JEAS

JOURNAL OF ENGINEERING AND APPLIED SCIENCES

Journal of Engineering and Applied Sciences, Volume 17, Number 2, December 2020, 207-217

Surface Thermodynamics Study and Spectrophotometric Data Analysis of Human Immunodeficiency Virus (HIV)-Antiviral Herbal Drug Coated Blood Interactions

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Abstract
The surface thermodynamics study and spectrophotometric data analysis of human immunodeficiency virus (HIV)-antiviral herbal drug-coated blood interactions was carried out. Data of thermodynamic parameters were collected to measure their efficacy in line with the absorbance characteristics of HIV infected and uninfected blood. The experimental method involved the serial dilution of the five different antiviral drugs (two single Herbal, three-in-one combined herbal, one Highly Active Antiretroviral Therapy/Fixed Dose Combination and one single antiretroviral drugs) and their subsequent incubation with the blood samples collected from ten HIV infected persons who had already commenced treatment with one or more antiretroviral drugs, and ten HIV negative persons. Absorbance measurements using a digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer at 25 set wavelengths were made and the peak absorbance data for various interacting systems were measured. The average peak absorbance values of different antiviral drugs interacted with HIV+ blood samples ranged from 1.82nm to 2.81nm at a constant 400Å. These fell within the visible range of the UV radiation which is 300-600 Angstrom. Average peak absorbance values of different antiviral drugs interacted with different blood components of HIV- blood samples ranged from 0.2nm to 2.05nm at a constant average of 350Å. The combined Hamaker coefficient ($A_{132abs.}$) for HIV+ of -0.0031 with drug 1 shows effectiveness in repelling a HIV particle with a coating efficacy shown in corresponding positive sign of $A_{131abs.}$of 0.1349 for HIV- samples. It is hoped that the use of the findings of this work in herbal drug design would yield good results in the fight against HIV and other viral diseases.

Keywords: Surface Thermodynamics, Human Immunodeficiency Virus, Antiviral Drugs, Blood Components, Absorbance

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1. Introduction

HIV/AIDS scourge is currently being managed clinically with the prescription of anti-retroviral therapy (ART) by medical experts. Anti-retroviral drugs have been discovered to be attacked and resisted by the HIV in the human system because they are DiNucleic Acid (DNA)-based while the HIV is RiboNucleic Acid (RNA)-based. Hence, the ineffectiveness of ARTs are as a result of the HIV developing resistance to the administered anti-retroviral drugs even when a combination Anti-Retroviral therapy (cART) or a highly active anti-retroviral therapy (HAART) has been prescribed for a HIV patient. The discovery and administration of highly active anti-retroviral therapy (HAART) to suppress HIV is sustaining the clinical management of the virus endemic. HIV is a rapidly mutating RNA-based virus. It lacks the ability to stop the genetic mutations that occurs during replication. This is further made worse by the HIV having the potential to develop resistance to antiretroviral drugs (Achebe, C. H., 2010).

Mbabuike (2018) had reported that, infection with the HIV, a pathogenic retrovirus, can cause acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983). Although macrophage, neuron and other cells

can be infected by HIV (Maddon et al., 1986), CD4+ lymphocytes are the major target cells for HIV (Dalgleish et al., 1985), because HIV has strong affinity to the CD4 molecules on the surfaces of CD4+ cells. HIV infection in a human body destroys so many CD4+ lymphocytes that the body begins to lose its natural immune function, therefore an AIDS patient is highly vulnerable to various infections like tuberculosis, neuronal dysfunction, tumors, and so on. Treatment success has been limited by poor tolerance of the treatments by patients and the emergence of resistant strains of HIV. A need thus exists for an effective HIV treatment that will be well tolerated and relatively cheap.

Over the years, efforts have been dedicated to remedial and preventive methods but there is no vaccine for total cure for HIV/AIDS yet. An ideal vaccine should be innocuous and capable of inducing neutralizing antibodies as well as persistent immune responses in the mucous membrane and the blood (Levy et. al., 1988). Drugs are being developed against new targets in different stages of HIV replication cycle. These drugs include new HIV reverse transcriptase inhibitors and HIV protease inhibitors, as well as new anti-HIV agents aimed at other targets (De, 2000). The use of antiretroviral drugs is quite expensive. Several therapies are currently available for initial therapy for HIV-infected patients but ongoing research is focused on additions to existing and novel drug classes that might have improved pharmacokinetic and tolerability profiles, as well as on new therapeutic combinations that might result in synergetic activity.

