Human Immunodeficiency Virus (HIV)-Blood Interactions: Surface Thermodynamics Approach

C.H. Achebe, *Member, IAENG*, S.N. Omenyi, O.P. Manafa, D. Okoli

Abstract - Sequel to the earlier works by Omenyi et al which established the role of surface thermodynamics in various biological processes from the electrostatic repulsion and van der Waals attraction mechanisms, HIV-blood interactions were modeled. This involved the use of the Hamaker coefficient approach as a thermodynamic tool in determining the interaction processes. It therefore became necessary to apply the Lifshitz derivation for van der Waals forces as an alternative to the contact angle approach which has been widely used in other biological systems. The methodology involved taking blood samples from HIV-infected and uninfected persons for absorbance measurement using Ultraviolet Visible Spectrophotometer. From the absorbance data various variables required for computations with the Lifshitz formula were derived. The Hamaker constants A_{11}, A_{22}, A_{33} and the combined Hamaker coefficients A_{132} were obtained using the values of the dielectric constant together with the Lifshitz equation. The absolute combined Hamaker coefficient, A_{132abs} for the infected blood samples gave the value of $0.2587x10^{-21}$Joule. The positive sense of this value implies net positive van der Waals forces indicating an attraction between the virus and the lymphocyte. A lower value of A_{131abs} = $0.1026x10^{-21}$ Joule obtained for the uninfected blood samples is also an indicator that a zero or negative absolute combined Hamaker coefficient is attainable.

Index Terms - Absorbance, Dielectric Constant, Hamaker Coeficient, Human Immunodeficiency Virus, Interaction Mechanisms, Lifshitz formula, Lymphocyte, Surface Thermodynamics, van der Waal.

I. INTRODUCTION

AT the 2001 Special Session of the UN General Assembly on AIDS, 189 nations agreed that AIDS was a national and international development issue of the highest priority [1]. Between December 2005 and March 2006, UNAIDS compiled data from reports obtained from 126 countries on HIV/AIDS prevalence. In sub-Saharan Africa the epidemic continued to ravage beyond limits that many experts believed impossible. Also, relatively new but rapidly growing epidemics in regions such as Eastern Europe and South-East Asia that may come to rival that of sub-Saharan Africa in scope had erupted [2].

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Over time diverse clinical approaches to the issue of HIV/AIDS have been employed to seek to proffer possible solutions to the threat. 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 [3].

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 roles in phenomena as diverse as the critical closing and opening of vessels in the microcirculation, cell adhesion, protein adsorption, antigen-antibody interactions, and phagocytosis [5].

In this study, we conceptualize HIV as a particle dispersed in a liquid (the serum) and interacting with another particle (CD4 lymphocyte). 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 of 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).

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 implicated. This concept will be employed in this research work to characterize the HIV-CD4 Lymphocyte 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 co-receptors that aid these interactions. The discovery and application of highly active anti-retroviral therapy (HAART) to suppress HIV has revolutionized the clinical

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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 [3] and the worldwide picture is one of increasing rates of infection [4].

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 [5].

II. THEORETICAL CONSIDERATIONS

A. Thermodynamic Approach to Particle-Particle Interaction

Consider the case where the virus, HIV conceived as a particle approaches the CD4 lymphocyte (also assumed to be a particle) and attaches itself on the surface of the lymphocyte dispersed in a serum, as shown in fig.1.

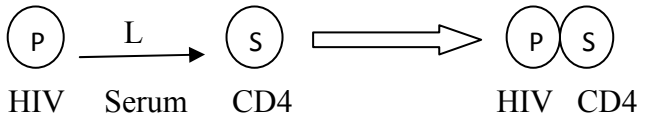

Fig. 1. Schematic of HIV-CD4 Lymphocyte Adhesion Process

The thermodynamic free energy of adhesion, ΔF^{adh} for the process shown in Fig.1 can be expressed as follows [19].

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

Where the subscript P stands for the virus, S stands for the blood cell and L the serum. ΔF^{adh} is the free energy of adhesion integrated from infinity to the equilibrium distance, d_o. For the virus to succeed in penetrating the membrane of the blood cells, the net free energy of engulfing (of the virus by the blood cell) will be given by;

$$\Delta F_{NET} = \gamma_{ps} - \gamma_{pl} < 0 \quad (2)$$

If ΔF_{NET} is greater than zero, the blood cell membrane will reject the virus.

