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The Role of Attractive Van der Waals Forces in the Interactions Between the Antiretroviral Drugs and the Blood Components

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Abstract: The mechanism by which antiretroviral drugs can block the HIV virus seems weak and hence it becomes necessary to study the interaction between the HIV and the drug-coated white blood cells. The effect of the Van der Waals forces were studied to determine and understand the interaction processes in the interaction between antiretroviral drugs and the blood components in human. The method adopted involved the serial dilution of the five different antiretroviral drugs (two HAART/FDC and three single drugs) used in this study and the subsequent incubation with the blood samples collected from HIV negative persons for the absorbance measurement using a digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer. The digital CD4 count machine (Cytoflowmeter) was equally used to obtain the CD4 counts of the blood samples used for this study. The variables required for the computations with the Lifshiftz formula were derived from the absorbance data. The MATLAB algorithm software tools were employed in the mathematical analysis of the very large body of data generated from the experiments. The Hamaker constants A_{11}, A_{22}, A_{33} and the combined Hamaker coefficients A_{131} of the various drugs interacting with the blood were obtained using the values of the dielectric constant together with the Lifshiftz equation. The absolute combined Hamaker coefficient A_{13Iabs} (a mean of all the values of the various Hamaker coefficients) for each antiretroviral drug on uninfected blood samples were also calculated. The absolute values for the combined Hamaker coefficient, A_{131abs} obtained for each of the five antiretroviral drugs interacting with uninfected blood samples are given thus: $D_1 = 0.36760 \times 10{-21}$ Joule, $D_2 = 0.46337 \times 10{-21}$ Joule, $D_3 = 0.53021 \times 10{-21}$ Joule, $D_4 = 0.50971 \times 10{-21}$ Joule, and $D_5 = 0.49599 \times 10{-21}$ Joule. The significance of this result are the positive senses of the absolute combined Hamaker coefficient which imply net positive van der Waals forces indicating an attraction between the antiretroviral drugs and the lymphocytes. This in effect suggests effective coating or binding of the lymphocytes with the drugs. An earlier study was conducted on the Role of Repulsive Van der Waals Interactions in the Treatment of Human Immunodeficiency Virus (HIV) Infections with Antiretroviral Drugs. This study looks the other way round.

Keywords: Absorbance, Transmittance, Dielectric Constant, Hamaker Constant, Hamaker Coefficient, Lifshitz Formula, Lymphocyte, Van der Waals Forces

1. Introduction

The increasing rate of HIV infection globally is blamed on the ineffectiveness of some available antiretroviral therapy to block or resist perfectly this virus from invading the uninfected white blood cells. Hence, the Hamaker coefficient of the HIV positive system is positive even in the presence of antiretroviral drugs. This shows that some of the antiretroviral drugs used in HIV treatment are not really effective since there is net attractive van der Waals force between the interacting particles. The mechanism by which these drugs can block the virus seems weak and hence it becomes necessary to study the interaction between the HIV and the drug-coated white blood cells. The HIV/AIDS cases have hitherto been managed

clinically with the discovery and administration of Highly Active Anti-retroviral Therapy (HAART). But these anti-retroviral drugs are heavily attacked and resisted by the HIV in the human system. The apparent ineffectiveness and failure of HAART is as a result of the ability and capacity of HIV to develop resistance to the administered anti-retroviral drugs. We felt it would be interesting to administer the antiretroviral drugs to uninfected blood and learn how much the drug coats the T4 lymphocytes, which are the components of the blood that attack the virus. Since some of the drugs act as blockers, the blocking would be effective if the drug completely coats the uninfected blood cells. The extent of the cell surface that is coated is important. The mechanism by which drugs can block the virus needs to be well understood to enable formulation of drugs that can effectively block the virus from penetrating the surface of the white blood cell. As HIV attaches itself on the surface of a given lymphocyte cell, some area of the surface of the cell is covered, showing that some change in surface energy has occurred.

Hamaker Coefficient is a significant thermodynamic tool used in determining the interaction processes and will be used in these HIV and drug-coated blood interactions. The Lifshiftz derivation for van der Waals forces is used to model the interaction to predict the nature of interaction between two solid particles suspended in a liquid medium (the serum). When the absolute combined Hamaker coefficient of the drug-blood interactions is positive, the attractive van der Waals forces between the drug and T lymphocyte in HIV negative system will prevail. This indicates effective coating of the surface of the lymphocytes with the antiretroviral drugs. Also, when the absolute combined Hamaker coefficient of the drug-blood interactions is negative in HIV positive system, the van der Waals forces become repulsive. This indicates that the drugs have effectively blocked the HIV from attacking the lymphocytes in the system (1).

