_{pl} is that between P and L and γ_{sl} that between S and L.

For the interaction between the individual components, similar equations can be written also;

$$\Delta F_{ps}^{adh}(d_1) = \gamma_{ps} - \gamma_{pv} - \gamma_{sv} \quad (2)$$

$$\Delta F_{sl}^{adh}(d_1) = \gamma_{sl} - \gamma_{sv} - \gamma_{lv} \quad (3)$$

$$\Delta F_{pl}^{adh}(d_1) = \gamma_{pl} - \gamma_{pv} - \gamma_{lv} \quad (4)$$

For a liquid, the force of cohesion, which is the interaction with itself is described by;

$$\Delta F_{ll}^{coh}(d_1) = -2\gamma_{lv} \quad (5)$$

ΔF^{adh} can be determined by several approaches, apart from the above surface free energy approach. The classical work of Hamaker [7] is very appropriate.

To explain the concept of Hamaker Constants, use is made of the van der Waals explanation of the derivations of the ideal gas law;

$$PV = RT \quad (6)$$

It was discovered that the kinetic energy of the molecules which strike the container wall is less than that of the bulk molecules. This effect was explained by the fact that the surface molecules are attracted by the bulk molecules (as in fig.3) even when the molecules have no permanent dipoles. It then follows that molecules can attract each other by some kind of cohesive force [8]. These forces have come to be known as van der Waals forces. van der Waals introduced the following corrections to eqn.(6);

$$\left[P + \frac{a}{V^2}\right](V - b) = RT \quad (7)$$

The correction term to the pressure, $\left(\frac{a}{V^2}\right)$ indicates that the kinetic energy of the molecules which strike the container wall is less than that of the bulk molecules. This signifies the earlier mentioned attraction between the surface molecules and the bulk molecules.

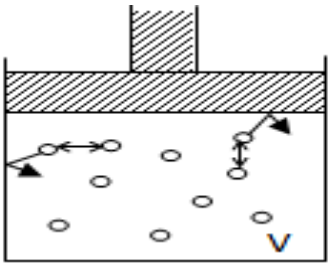

Fig. 3: Attraction of Surface Molecules by Bulk Molecules in a Container of Volume, V [9]

After the development of the theory of quantum mechanics, London quantified the van der Waals statement for molecules without a dipole and so molecular attraction forces began to be known as London/van der Waals forces [10]. London stated that the mutual attraction energy, V_A of two molecules in a vacuum can be given by the equation;

$$V_A = -\frac{3}{4}h\upsilon_0\left[\frac{\alpha^2}{H^6}\right] = -\left[\frac{\beta_{11}}{H^6}\right] \quad (8)$$

Where; h = Planck's constant, υ_o = the characteristic frequency of the molecule, α = the polarizability of the molecule, H = their separation

The interaction of two identical molecules of a material 1 is shown in fig.4 below.

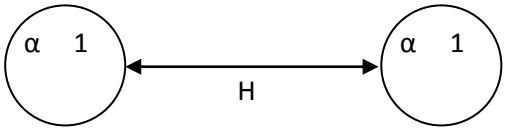

Fig. 4: Interaction of Two Identical Molecules of Materials, 1 and Polarizability, α, at a Separation, H [9]

Hamaker made an essential step in 1937 from the mutual attraction of two molecules. He deduced that assemblies of molecules as in a solid body must attract other assemblies. The interaction energy can be obtained by the summation of all the interaction energies of all molecules present as in fig.5 below.