The solid-liquid interfacial tension (γ_{sl}) plays an important role in a wide range of problems in the field of science and applied science. Thermodynamic models based on interfacial tensions have successfully been used to explain many phenomena such as cell adhesion, protein adsorption, phagocytosis, etc [5].

Unlike fluid-fluid interfacial tensions, interfacial tensions involving a solid phase are difficult, if not impossible, to measure directly [6]. The most common approach for estimating these interfacial tensions involve interpretation of contact angle data. The attractiveness of using contact angles to estimate solid-liquid (γ_{sl}), and solid-vapour (γ_{sv}) interfacial tensions arises from the fact that contact angles can in principle, be measured relatively easily on suitably prepared solid surfaces[5]. In order to estimate γ_{sl} and γ_{sv} from contact angle data, Young's equation which only gives the difference; $\gamma_{sv} - \gamma_{sl}$,

$$\text{i.e., } \gamma_{sv} - \gamma_{sl} = \gamma_{lv} \cos\theta \quad (3)$$

must be used. The drawback in the use of eqn.(3) is the difficulty in measuring γ_{sl}. Neuman [7] in his rigorous work solved the problem by providing an empirical expression given in eqn.(4).

$$\gamma_{sl} = \frac{\left(\gamma_{sv}^{1/2} - \gamma_{lv}^{1/2}\right)^2}{1 - 0.015\left(\gamma_{sv}\gamma_{lv}\right)^{1/2}} \quad (4)$$

The equation of state, eqn.(4), in conjunction with eqn.(3) can be used to determine γ_{sl} and γ_{sv} from contact angle data θ.

There are available several other interfacial tension equations based on surface tension components approach [8], in terms of the dispersion and polar interactions.

If in this study we assume that the interfacial free energies are dispersive, then the polar contributions to the particle interfacial tension play no role in determining the interfacial tensions. The total free energy of adhesion will be given solely by the dispersion interactions, and can be obtained from Lifshitz theory considerations. The advantage of using Lifshitz-van der Waals interactions instead of the interfacial tension components equations was stated earlier. Briefly the calculation of dispersion interactions from Liftshitz theory is independent of contact angle data and these interactions are not approximated by geometric means as is the case when surface tension component equations are used [6].

B. Lifshitz Theory

The free energy of interaction for a system consisting of two plane, semi-infinite, parallel bodies of materials 1 and 2 separated by a material 3, of thickness L is given by;

$$\Delta F_{132} = -\left[\frac{-A_{132}}{12\pi L^2}\right] \quad (5)$$

Where, A_{132} is the Hamaker constant for the system. The Hamaker constant can be calculated through the pair-wise additivity approach as originally proposed by Hamaker [9] or by the macroscopic approach of Lifshitz [13].

The major problem associated with using a pair-wise summation of the interactions between molecules is that the influence of neighbouring atoms on these interactions is ignored. While this influence may not be significant for very disperse media, such as gases, it becomes important in condensed media [6]. The macroscopic approach of Lifshitz avoids this problem because the actual atomic structures of the materials are ignored and the interactions between macroscopic bodies are calculated from bulk material properties, such as the dielectric permittivity $\varepsilon(i\xi)$ and refractive indices. The dielectric permittivity is the macroscopic manifestation of the microscopic polarizability of the constituent atoms of the material. The Hamaker coefficient is therefore, the macroscopic resultant of the interactions due to the polarization of the different atoms in the material [6].

The Hamaker coefficient, according to Lifshitz theory is given by:

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$$A_{PLS}=\frac{3}{4}\pi\hbar\int_0^{\infty}\left[\frac{\varepsilon_i(i\zeta)-\varepsilon_k(i\zeta)}{\varepsilon_i(i\zeta)+\varepsilon_k(i\zeta)}\right]\left[\frac{\varepsilon_j(i\zeta)-\varepsilon_k(i\zeta)}{\varepsilon_j(i\zeta)+\varepsilon_k(i\zeta)}\right]d\zeta \quad (6)$$

Where $\varepsilon_j(i\zeta)$ is the dielectric constant of material, j along the imaginary frequency axis and $\hbar$ is Planck's constant.