2. Methodology

The aim of this study is to employ the van der Waals interactions mechanism to study the interaction between the blood cells and the antiretroviral drugs (single drugs or Highly Active Antiretroviral Therapy, HAART/Fixed-Dose Combination, FDC) as a way to understand the use of these drugs in the proffering solution to the HIV/AIDS pandemic.

2.1. Collection of Samples

This research work involved the collection of popular and commonly used Antiretroviral drugs (three single tablets and two HAART). The samples were collected from the University of Nigeria Teaching Hospital (UNTH) APIN CENTRE PEPFAR, Ituku – Ozalla, Nigeria, and the collection of blood from ten HIV negative persons. The blood samples were collected from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and Anambra State Teaching Hospital, Amaku, Nigeria. Anticoagulant test tubes and ice packs were used to ensure the freshness of the collected samples and to avoid the samples becoming lysed (spoilt). Storage facilities like refrigerators regulated at the temperature range of -4 to 2°C, were also used to ensure that the samples were healthy enough so as to obtain good results.

Figure 1. The Tablets of the five different antiretroviral drugs used.

Table 1. The details of the five different Antiretroviral Drugs used in the Study.

Drug Number	Tablets	Abbrevi Ation	Size	Batch Number	Expiration Date	Pharmaceutical Company
1	Lamivudine, Nevirapine & Zidovudine	3TC + NVP + ZDV	150mg/ 200mg/ 300mg	7220929	01/2016	Strides Arcolab Limited
2	Tenofovir, Lamivudine & Efavirenz	TDF + 3TC + EFV	300mg/ 300mg/ 600mg	3018522	09/ 2015	Mylan Laboratories Limited
3	Nevirapine	NVP	200mg	7216348	04/2015	Strides Arcolab Limited
4	Efavirenz	EFV	600mg	E121035A	07/2015	HETERO LABS LIMITED
5	Lamivudine	3TC	150mg	LEX –023	04/ 2016	MCNEIL & DRUGS Pharmaceuticals Ltd.

Table 1 shows the details of the five different antiretroviral drugs used in the study. Drugs 1 and 2 are both Highly Active Antiretroviral Therapy (HAART) as well as Fixed Dose Combination (FDC), while drugs 3, 4 and 5 are single antiretroviral drugs. Drugs 1, 3 and 5 are administered to HIV patients twice daily while drugs 2 and 4 are taken once a day. It is worthy to note that all the antiretroviral drugs used were not yet expired during the period of the experiments.

2.2. Preparation of Samples

The drugs passed through serial dilution in the Laboratory,

in order to get the right concentration of drug in the blood. After the serial dilutions to 10^{-2}, the drug solution mixed with the blood was incubated at normal body temperature ($37^{\circ}C$) to facilitate drug – blood interactions (This is an in vitro experiment). The knowledge of the onset and duration of action of each drug was used in administering the stat dose and the maintenance dose in the blood samples. These collected samples with drug concentrations were loaded into a centrifugal separator and the blood components were separated. The blood samples were spun at the speed of 3000 rpm for 5 minutes in the centrifuge. It works by the principle of centrifugal force which is the outward pull due to rotation exerted by the centrifuge which is greater than the force of gravity thereby causing the particles in the fluid to sediment. The intension is to achieve clear separation of blood components namely components as White Blood Cells (WBC) also called the Lymphocytes, Red Blood Cells (RBC), and the Plasma or Serum each sample at a time. 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. About 200 slides were successfully prepared in the laboratory.

2.3. Measurement of Samples

The CD4 cells count of the blood samples collected were obtained using a digital CD4 count machine which is known as Flow cytometry instrument. Absorbance measurements were done on all the different components of all ten samples (HIV negative blood samples). A digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer was used in the measurements. The absorbance values of the samples were measured over a range of wavelength spanning between 230 and 800 Hertz alongside with their corresponding transmittance values. The data collected were used to obtain the plots as presented in this research work.

These processes were repeatedly done to ensure reliability of the collected data. The collected samples of popular and unexpired antiretroviral drugs were preserved at ambient temperature. To prevent the collected blood samples getting lysed (spoilt) especially whole blood samples and the isolated red blood cells which are readily susceptible to bacterial and thermal attacks adequate refrigeration was employed.