The persistent case of HIV infection globally could be as a result of the ineffectiveness of some available antiretroviral therapy to block or resist perfectly this virus from invading healthy lymphocytes (white blood cells). It has becomes necessary to study the interaction between the HIV particle and the drug-coated lymphocyte as to understand the mechanism by which drugs can coat the lymphocyte thereby blocking the virus.

The challenge of producing synthetic and herbal drugs that can eliminate the HIV still remains. The research as to how effective the available antiretroviral drugs are, becomes pertinent and a discovery from such venture to can be found by studying surface effects in HIV-Drug interactions. Admittedly, there are several classes of drugs, which are recommended to be used in combination to treat HIV infection but, the use of herbal extracts which are yet to be formulated as less harmful compounds in the body, is gradually gaining global interests. Ethnomedicine refers to the study of traditional medicine practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare-seeking process and healing practices (Lowe, et.al 2000; LAD, 2006). Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mann, 1978; Njoku, 2009).

Since some of the drugs act as inhibitors, blocking the virus against penetration would be effective if a drug completely coats a lymphocyte cells. With absorbance as a surface phenomenon, Achebe and Omenyi(2013), have shown that its peak value, of the surface of the lymphocyte blood component, was reduced by the presence of the virus.The extent, to which a lymphocyte cell surface is effectively coated, is central to the blocking of the HIV from making contact with a lymphocyte cell.

In this paper, the concept of absorbance, as the basic thermodynamic property that determines surface coating is proved to be responsible for binding or coating. The trust of this publication is to find, to what extent the peak absorbance of the surface of a given HIV infected blood component, is changed by the administration of the antiviral herbal drugs when compared to antiretroviral synthetic drugs.

2. Methodology

2.1 Sample Collection

Table 1shows the details of the five different antiviral drugs used in the study. Drugs 1, 2 are antiviral crude herbal extracts (see Figures 1a-b) while drug 3 is a Combined antiviral crude herbal extracts (see Figures 1d). Drugs 4 and 5 are single antiretroviral drugs Highly Active Antiviral Therapy (HAART) as well as Fixed Dose Combination (FDC) respectively (Figure 1e). It is worthy to note that all the antiviral drugs used were not expired or contaminated during the period of the experiments. Blood samples were collected from Chukwuemeka Odimegwu

Ojukwu Teaching Hospital, Awka Anambra State from ten infected persons that had already commenced treatment with one or more antiretroviral drugs and from ten HIV negative persons.
Altogether, a total of twenty samples from different individuals were collected and screened to determine the infection status and stored in anticoagulant test tubes and ice packs to ensure the freshness and to avoid the samples becoming lysed. The samples were thereafter stored in a refrigerator for proper preservation.

2.2. Sample Preparation

The drugs passed through serial dilution at Mater Misericordae Hospital laboratory, Afikpo in order to get the right concentration of drugs used in inoculating the bloods. The three major herbal drug materials that were of research interest in this report are the plant materials of: Garcinia kola, Azaradichtaindica and Mangiferaindica

Table 1: Details of the Five Antiviral drugs used in the study (Mbabuike, 2018)

Drug No.	Tablets or Powder	Abbreviation	Size or Quantity	Type of Drug	Expiration Date	Batch Number	Pharma-ceutical Company
1	**G**arcinia**K**ola	GK	790mg	Herbal extract	Not determined	-	Locally produced
2	**A**zadirachta**I**ndica	AI	970MG	Herbal extract	Not determined	-	Locally produced
3	**G**arcinia, **A**zadirachta, **M**angifera	GAM	2800mg (G.800mg, A.1000mg, M.1000mg)	Herbal extract	Not determined	-	Locally produced
4	**Efa**virenz	Efv	600mg	Single dose	07/2018	E121047	HETERO LABS LIMITED
5	**E**favirenz, **L**amivudine, **T**enofovir	ELT	1200mg (E.600mg, L.300mg, T.300mg)	HAART and FDC	08/2018	E141689	HETERO LABS LIMITED

After the serial dilutions to 10^{-2}, the drug solution mixed with the blood was incubated for 24 hours at normal body temperature (37˚C) to facilitate Drug-Blood interactions. The collected samples with drugs were loaded into a centrifugal separator and the blood components were separated. This helped to see the separation of such components as White Blood Cells (WBC) also called the Lymphocytes, Red Blood Cells (RBC) also called the Erythrocyte, and the Plasma or Serum, for each sample. Glass slides were prepared and smeared with the samples, dried at room temperature and ready for absorbance measurements. The slide preparations and sample smearing were carried out diligently at the same laboratory. About 505 slides were successfully prepared.