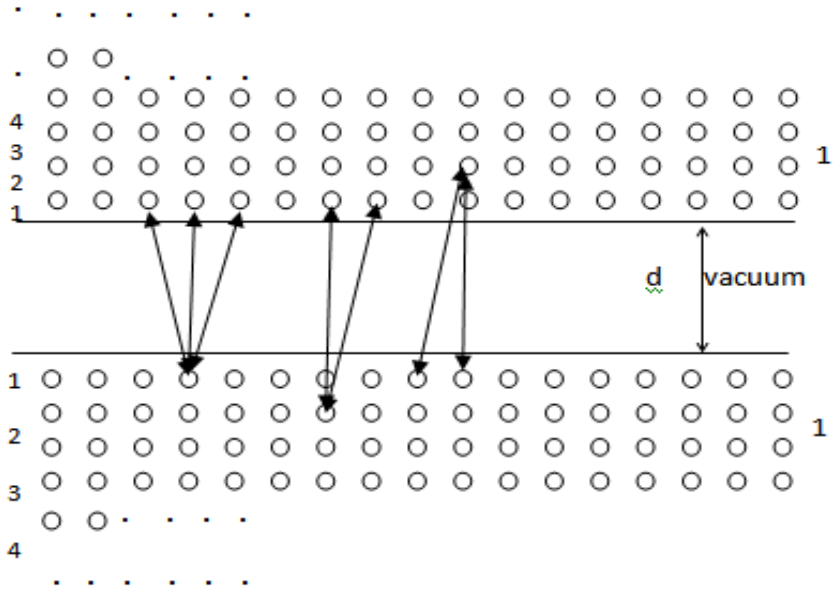

Fig. 5 Interaction of Two Semi-infinite Solid Bodies, 1 at a Separation, d in Vacuum [9]

B. Mathematical Model for the Interactions between the Lymphocyte and the Virus

The mutual attraction energy, V_A of two molecules in a vacuum is given by;

$$V_A = -\frac{3}{4} h v_0 \left[\frac{\alpha^2}{H^6}\right] = -\left[\frac{\beta_{11}}{H^6}\right] \quad (9)$$

The assemblies of molecules as in a solid body have interaction energy as the summation of all the interaction energies of all the molecules present and the van der Waals pressure, P_{vdw} as follows;

$$P_{vdw} = \left[\frac{A_{11}}{6\pi d^3}\right] \quad (10)$$

For a sphere of radius, R and a semi-infinite body at a maximum separation distance, d the van der Waals force of attraction, F_{vdw} is given as;

$$F_{vdw} = \left[\frac{A_{11} R}{6 d^2}\right] \quad (11)$$

Where A_{11} = Hamaker constant

$$A_{11} = \pi^2 q_1^{\,2} \beta_{11} \quad (12)$$

Where q_1 = number of atoms per cm^3, β_{11} = London-van der Waals constant

Given two dissimilar condensed bodies of given geometry with a separation distance, d, the corresponding van der Waals force between them can be determined. For the system under study, the interacting bodies are the lymphocytes, 1 and the virus, 2.

Fig. 6: Interaction of Two Un-identical Molecules of Lymphocyte, 1 and Virus (HIV), 2, at a Separation, d

The van der Waals force between the lymphocyte, 1 and the virus, 2 is given by the relations;

$$F_{vdw} = -\left[\frac{A_{12}R_{12}}{6d^2}\right] \quad (13)$$

Where; $A_{11} = \pi^2 q_1^{\,2}\beta_{11}$ = Hamaker constant for lymphocyte

$A_{22} = \pi^2 q_2^{\,2}\beta_{22}$ = Hamaker constant for the virus (HIV)

$A_{12} = \pi^2 q_{12}^{\,2}\beta_{12}$ = Hamaker constant for both materials (i.e. lymphocyte and the virus)

Where; $\beta_{12} = \sqrt{\beta_{11}\beta_{22}}$

Thus the Hamaker constant becomes;

$$A_{12} = \sqrt{\left(\pi^2 q_1^{\,2}\beta_{11}\right)\left(\pi^2 q_2^{\,2}\beta_{22}\right)} \quad (14)$$

$$A_{12} = \sqrt{A_{11}A_{22}} \quad (15)$$

For a combination of our two dissimilar materials (i.e. lymphocyte 1, and the virus 2) with the gap between them filled with plasma or serum as the medium 3 the combined Hamaker coefficient will be given by;

$$A_{132} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)\left(\sqrt{A_{22}} - \sqrt{A_{33}}\right) \quad (16)$$