2.4. Theoretical Considerations

2.4.1. The Concept of Interfacial Free Energy

Consider the work done by a force F to move a flat plate along another surface by a distance dx is given, for a reversible process, given by (1) below;
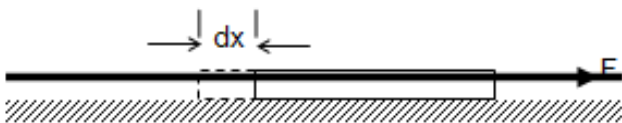
$$\delta w = Fdx \qquad (1)$$

Figure 2. *Schematic Diagram Showing Application of a Force F, on a Surface (Source: 2).*

However, the force F is given by; $F = L\gamma$ (2)

Where L is the width of the plate and γ is the surface free energy per unit surface area (interfacial free energy)

$$\text{Hence; } \delta w = L\gamma dx \qquad (3)$$

$$\text{But; } \delta A = Ldx \qquad (4)$$

$$\text{Therefore; } \delta w = \gamma dA \qquad (5)$$

This is the work required to form a new surface of area dA. For pure materials, γ is a function of T only, and the surface is considered a thermodynamic system for which the coordinates are γ, A and T. The unit of γ is J/m^2. In many processes that involve surface area changes, the concept of interfacial free energy is applicable.

2.4.2. 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_O (3), is given by;

$$\Delta F_{PLS}^{adh}\left(d_O\right) = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \qquad (6)$$

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;

$$\Delta F_{ps}^{adh} = \gamma_{ps} - \gamma_{pv} - \gamma_{sv} \qquad (7)$$

$$\Delta F_{sl}^{adh}\left(d_1\right) = \gamma_{sl} - \gamma_{sv} - \gamma_{lv} \qquad (8)$$

$$\Delta F_{pl}^{adh}\left(d_1\right) = \gamma_{pl} - \gamma_{pv} - \gamma_{lv} \qquad (9)$$

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

$$\Delta F_{11}^{coh}\left(d_1\right) = -2\gamma_{lv} \qquad (10)$$

ΔF^{adh} can be determined by several approaches, apart from the above surface free energy approach. The classical work of Hamaker (4) is very appropriate.

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

$$PV = RT \qquad (11)$$

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 figure 3 below) even when the molecules have no permanent dipoles. It then follows that molecules can attract each other

by some kind of cohesive force (5). These forces have come to be known as van der Waals forces. Van der Waals introduced the following corrections to (11);
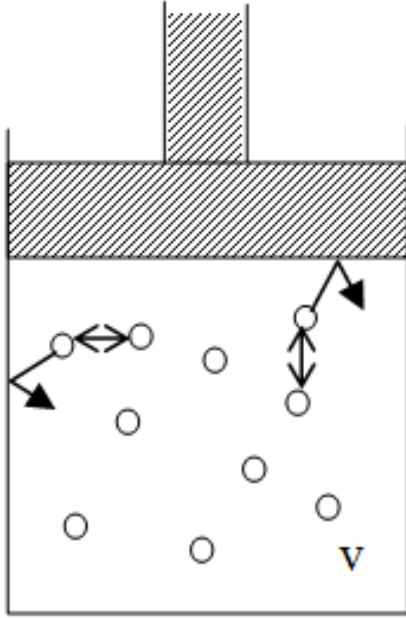
$$\left[P+\frac{a}{V^2}\right](V-b)=RT \qquad (12)$$

Figure 3. *Attractions of Surface Molecules by Bulk Molecules in a Container of Volume V (Source: 6).*

The correction term to the pressure, $\left(\dfrac{a}{V^2}\right)$ 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.

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 (7). London stated that the mutual attraction energy, V_A of two molecules in a vacuum can be given by the equation;

$$V_A=-\frac{3}{4}h\upsilon_0\left[\frac{\alpha^2}{H^6}\right]=-\left[\frac{\beta_{11}}{H^6}\right] \qquad (13)$$

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 figure below.