2.3. Measurements

Absorbance measurements were done on all the different components of twenty samples (HIV infected blood of those that had started ARV treatment and uninfected blood samples). A digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer was used at the laboratory of the Department of Mechanical Engineering, Nnamdi Azikiwe University, Awka for the measurements. The absorbance values of the samples were measured over a range of wavelength between 230 and 950 Hertz alongside with their corresponding transmittance values.

3. Results and Discussion

3.1. The Absorbance values

The absorbance values for each drug were plotted as a function of the wavelength as given in Figure 1a-e.

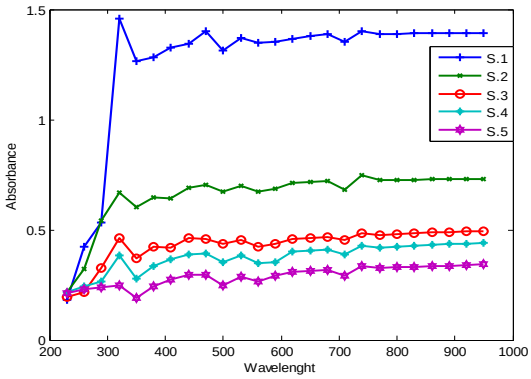

Fig.1a: (D1-D5) in 1ml conc. of sterile water

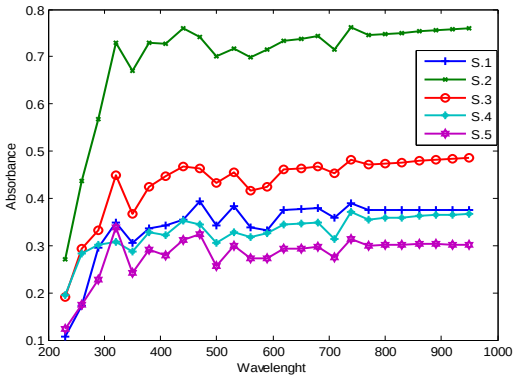

Fig.1b: (D1-D5) in 2ml conc. of sterile water

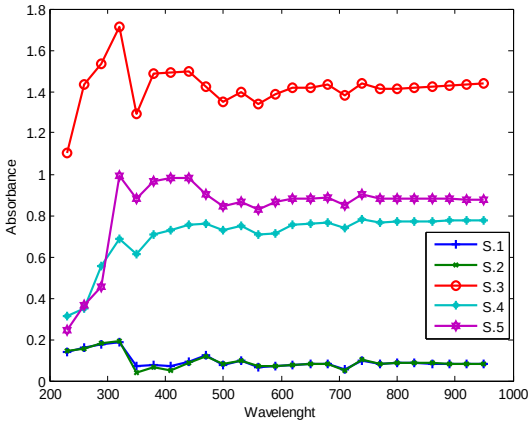

Fig.1c: (D1-D5) in 3ml conc. of sterile water

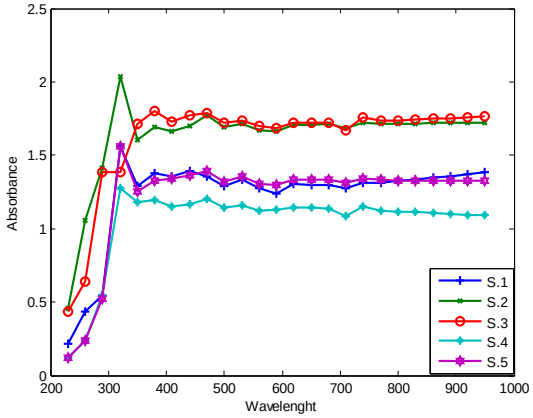

Fig.1d: (D1-D5) in 4ml conc. of sterile water

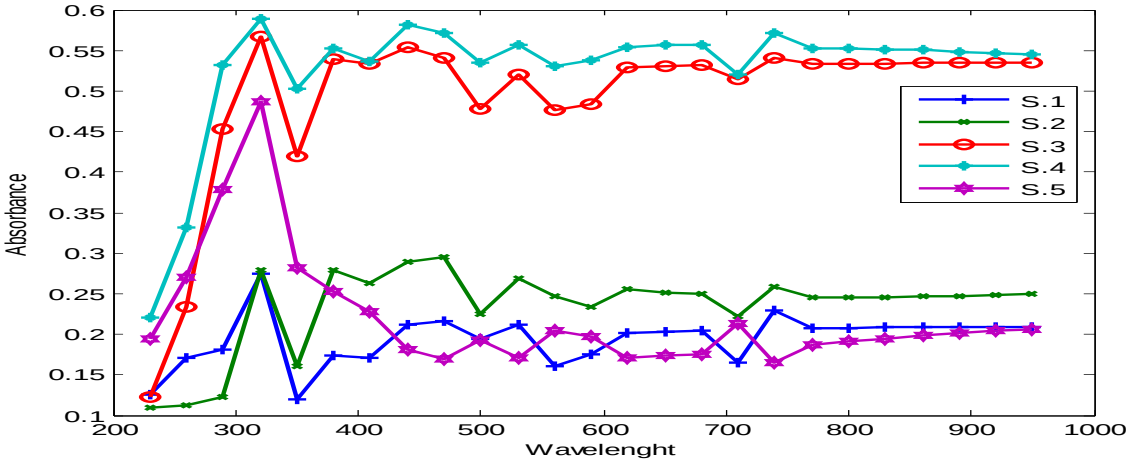

Fig.1e: (D1-D5) in 5ml conc. of sterile water

3.1.1. Antiretroviral Drugs in Air (Sterile H_2O)