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad (17)$$

A_{33} = Hamaker constant for serum (plasma)
A_{13} = Hamaker constant for both materials (i.e. lymphocyte and plasma)
A_{23} = Hamaker constant for both materials (i.e. the virus and plasma)
A mean of all the values of the combined Hamaker coefficient, A_{132} gives an absolute value for the coefficient denoted by A_{132abs};

$$A_{132abs} = \frac{3}{4}\pi\hbar\int_0^{\infty}\left[\frac{\varepsilon_1(i\zeta)-\varepsilon_3(i\zeta)}{\varepsilon_1(i\zeta)+\varepsilon_3(i\zeta)}\right]\left[\frac{\varepsilon_2(i\zeta)-\varepsilon_3(i\zeta)}{\varepsilon_2(i\zeta)+\varepsilon_3(i\zeta)}\right]d\zeta \quad (18)$$

Applying the limits of integration (for the minimum and maximum values of A_{132} respectively), the absolute value for the combined Hamaker coefficient could thus be derived from;

$$A_{132abs} = \frac{\sum_0^N (A_{132})}{N} \quad (19)$$

IV. DATA ANALYSIS

A. Relevant Mathematical Applications

From the knowledge of light absorbance, reflection and transmittance, it could be noted that;

$\bar{\mathbf{a}} + T + R = 1$ (20)

Where; $\bar{\mathbf{a}}$ is absorbance, T is transmittance, and R is reflectance

Also; $T = \exp^{-\bar{\mathbf{a}}}$ (21)

With the values of $\bar{\mathbf{a}}$ and T ascertained, R could easily be derived by substituting into eqn.(20).
The next step is to find a value for the refractive index, *n* employing the mathematical relation [11]

$$n = \left[\frac{1-R^{1/2}}{1+R^{1/2}}\right] \quad (22)$$

A value for the extinction coefficient, *k* is obtained from the equation;

$$k = \left[\frac{\alpha\lambda\times10^{-9}}{4\pi}\right] \quad (23)$$

Where; α is the absorption coefficient defined as follows;

$$\alpha = \left[\frac{\bar{a}}{\lambda\times10^{-9}}\right] \quad (24)$$

The dielectric constant, ε could thus be given by the formula [12]

For the real part; $\varepsilon_1 = n^2 - k^2$ (25)

For the imaginary part; $\varepsilon_2 = 2nk$ (26)

With these values, it is possible to use the approximate approach of eqn.(27) to determine A_{11}.

$$A_{11} = 2.5\left[\frac{\varepsilon_{10} - 1}{\varepsilon_{10} + 1}\right]^2 = 2.5\left[\frac{n_1^{\,2} - 1}{n_1^{\,2} + 1}\right]^2 \quad (27)$$

This gives a value to the Hamaker constant $A_{11,}$ and by extension to other Hamaker constants A_{22} and $A_{33.}$ Thus, the Hamaker coefficient, A_{132} could readily be gotten from the relations as in eqns.(28) and (29);

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad (28)$$

Alternatively; $$A_{132} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)\left(\sqrt{A_{22}} - \sqrt{A_{33}}\right) \quad (29)$$

B. Comparison between the Peak Absorbance Values of HIV Positive and Negative Blood Components

It could be seen that the peak absorbance values of the various blood samples and components vary in magnitude revealing the notable effect of the virus on them. A quick comparison between the positive and negative samples of the lymphocytes is very crucial to this research. This is because the human immunodeficiency virus actually attacks the lymphocytes by attaching itself to the CD4+ cells. The table 1 below reveals the degree of variation between similar infected and uninfected blood components at a glance for a clearer understanding.