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 shown in figure 5 below.
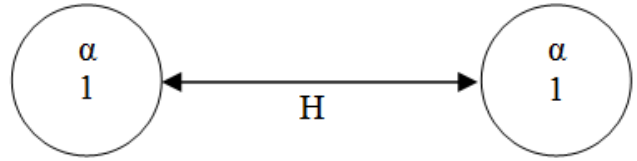

Figure 4. *Interactions of Two Identical Molecules of Materials, 1 and Polarizability, α, at a Separation, H (Source: 6).*
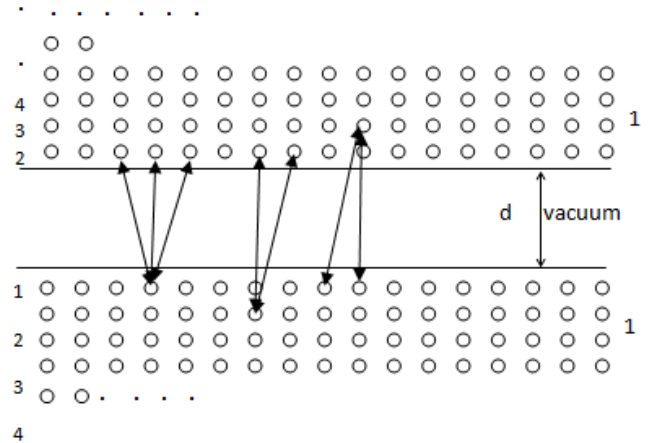

Figure 5. *Interactions of Two Semi-infinite Solid Bodies, 1 at a Separation, d in Vacuum (Source: 6).*

2.4.3. Mathematical Model for the Interactions Mechanism

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

$$V_A=-\frac{3}{4}h v_0\left[\frac{\alpha^2}{H^6}\right]=-\left[\frac{\beta_{11}}{H^6}\right] \qquad (14)$$

Where; h = Planck's constant
v_0 = characteristic frequency of the molecule
α = polarizability of the molecule
H = their separation distance
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;

$$P_{vdw}=\left[\frac{A_{11}}{6\pi d^3}\right] \qquad (15)$$

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;

$$F_{vdw}=\left[\frac{A_{11}R}{6d^2}\right] \qquad (16)$$

Where A_{11} = Hamaker constant

$$A_{11}=\pi^2 q_1^2\beta_{11} \qquad (17)$$

Where q_1 = number of atoms per cm^3

β_{11} = London-van der Waals constant

2.5. Data Analysis

2.5.1. Relevant Mathematical Applications

Applying the following relations to the obtained raw data the Hamaker coefficient could be derived;

$$\bar{a} + T + R = 1 \tag{25}$$

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

$$\text{Also; } T = 10^{-\bar{a}} \tag{26}$$

With the values of $\bar{a}$ and T ascertained, R could easily be derived by substituting into (25).

The next step is to find a value for the refractive index, *n* employing the mathematical relation

$$n = \left[\frac{1 - R^{\frac{1}{2}}}{1 + R^{\frac{1}{2}}} \right] \tag{27}$$

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

$$k = \left[\frac{\alpha \lambda \times 10^{-9}}{4\pi} \right] \tag{28}$$

Where; α is the absorption coefficient defined as follows;

$$\alpha = \left[\frac{\bar{a}}{\lambda \times 10^{-9}} \right] \tag{29}$$

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

$$\text{For the real part; } \varepsilon_1 = n^2 - k^2 \tag{30}$$

$$\text{For the imaginary part; } \varepsilon_2 = 2nk \tag{31}$$

Also A_{11} is given as

$$A_{11} = 2.5 \left[\frac{\varepsilon_{10} - 1}{\varepsilon_{10} + 1} \right]^2 = 2.5 \left[\frac{n_1^2 - 1}{n_1^2 + 1} \right]^2 \tag{32}$$

This gives a value to the Hamaker constant A_{11}, and by extension to other Hamaker constant A_{33}.

For a combination of two identical materials when the gap between 1 and 1 is filled with a medium 3, from Hamaker's calculations;

$$A_{131} = A_{11} + A_{33} - 2A_{13} \tag{34}$$

Rewriting these equations will give;

$$A_{131} = \left(\sqrt{A_{11}} - \sqrt{A_{33}} \right)^2 \tag{36}$$

2.6. Computation of the Hamaker Coefficients

This involves the computation of the Hamaker coefficients of the antiretroviral drugs interacting with HIV negative blood.

2.6.1. Computation of the Hamaker Coefficients of Drugs on Uninfected Blood

To obtain a value for the combined Hamaker coefficient A_{131} for the drugs interacting with the HIV negative blood the relations of (34) and (36) are employed, with

A_{33} = Hamaker constant, A_{11} values for drug – coated HIV negative plasma (serum) as the intervening medium.

$$A_{131abs} = \frac{\sum_{0}^{N} (A_{131})}{N} \tag{44}$$

Table 2. *Comparison between the absolute values of A_{131} for Uninfected blood for the five different antiretroviral drugs.*

Variable ($\times 10^{-21}$ Joule)	D1	D2	D3	D4	D5
A_{131}	0.367603	0.463371	0.530208	0.509707	0.495986

Table 2 above reveals that the interaction energy of the lymphocytes, A_{131}. The absolute combined Hamaker coefficients A_{131abs} of each of the drugs in HIV negative blood gave a positive value. This indicates that the van der Waals forces are attractive; hence there is attraction between the administered antiretroviral drugs and T cells. This shows that the antiretroviral drugs can be used for the surface coating or binding of the lymphocytes for systematic blocking of HIV's attacks on the human immune system.