Eqn. (5) when evaluated at molecular contact (L=0), should be equivalent to the thermodynamics free energy of adhesion, eqn.(1). Due to the finite size of the constituent molecules, two macroscopic surfaces cannot approach to L=0. Isrealachvili [15] introduced a cut-off distance parameters, d_o, which represents the closest distance that two surfaces can approach.

When the surfaces are at a distance d_o, they are considered to be in molecular contact. The parameter d_o, therefore eliminates the divergence in Lifshitz theory. The free energy of adhesion, using the concept of d_o, is related to eqn. (5) via:

$$\Delta F^{adh}=-\left[\frac{A_{PLS}}{12\pi d_0^2}\right] \quad (7)$$

Hough and White [6] found that a value of $1.6\text{x}10^{-10}$m gave satisfactory estimates of interfacial tensions of liquid alkanes while Omenyi et al found that a value of $1.82\text{x}10^{-10}$m was satisfactory in various particle interaction processes [3].

Eqn. (7) implies that if the Hamaker coeeficient A_{PLS} for a particle/liquid/solid system is known, then it is possible to estimate the total dispersion or London-van der Waals interactions for such a system.

The lifshitz-van der Waals constant A_{132} otherwise known as Hamaker coefficient could be negative. In such instance a repulsive electrostatic force will be developed which impairs contact between the interacting particles. The effect here is repulsion rather than attraction among the particles.

TABLE 1
COMBINATIONS OF MATERIALS FOR WHICH NEGATIVE LIFSHITZ-VAN DER WAALS CONSTANT A_{132} IS FOUND [25]

System	A_{132} /eV
Si/Al_2O_3	- 0.19
Ge/Cds/olystyrene	- 0.28
Cu/MgO/KCl	- 0.17
Au/Si/KCl	- 0.81
Au/Polystyrene/H_2O	- 0.14

The table1 above consists in systems that the individual Lifshtiz-Hamaker constants obey. This implies that in the macroscopic theory of van der Waals forces there are situations where the van der Waals force of three different materials can be negative. This therefore demonstrates that the concept of negative van der Waals force is physically sound. For a system of a particle of radius R, interacting with a plane solid surfaces in a liquid medium, eqn.(7) can be written as;

$$\Delta F^{adh}=-\left[\frac{A_{PLS}R}{6\pi d_0^2}\right] \quad (8)$$

Because of the problem of establishing the radius R of the virus, in this preliminary study, it is assumed that on a molecular level, the two surfaces approaching themselves appear plane to each other, so that eqn.(7) can be used. The Hamaker coefficient is related to the interfacial free energies by;

$A_{PLS} = -12\pi\, d_o^2\,(\gamma_{PS} - \gamma_{PL} - \gamma_{SL})$ (9)

Obtained by combining eqn.(1) with eqn.(7). It is worth noting that for interaction of a particle against itself, then from eqn.(6);

$$A_{ij}=\frac{3}{4}\pi\hbar\int_0^{2}\left[\frac{\varepsilon_i(i\zeta)-\varepsilon_j(i\zeta)}{\varepsilon_i(i\zeta)+\varepsilon_j(i\zeta)}\right]^2 d\zeta \quad (10)$$

Thus, for our system,

$A_{PLP} = A_{PP} + A_{LL} - 2A_{PL} = (\sqrt{A_{PP}} - \sqrt{A_{LL}})^2$ (11)

$A_{PLS} = A_{PS} + A_{LL} - A_{PL} - A_{SL} = (\sqrt{A_{PP}} - \sqrt{A_{LL}})\,(\sqrt{A_{SS}} - \sqrt{A_{LL}})$ (12)