Table 2 shows the average peak absorbance and the corresponding average peak wavelength for the five antiviral drugs in 1 – 5mls of sterile water. The table equally shows the average peak absorbance values of the different antiviral drugs in air ranging from 0.6402 to 0.9548 and their corresponding average peak wavelengths range from 320 to 344 Ångstrom. This falls within the visible range of the ultraviolet radiation which is 300 - 600 Ångstrom.

Table 2: Avg. Peak ā and λ values for all the antiviral drugs in sterile water (Mbabuike, 2018).

Drug Number	Avg. Peak ā value (nm)	Avg. Peak λ value (Å)
D1 (GK.)	0.7592	322
D2 (AI)	0.7762	320
D3 (GAM)	0.9548	326
D4 (Efv.)	0.6402	344
D5 (ELT)	0.7420	344

3.1.2. Infected Persons That Had Already Commenced Treatment with Antiretroviral Drugs

The absorbance values measured for each of the ten samples were averaged; it was the average values for each blood component incubated in each antiviral drug for those that had previously started drug treatment that were plotted as a function of the wavelength, as given in Figure 2.

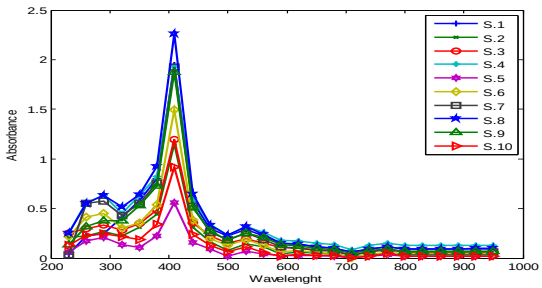

Fig.2a: Infected LYMPHOS (with drug 1).

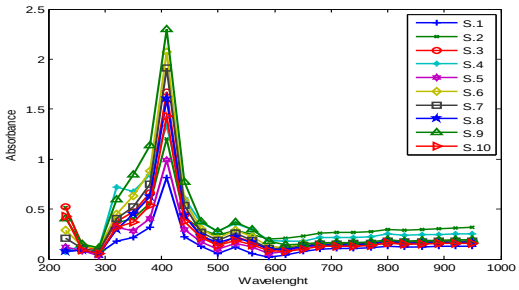

Fig.2b: Infected LYMPHOS (with drug 2).

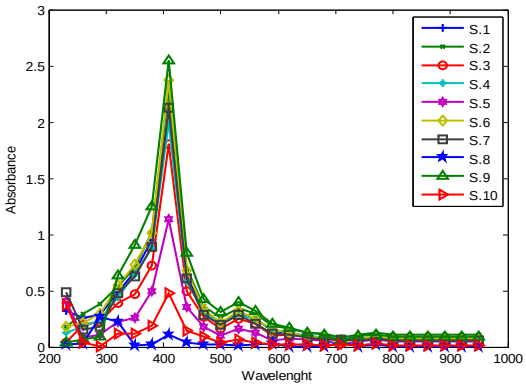

Fig.2c: Infected LYMPHOS (with drug 3).

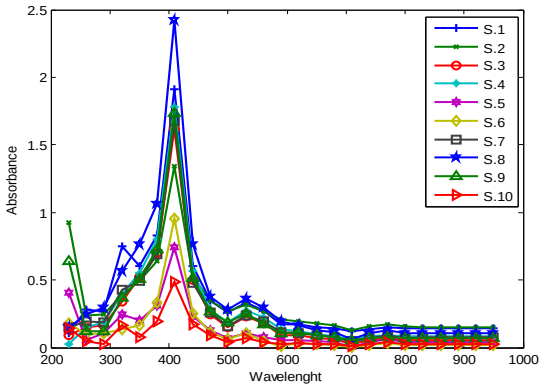

Fig.2d: Infected LYMPHOS (with drug 4).