Table 3 above is the summary of the results of HIV - drug coated blood interactions. The various antiretroviral drugs are shown alongside with their respective abbreviations and sizes. The absolute combined Hamaker coefficients A_{131abs} of each of the drugs in HIV negative blood gave a positive value. This indicates that the van der Waals forces are attractive; hence there is attraction between the administered antiretroviral drugs and T cells. This shows that the antiretroviral drugs can be used for the surface coating or binding of the lymphocytes for systematic blocking of HIV's attacks on the human immune system.

3. Results and Discussion

Figure 6 above gives the results for the five antiretroviral drugs in Whole blood. The absorbance of the interacting systems significantly increased as the wavelenghts increased until a peak wavelength 410Å. Further increase in the wavelength gave sharp decrease in the absorbance values which remained almost constant between wavelenghts 600 and 800Å. The peak values fall within the visible range of the ultraviolet radiation which is 300 – 600Å. The peak absorbance values range from 1.20 and 1.40.

Figure 7 above gives the results for the five antiretroviral drugs in Red blood cells. The absorbance of the interacting

systems significantly increased as the wavelenghts increased until a peak wavelength 410Å. Further increase in the wavelength gave sharp decrease in the absorbance values which was almost constant between wavelenghts 410 and 800Å. The peak values fall within the visible range of the ultraviolet radiation which is 300 – 600Å. The peak absorbance values range from 1.49 and 2.31.

***Table 3.** Summary of the results of HIV - Drug Coated Blood Interactions.*

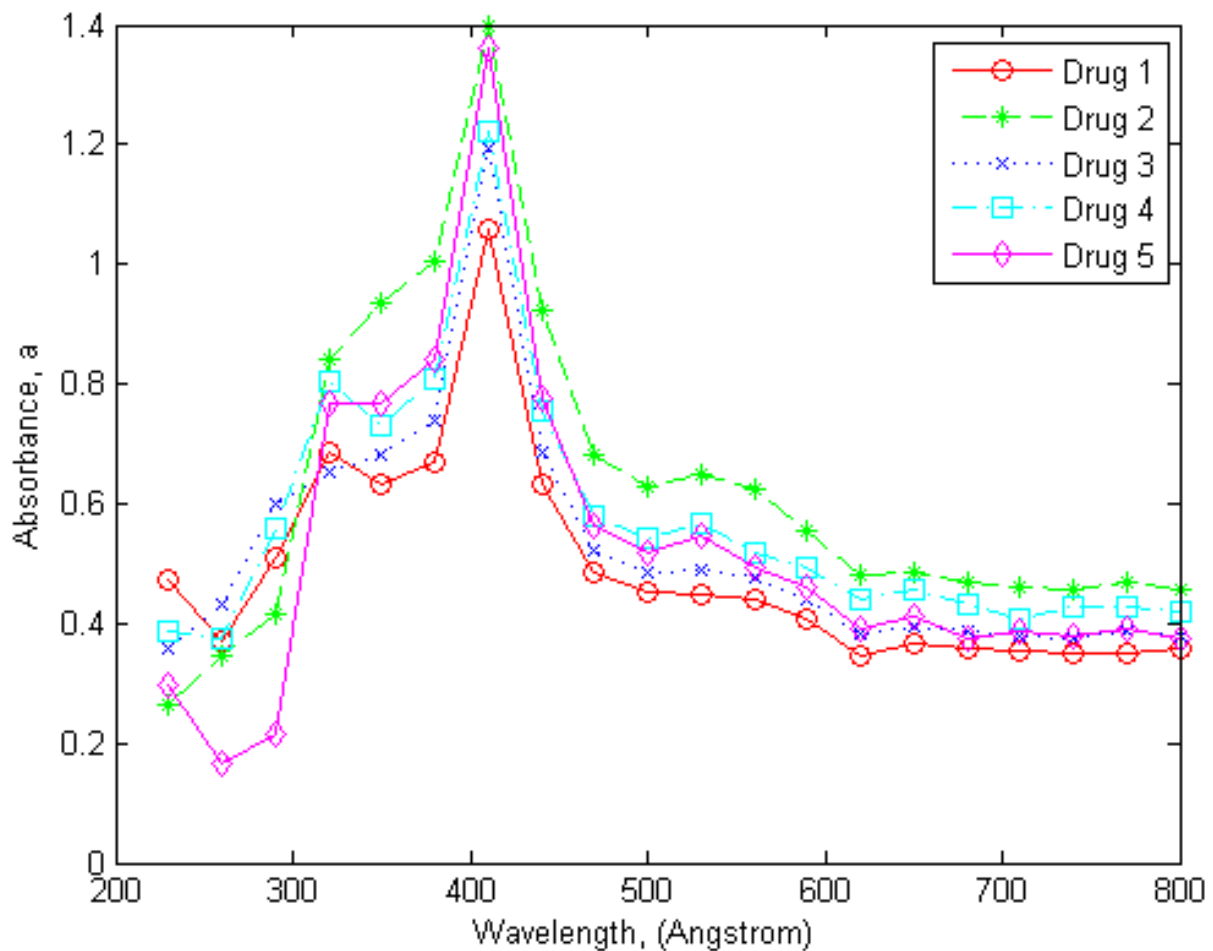| Drug Number	Type of Drug	ARV	Abbr.	Size	Absolute Combined Hamaker coefficient A_{131abs} (×10^{-21} Joule) of HIV Negative Blood	Sign of A_{131abs} obtained	Van der Waals Forces	Nature of Interaction
Drug 1	HAART and FDC	Lamivudine, Nevirapine & Zidovudine	3TC + NVP+ ZDV or AZT	150mg/ 200mg/ 300mg	0.367603	+ve	Attractive	Attraction (surface coating)
Drug 2	HARRT and FDC	Tenofovir, Lamivudine & Efavirenz	TDF + 3TC + EFV	300mg/ 300mg/ 600mg	0.463371	+ ve	Attractive	Attraction (surface coating)
Drug 3	Single Drug	Nevirapine	NVP	200mg	0.530208	+ ve	Attractive	Attraction (surface coating)
Drug 4	Single Drug	Efavirenz	EFV	600mg	0.509707	+ ve	Attractive	Attraction (surface coating)
Drug 5	Single Drug	Lamivudine	3TC	150mg	0.495986	+ ve	Attractive	Attraction (surface coating)