TABLE 2
VALUES OF DIVERS VARIABLES FOR BOTH INFECTED AND UNINFECTED BLOOD COMPONENTS

Blood Component	Wave-length at Peak Absorb-ance (Hz)	Ranges of Peak Absorb-ance	Cell Count ($\text{x}10^{10}$ Cells/l)	A_{ii} ($\text{x}10^{-21}$ J)
HIV Infected RBC	410	0.424 to 1.832	230 to 450	2.9575
Uninfected RBC	410	0.474 to 3.000	470 to 560	3.1212
HIV Infected CD4 Lymphocyte	290	0.019 to 0.163	0.009 to 0.124	0.9868
Uninfected CD4 Lymphocyte	290	0.040 to 0.197	0.080 to 0.180	0.9659
HIV Infected Serum	290	0.018 to 0.074	-	0.2486
Uninfected Serum	290	0.021 to 0.114	-	0.4388

To determine the combined Hamaker coefficient using the Lifshitz theorem of eqn. (10), there is a need to evaluate the dielectric constant ε of that equation. This could be done through the measurement of the absorbance for each sample of infected and uninfected blood.

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

$\bar{\mathbf{a}} + T + R = 1$ (13)

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

Also; $T = \exp^{-\bar{\mathbf{a}}}$ (14)

With the values of $\bar{\mathbf{a}}$ and T ascertained, R could easily be derived by substituting into eqn.(27).

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The next step is to find a value for the refractive index, n employing the mathematical relation [22].

$$n = \left[\frac{1 - R^{1/2}}{1 + R^{1/2}}\right] \quad (15)$$

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

$$k = \left[\frac{\alpha\lambda \times 10^{-9}}{4\pi}\right] \quad (16)$$

Where; α is the absorption coefficient defined as follows;

$$\alpha = \left[\frac{\bar{a}}{\lambda \times 10^{-9}}\right] \quad (17)$$

The dielectric constant, ε could thus be given by the formula [21].

For the real part; $\varepsilon_1 = n^2 - k^2$ (18)

For the imaginary part; $\varepsilon_2 = 2nk$ (19)

With these values, it is possible to calculate A_{PLS} or A_{ij} using the relevant equations.

III. EXPERIMENTAL PROCEDURES

A. Sample Collection

This research work involved collection of blood samples from twenty HIV infected and twenty uninfected persons from the Nnamdi Azikiwe University Teaching Hospital. The collected blood samples were screened to determine the infection status. Each sample was mixed with some anticoagulants in test tubes to ensure the freshness of the collected samples and to avoid the samples becoming lysed (spoilt). The samples were refrigerated pending usage to avoid spoilage. Since it was not possible to separate HIV from infected cells, thus the infected cells were assumed to represent the HIV. This assumption is acceptable owing to the manner of infection which is viewed as a surface effect thus a particle-particle interactions mechanism.

B. Sample Preparation

The collected samples were loaded into a centrifugal separator and the blood components were separated. 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. Glass slides were prepared and smeared with the samples for absorbance measurements. The slide preparations and sample smearing were done at the same laboratory with proper safety measures to avoid contact with the skin.

C. Measurements

The CD4+ counts of the blood samples were obtained using a digital CD4+ Counter and results presented on table 1. 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 for red blood cells obtained were plotted in fig.4 for 20 infected patients and fig.3 for 20 uninfected persons.

IV. DATA PRESENTATION AND ANALYSIS

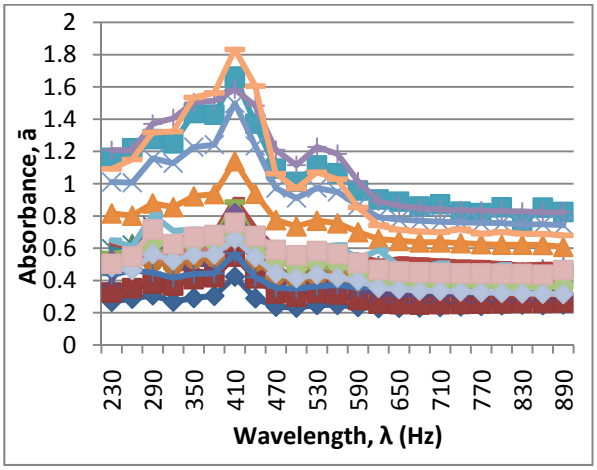

Fig. 2. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Infected Red Blood Cells

Fig. 2 reveals an interesting pattern for HIV positive red blood cells. The absorbance of the respective twenty HIV infected blood samples steeply increased as the wavelength increased until a critical wavelength of 410Hz, where a peak value was attained. A further increase in the wavelength saw at first a steep and latter a gradual decrease in the absorbance values. This peak value falls within the visible range of the ultraviolet radiation which is between 300 - 600Hz. The peak values of absorbance ranges between 0.424 and 1.832.