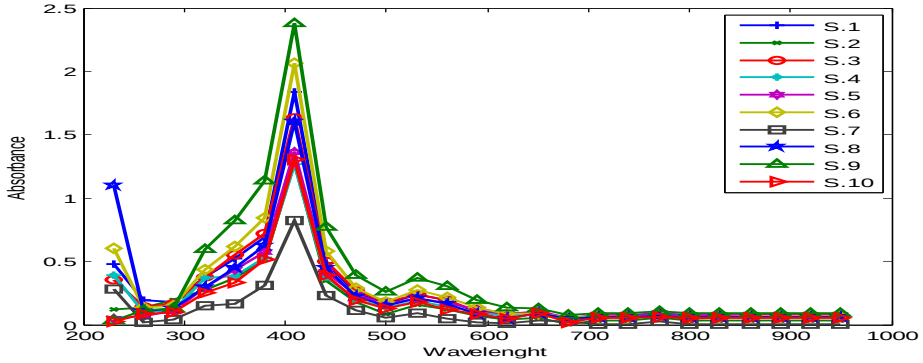

Fig.2e: Infected LYMPHOS. (with drug 5).

Figure 2 (a – e)shows the absorbance-wavelength plots for drugs 1 to 5 in the HIV positive lymphocyte blood component with prior treatment with antiretroviral drug. The absorbance of the interacting systems significantly increased as the wavelengths increased until a peak absorbance was reached at 400 Å for the Lymphocytes. Further increase in the wavelength gave rise to a decrease in the absorbance values which became almost constant between wavelengths 750 and 900 Å. The peak values fall within the visible range of the ultraviolet radiation. The peak absorbance values are presented in Table 3together with the corresponding wavelengths.

Table 3 shows average Peak (Max.) ā values of drugs$_{1\text{-}5}$ and corresponding average Peak (Max.) λ values of different antiviral drugs interacted with different blood components$_{1\text{-}4}$ of HIV+. The average peak absorbance values of different antiviral drugs interacted with different blood components of HIV+ ranged from 1.82nm to 2.81nm at a constant 400Å. These fell within the visible range of the UV radiation which is 300-600 Angstrom.

Table 3: Avg. Peak (Max.) ā values of drugs and corresponding avg. Peak (Max.) λ values of different antiviral drugs$_{1\text{-}5}$ interacted with different blood components$_{1\text{-}4}$ of HIV+ as depicted in the plots above.

Blood Component		Avg. Peak (Max.) ā values of drugs$_{1\text{-}5}$ and corresponding avg. Peak (Max.) λ values HIV Positive blood components$_{1\text{-}4}$ interacted with different Drug$_{1\text{-}5}$ samples				
		D1	**D2**	**D3**	**D4**	**D5**
Serum	ā	2.5000	2.0000	2.3100	2.0000	1.8200
	λ	400	400	400	400	400
White	ā	2.3000	2.4200	2.6100	2.400	2.400
	λ	400	400	400	400	400
Red	ā	2.8000	2.5200	2.3100	2.6200	2.8100
	λ	400	400	400	400	400
Whole	ā	1.8200	2.3100	2.3100	2.500	2.1300
	λ	400	400	400	400	400

3.1.3: HIV Negative Persons

The absorbance values measured for each of the ten samples were averaged; it was the average values for each blood component incubated in each antiviral drug for HIV negative patients that were plotted as a function of the wavelength as given by (Mbabuike, 2018). Figures 3 (a – e) shows absorbance Vs wavelength plots for Uninfected Lymphocytes with D$_{1\text{-}5}$.