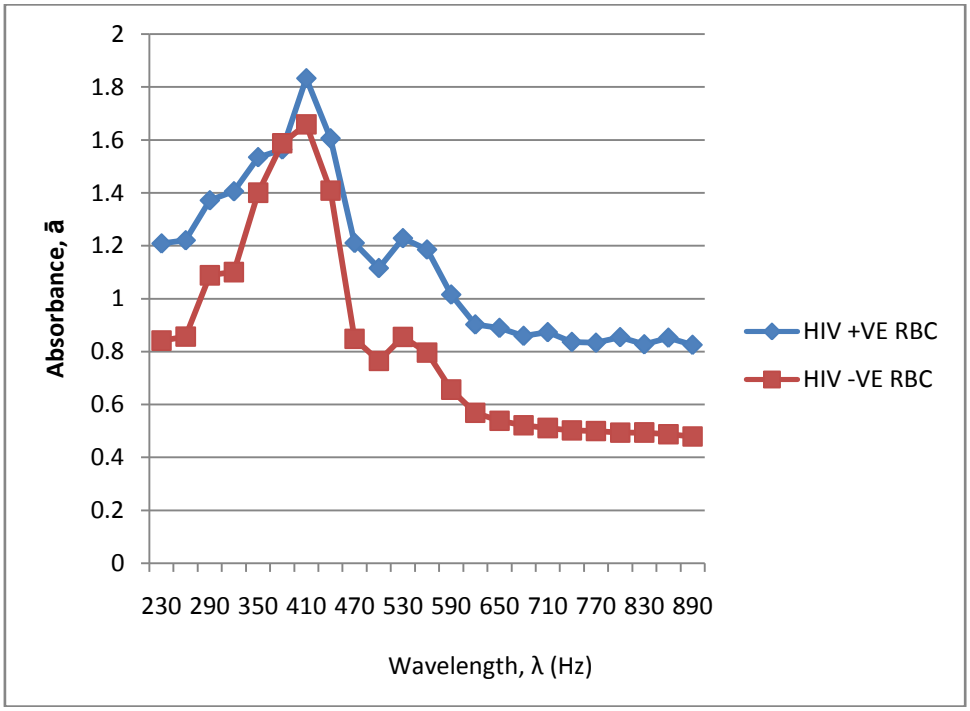

Fig. 7: Plot of Absorbance Vs Wavelength for HIV +ve and –ve Red Blood Cells

A peak absorbance value of greater than 1.8 and 1.6 for HIV positive and negative Red Blood cells respectively were recorded at wavelength of 410Hz. This is significant as a reference point in the study of the viral infection mechanism and may be of interest in determining the critical Hamaker constant that favours repulsion between the virus and the lymphocyte. It could be noted that the infected Red Blood cells have higher absorbance values than the uninfected ones. This also indicates that the Serum/Plasma holds the key to energy change of the interacting system as it shows quite the reverse of the trend obtained in the other cases.