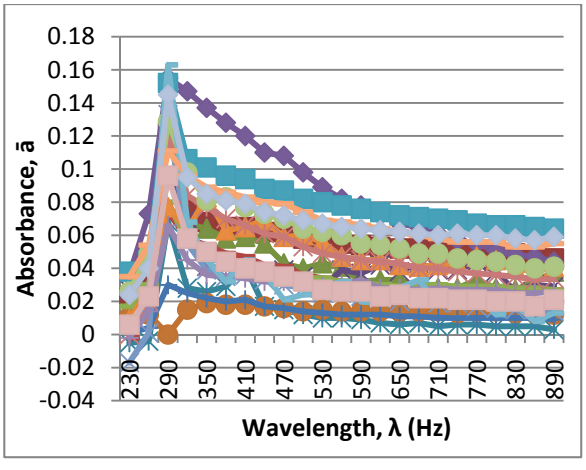

Fig. 3. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Positive Lymphocytes [24]

Fig. 3 shows a similar pattern as that of fig.2 with the peak value occurring at the wavelength of 290Hz for infected patients. However, the peak absorbance values are of the range $0.019 \leq \bar{a} \leq 0.163$.

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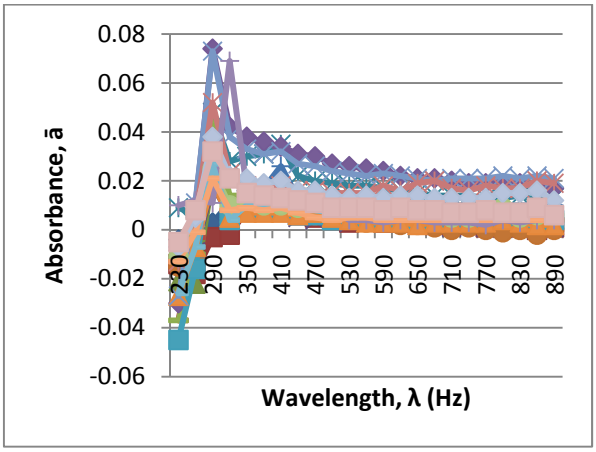

Fig. 4. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Positive Plasma (Serum)

Here once again the plot followed the initial pattern exhibited by other blood components. The peak value here occurred at a wavelength of 290Hz which corresponds exactly with that of the lymphocytes. However, the peak absorbance range is between 0.018 and 0.074. It is interesting though, that at the lower wavelengths of between 230–260Hz negative absorbance values were recorded.

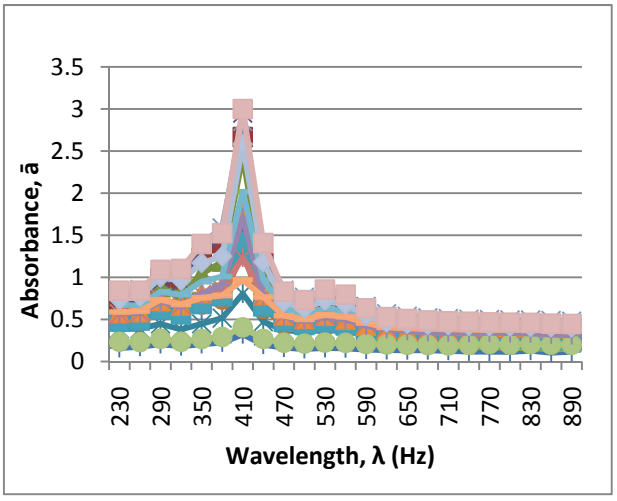

Fig. 5. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Negative Red Blood Cells (RBC)

The peak value of the absorbance for HIV negative red blood cells was obtained at the wavelength of 410Hz and ranges as follows $0.473 \leq \bar{a} > 3$. The reaction here also follows the earlier patterns with all the various twenty samples showing moderately conformed characteristics.