***Figure 6.** The relationship between Absorbance, á and Wavelength, λ for five different antiretroviral drugs on ten samples of Whole blood.*
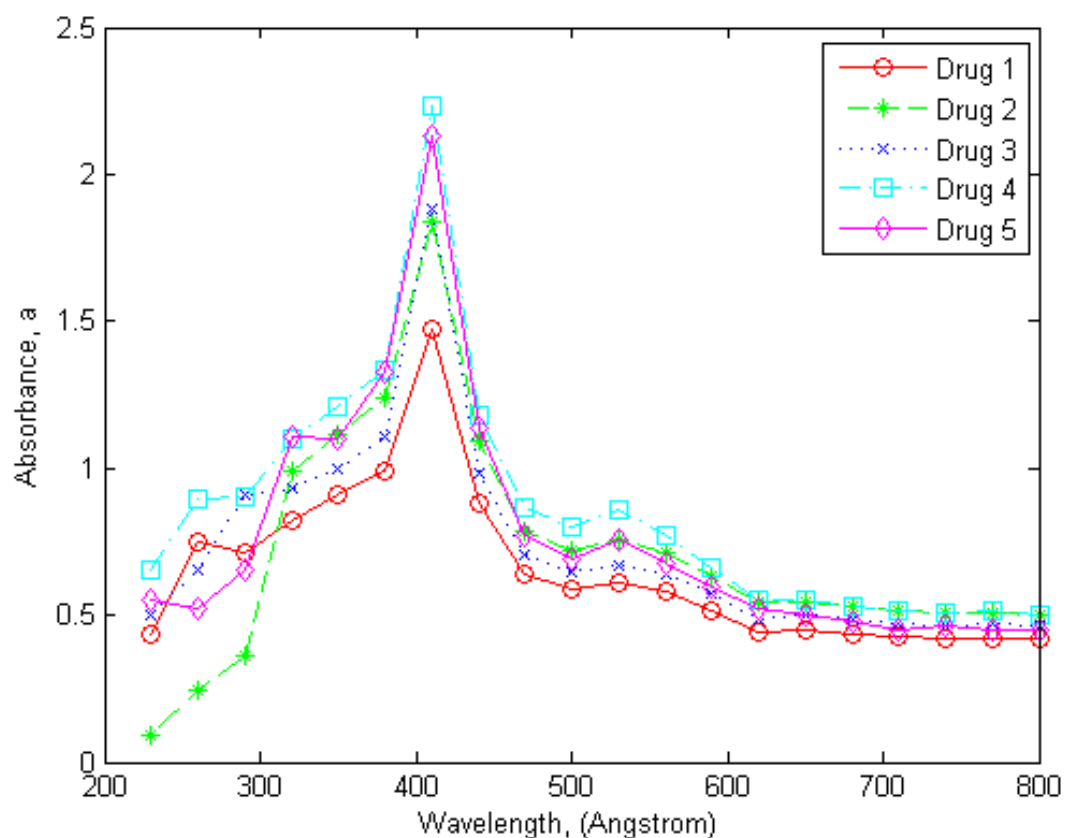
Figure 7. Variation between Absorbance, á and Wavelength, λ for five different antiretroviral drugs on ten samples of Red blood cells.
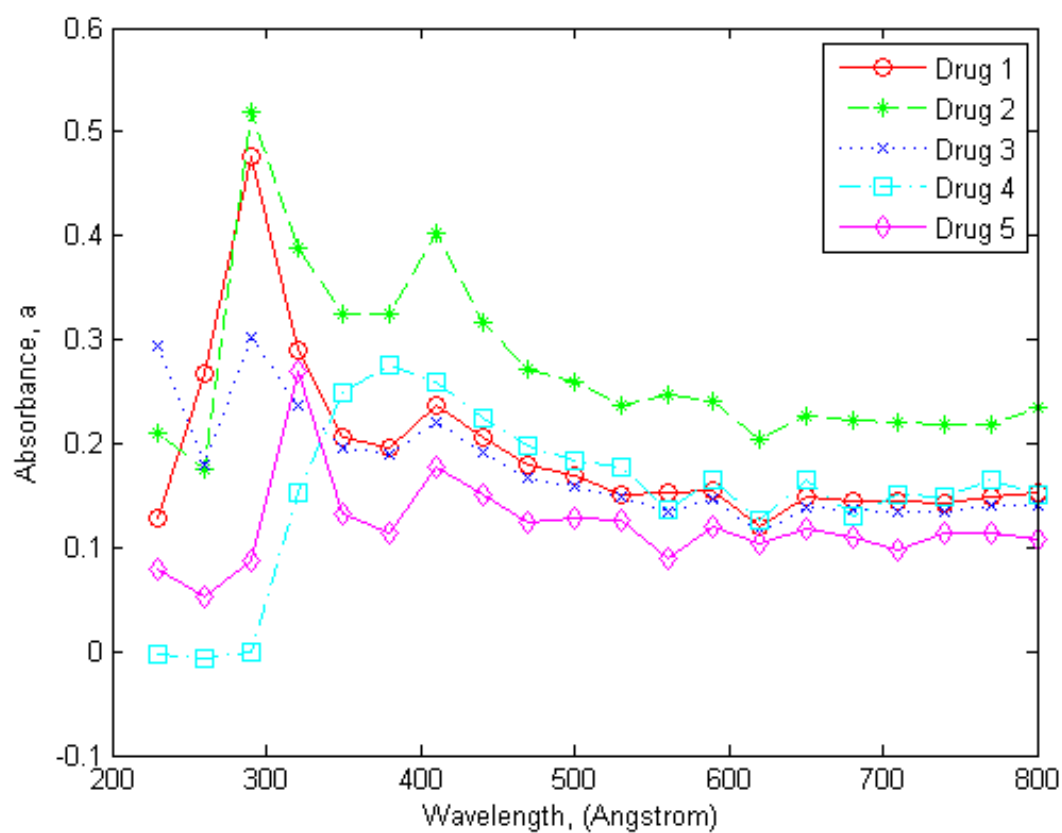