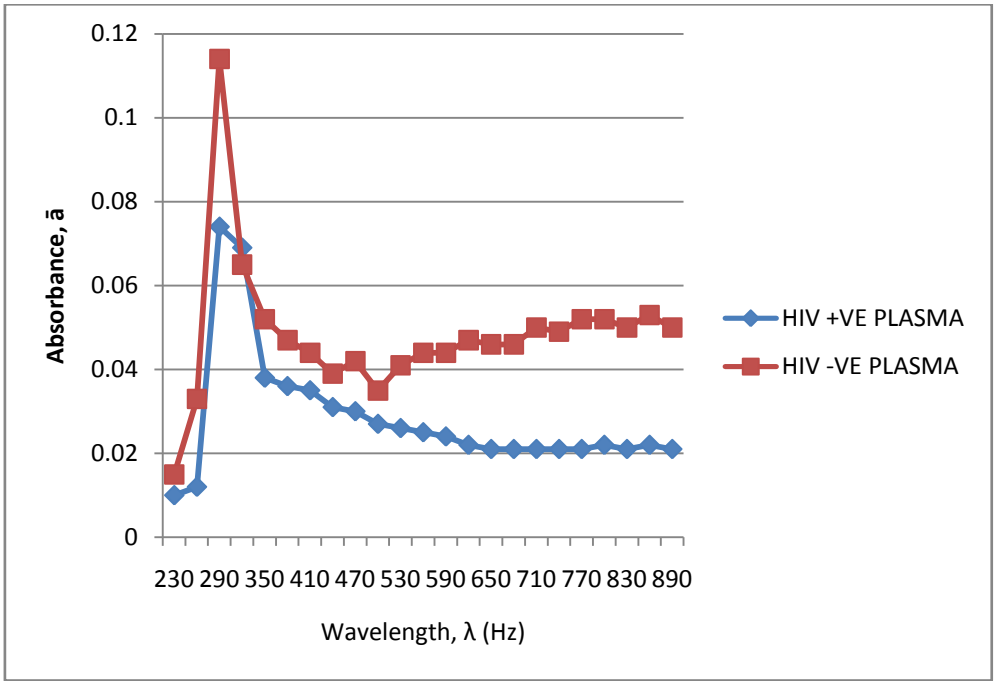

Fig. 8: Plot of Absorbance Vs Wavelength for HIV +ve and –ve Plasma

The trend here shows the opposite as the uninfected plasma reveals a higher absorbance values at all wavelengths. This indicates that a shift in the energy equation of the system is tenable by some alteration to the serum as an intervening medium in the HIV-Lymphocyte interaction. It is then suggests a possibility of attaining repulsion between the HIV and the White Blood cells by some additives to the Serum.

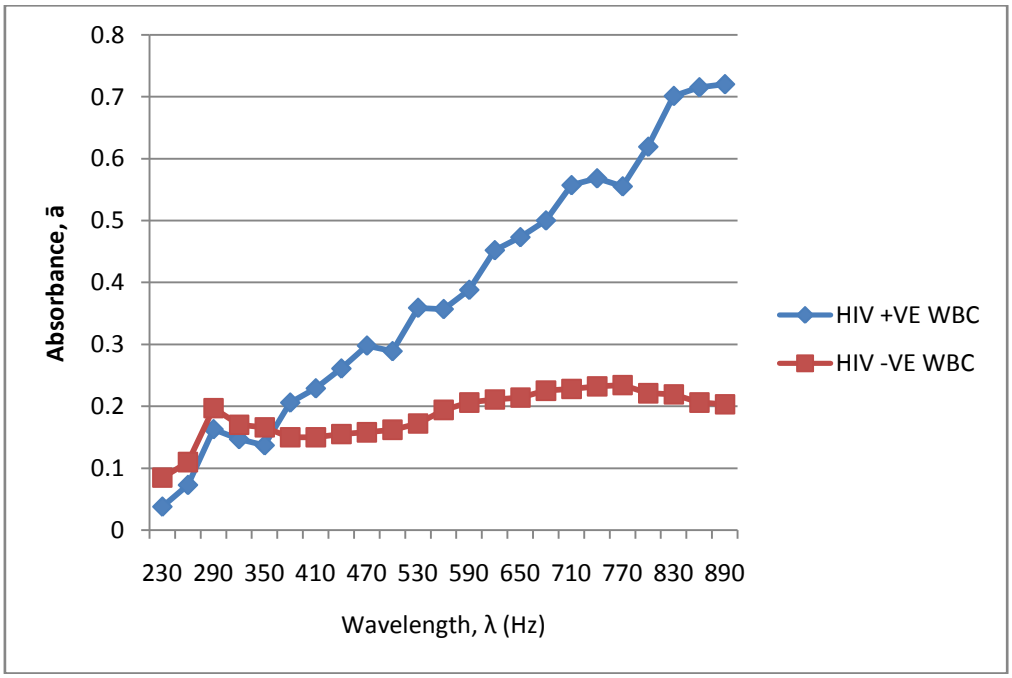

Fig. 9: Plot of Absorbance Vs Wavelength for HIV +ve and –ve White Blood Cells

The absorbance values of both the HIV positive and negative Lymphocytes (White Blood cells) were increasing with increase in wavelength. This is the opposite of the results obtained with the Red Blood cells and the Plasma. This may be explained away by the fact of a higher energy level of these cells. The HIV infected lymphocytes also gave higher absorbance values than the uninfected ones at wavelengths greater than 350Hz. This is a clear indicator that infection had occurred and shows the alteration in absorbance values due to HIV infection.