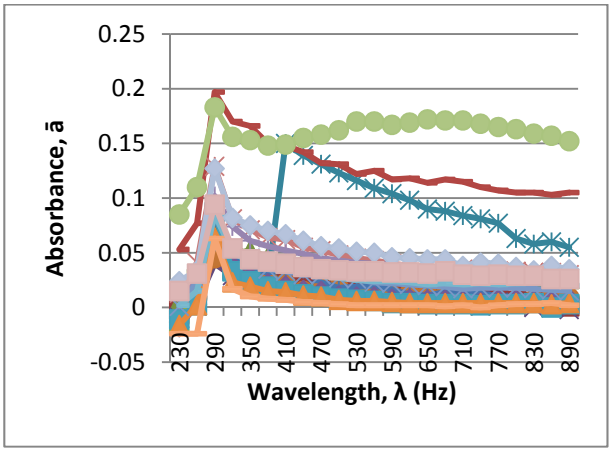

Fig. 6. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Negative Lymphocytes

The plot for the samples of HIV negative lymphocytes reveals similar characteristics as their counterparts, however with the peak values occurring at 290Hz. The absorbance values at this peak point are of the magnitude between 0.040 – 0.197.

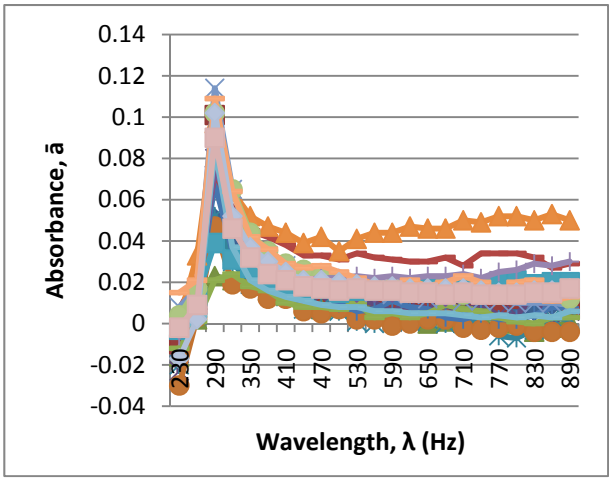

Fig. 7. Plot of Absorbance, $\bar{a}$ versus Wavelength, λ for Twenty Samples of HIV Negative Plasma (Serum)

For the HIV negative plasma samples, a small portion of the absorbance falls within the negative band. The characteristics of the plot follow the usual pattern once more with the peak value obtained at a wavelength of 290Hz. The absorbance values at this peak point fall within the range of 0.021 and 0.114.

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A. Computation of the Hamaker Coefficients

Eqn.(10) was used to obtain for each interacting systems, A_{ij} (A_{11}, A_{22}, A_{33}, A_{12}, A_{13}, A_{23}) by appropriate change of variables. MATLAB computation tools were used. This involved the numerical integration of eqn.(10) for each wavelength from 230 to 890 for all the twenty samples in each category. The results are given also on table 2.

The infected lymphocytes are used in lieu of the virus because there is currently no known means of isolating the virus. The assumption here is that the infected lymphocyte is an approximation of the actual virus owing to the manner of the infection. The mechanism of the viral infection is such that it actually attaches its CD8+ cells on the wild CCR5 dendrites of the blood CD4+ T4 cells while changing the nature of the cells.

Ultimately a single value of the Hamaker coefficient is essential in determining the final outcome. This is known as the absolute combined Hamaker coefficient, A_{132abs}. It is obtained by deriving a mean for all the values of the Hamaker coefficient to give a single value.

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.

The positive value of the absolute combined Hamaker coefficient $A_{132abs}=0.2587x10^{-21}$Joule obtained for the HIV positive samples is an affirmation that the blood samples were actually infected. This when compared with the value of $A_{131abs}=0.1026x10^{-21}$Joule obtained for the uninfected blood samples is conclusive of the fact of the relevance of the concept of Hamaker coefficient to HIV-blood interactions.

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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