Figure 8. Variation of Absorbance, á with Wavelength, λ for five different antiretroviral drugs on ten samples of Lymphocytes.
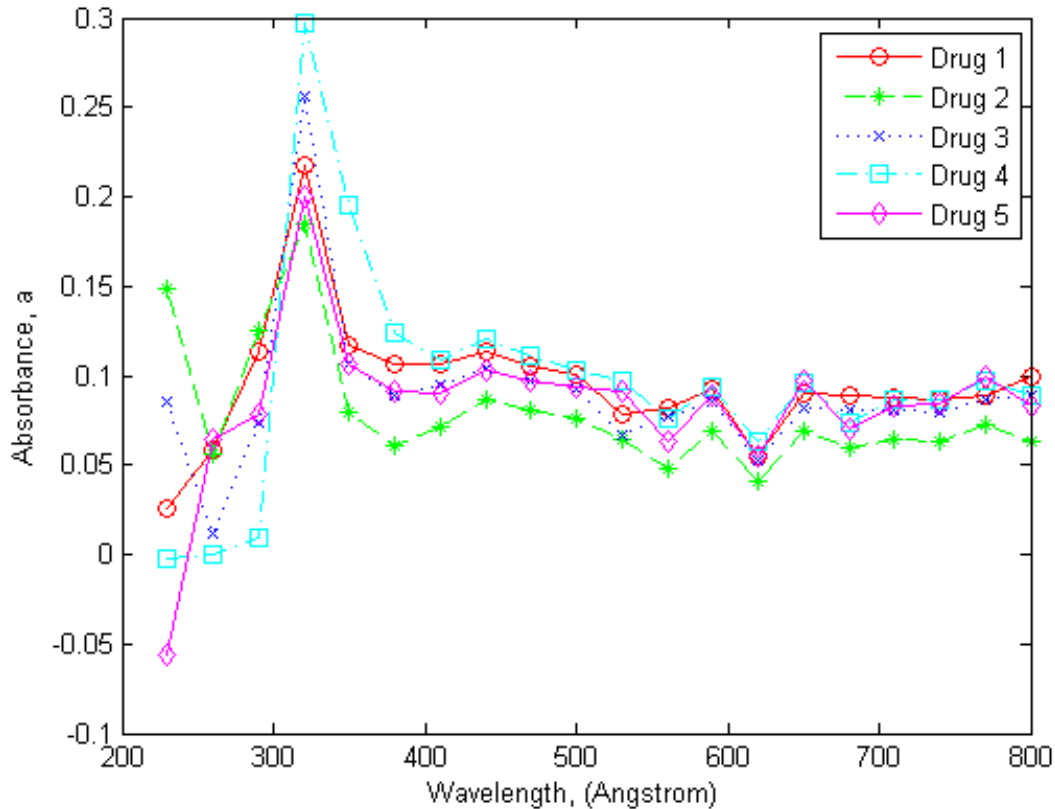
Figure 9. *Variation of Absorbance, á with Wavelength, λ for five different antiretroviral drugs on ten samples of Plasma.*