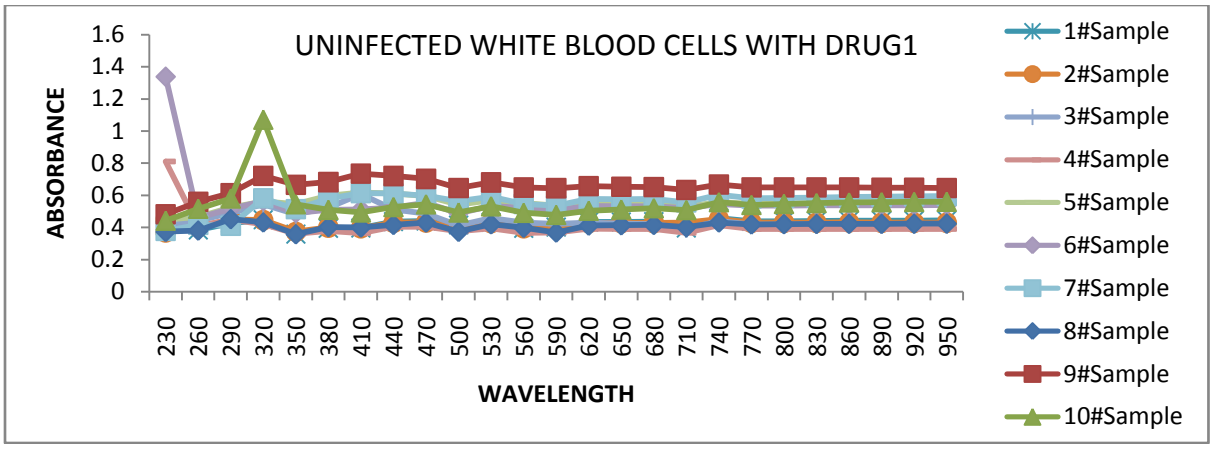

Fig.3(a)

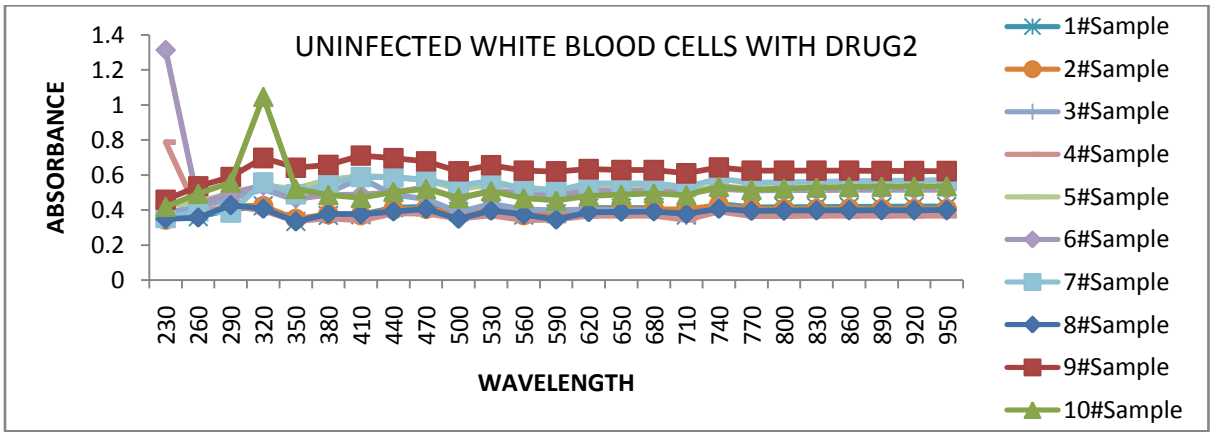

Fig.3(b)

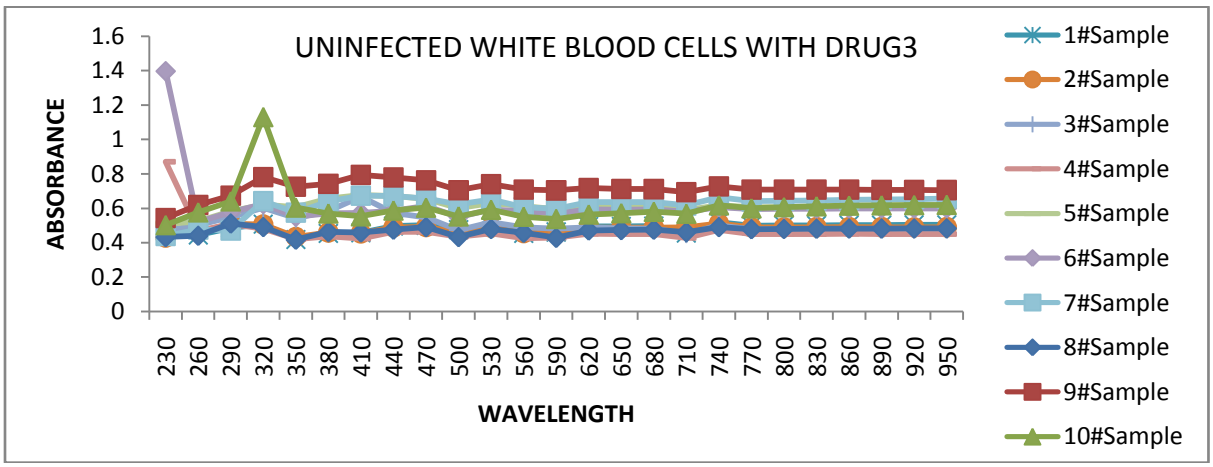

Fig.3(c)