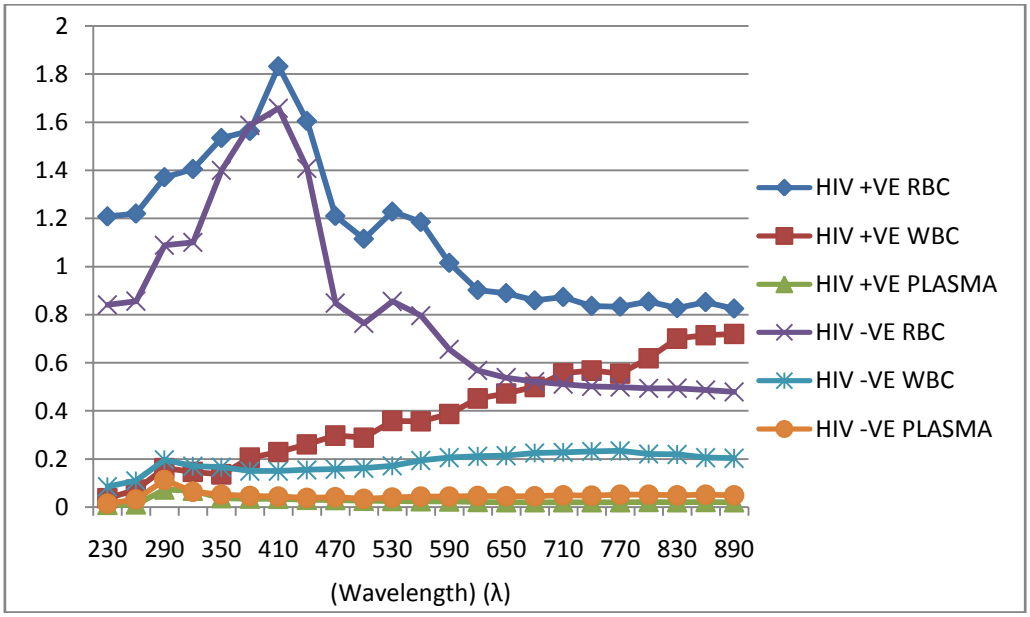

Fig. 10: Combined Plot of Absorbance Vs Wavelength for all Three Blood Components

Table 1: Comparison between Peak Absorbance Values of HIV Positive and Negative Blood Components Respectively [13], [14]

Sample Type	Wavelength, λ (Hz) (Peak Values)	Absorbance, ā (Peak Values)		
		HIV Positive	HIV Negative	Variance
Lymphocytes (WBC)	**290**	**0.019 – 0.163**	**0.040 – 0.197**	**0.021 – 0.034**
Plasma (Serum)	**290**	**0.018 – 0.074**	**0.021 – 0.114**	**0.003 – 0.040**
Red blood cells (RBC)	**410**	**0.424 – 1.832**	**0.473 - >3.000**	**0.049 - >1.168**

The disparity between the peak absorbance values of HIV positive and negative blood components respectively is an indication of how the virus affects the properties of blood. The trend is such that the absorbance values of the HIV positive samples is generally decreased by a significant factor as shown in table 1 above. The lymphocytes are of particular interest to this research since the virus attacks this blood component by being attached to the CD4+ cells which serve as receptor cells. As could be seen from the table, the variation between the peak values of absorbance of the various blood components is such that the lymphocytes vary with a magnitude of 0.021 to 0.034, the red blood cells differ by a factor of 0.049 to >1.168 while the plasma had a difference of between 0.003 and 0.040. The decrease in the absorbance of the HIV infected blood samples reveals the role of the virus in significantly affecting the surface properties of the infected blood cells and specimens.

V. CONCLUSION

This research work on HIV-blood interaction has further buttressed the place of the relevance of engineering thermodynamics or at least quasi-thermodynamics in finding solution to various scientific and biological processes.

The indispensible fact of inter-relativity of diverse disciplines and the prime place of engineering to this end cannot be overemphasized. This in some way goes to speak of concurrent engineering and its vital role in the twenty first century research.

This research concludes that there is a possibility of finding an antidote/cure for the HIV-AIDS pandemic if further work towards defining the conditions of the system that could render the absolute combined Hamaker coefficient negative and the additive(s) to the serum (in form of drugs) as the intervening medium that could achieve this condition. That predictably may be the much desired solution.

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