Figure 8 above gives the results for the five antiretroviral drugs in the Lymphocytes. The absorbance of the interacting systems significantly increased as the wavelenghts increased until a peak wavelength. Further increase in the wavelength gave sharp decrease in the absorbance values which remained almost constant between wavelenghts 600 and 800Å. The peak values fall within the visible range of the ultraviolet radiation which is 300 – 600Å.

Figure 9 above gives the results for the five antiretroviral drugs Plasma. The absorbance of the interacting systems significantly increased as the wavelenghts increased until a peak wavelength 320Å. Further increase in the wavelength gave sharp decrease in the absorbance values with some negative absorbance values occuring between wavelenghts 500 and 800Å. The absorbance values were near zero and remained constant between wavelenghts 500 and 800Å. The peak values fall within the visible range of the ultraviolet radiation which is 300 – 600 Å. The peak absorbance values range from 0.19 and 0.30.

4. Conclusions

The significance of engineering thermodynamics in proffering solutions to various biological processes is an interesting phenomenon. In the twenty first century research works, there is a growing need to achieve a more reliable research result through a synergy between engineers and biological researchers. The absolute values for the combined Hamaker coefficient, A_{131abs} obtained for each of the five antiretroviral drugs interacting with uninfected blood are given thus: D1 = 0.36760×10^{-21}Joule, D2 = 0.46337×10^{-21}Joule, D3 = 0.53021×10^{-21}Joule, D4 = 0.50971×10^{-21}Joule, and D5 = 0.49599×10^{-21}Joule. The positive values of the absolute combined Hamaker coefficient, A_{131abs} obtained for the five antiretroviral drugs interacting with HIV negative blood samples are in affirmation that there is a surface coating or binding of the drugs on the surface of the lymphocytes. The positive sense of the absolute combined Hamaker coefficient shows that there is an attraction between the interacting bodies or particles (drug - lymphocyte interactions). Hence, the van der Waals forces of the interacting process are attractive.

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