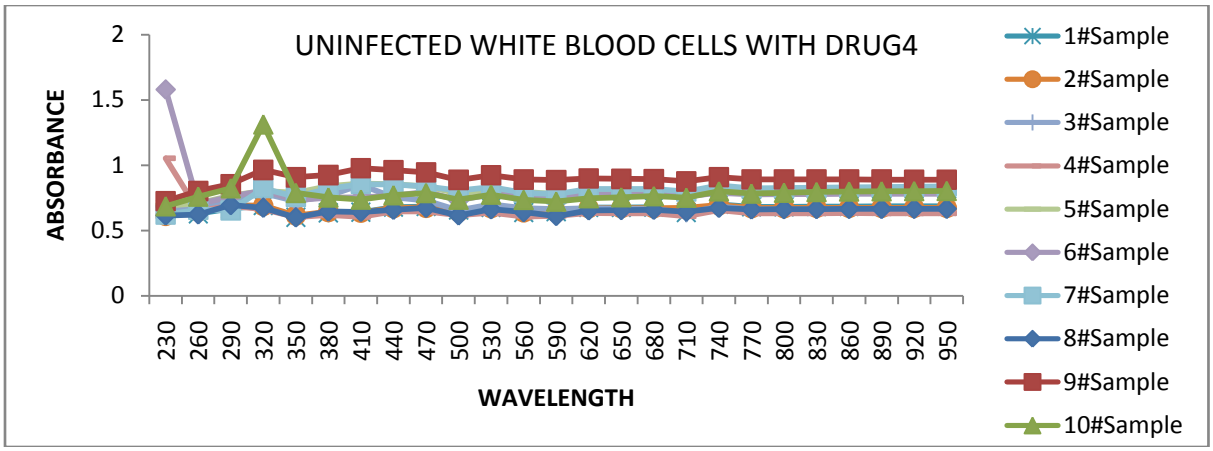

Fig.3(d)

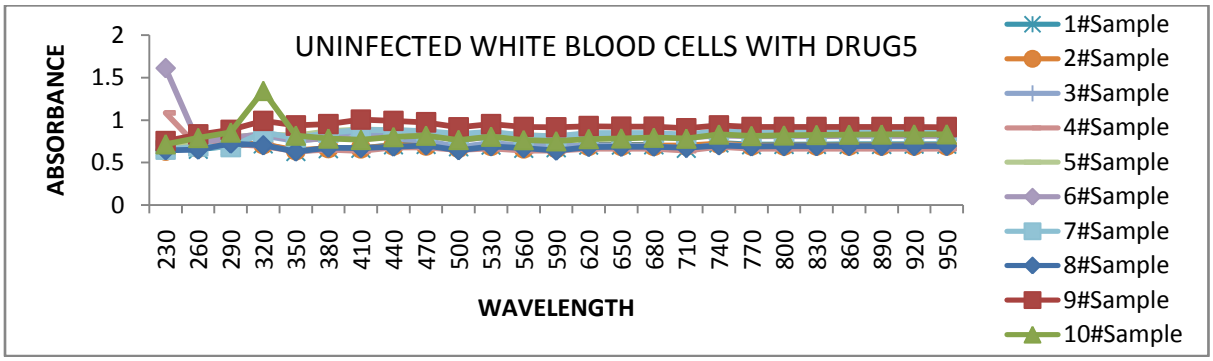

Fig.3(e)

Figures 3 (a – e), shows the results for drugs 1 to 5 in the HIV negative lymphocyte blood component. As observed, the absorbance values significantly increased as the wavelengths increased until a peak absorbance was attained. Further increase in the wavelength gave sharp decrease in the absorbance values which remained almost constant between wavelengths 600 and 800 Å. The peak absorbance values for the five antiretroviral drugs on lymphocytes range from 0.27 to 0.52, on the Plasma they range from 0.18 to 0.30, on Red blood cells they range from 1.05 to 1.40, on Whole blood they range from 1.05 to 1.40, falling within the visible range of the ultraviolet radiation which is 300 - 600 Å; these values are presented in Table 4.

Table 4 shows average Peak (Max.) ā values of drugs$_{1\text{-}5}$ and corresponding average Peak (Max.) λ values of different antiviral drugs interacted with different blood components$_{1\text{-}4}$ of HIV-. The average peak absorbance values of different antiviral drugs interacted with different blood components of HIV+ ranged from 0.2nm to 2.05nm at a constant average of 350Å. These fell within the visible range of the UV radiation which is 300-600 Angstrom.

Table 4: Avg. Peak (Max.) ā values of drugs and corresponding avg. Peak (Max.) λ values of different antiviral drugs$_{1-5}$ interacted with different blood components$_{1-4}$ of HIV- as depicted in the plots above (Mbabuike, 2018).

Blood Component		Avg. Peak (Max.) ā values of drugs$_{1-5}$ and corresponding avg. Peak (Max.) λ values HIV Negative blood components$_{1-4}$ interacted with different Drug$_{1-5}$ samples				
		D1	**D2**	**D3**	**D4**	**D5**
Serum	ā	1.5	0.45	0.51	0.7	0.72
	λ	300	300	300	300	300
White	ā	0.72	0.71	0.76	0.2	0.97
	λ	300	300	300	300	300
Red	ā	1.8	1.78	1.86	2.05	2.03
	λ	390	400	400	400	400
Whole	ā	1.65	1.65	1.68	1.95	1.95
	λ	400	400	400	400	400

From table 5, it could be calculated that the average absorbance value of an uninfected lymphocyte human blood component is 0.1359 (between 0.0493 and 0.2941). The average peak absorbance values of the lymphocyte blood components of patients that were HIV positive with antiviral drugs and the average peak absorbance values of the lymphocyte blood components of patients that were HIV negative with antiviral drugs are higher than the average peak absorbance of blood components of patients that were HIV negative without drugs when table 3 and 4are compared with Table 5. This indicates that the antiviral drugs have the effect of increasing the peak absorbance values of the blood components, i.e., the drugs increase the light absorption capacity of the blood cells. Previous researches (Achebe, 2010) have shown that the virus reduces the peak absorbance values of the blood components.

Table 5: Average absorbance values (nm) for Lymphocyte blood samples of ten patients without HIV (Mbabuike, 2018).

1	2	3	4	5	6	7	8	9	10
0.066	0.0923	0.0923	0.0493	0.1922	0.2002	0.1998	0.0512	0.2941	0.1906

This paper compared the peak absorbance values of HIV negative lymphocyte blood without drugs and HIV positive lymphocyte blood with drugs and HIV negative lymphocyte blood with drugs and reported that the absorbance values of infected lymphocyte blood components with drugs and those of uninfected lymphocyte blood components with drugs were higher than those of uninfected lymphocyte blood components without drugs. The absorbance values of blood samples with drugs are generally increased by a significant factor. The relative increase in the absorbance of the lymphocyte blood samples with drugs reveals the role of the drugs in significantly affecting the surface properties of the infected blood cells (Achebe et al., 2013). It was also reported by Ozoihu (2014) that HIV has the capacity to reduce the surface energy of blood. Ani (2015) verified the efficacy of antiretroviral pharmaceutical drugs against the HIV particle using the Hamaker concepts of the surface energetics. The negative values of the absolute combined Hamaker coefficient for infected blood-drug interactions he recorded, implies

repulsion or blocking of the invading virus by the drug-coated lymphocyte thus, confirming (Chukwuneke, 2015). The positive values of the absolute combined Hamaker coefficient for uninfected blood-drug interactions as he recorded implies attraction or coating of a lymphocyte particle by a drug particle. Therefore, the power of effecting functional cure is the potency of the antiretroviral drugs and this have been quantitatively and qualitatively verified thermodynamically by (Ani, 2015), (Ani, et al., 2015). However, the restorative action of antiviral drugs is a positive sign to the reduction of the virus effect. This work is devoted to showing that the surface property of a blood component can be significantly changed by the antiviral drugs and that this effect can possibly be used in drug design.

3.1.4: Test of Significance

Table 6: Analysis of the absolute combined Hamaker coefficients (interactive terms) for blood samples (Mbabuike, 2018).

Hamaker variable	Control (without Drug)	D1	D2	D3	D4	D5	$LSD_{0.05}$
A_{132} HIV+	0.0144^{2}_{1}	-0.0031^{1}_{1}	$0.0055^{1,2}_{1}$	$0.0074^{1,2}_{1}$	$0.0081^{1,2}_{1}$	$0.0134^{1,2}_{1}$	***0.0152***
A_{131} HIV-	0.0548^{1}_{1}	0.1349^{2}_{2}	0.1234^{2}_{2}	0.1333^{2}_{2}	0.0144^{2}_{2}	0.01442^{2}_{2}	***0.0195***
A_{232} HIV+	0.3645^{2}_{2}	0.0040^{1}_{1}	0.0058^{1}_{1}	0.0108^{1}_{1}	0.0037^{1}_{1}	0.0042^{1}_{1}	***0.0970***
$LSD_{0.05}$	***0.1377***	***0.0134***	***0.0134***	***0.0165***	***0.0144***	***0.0158***	

$A_{132} \Rightarrow$ Hamaker coefficient of uninfected lymphocyte (1), uninfected serum (3) and infected lymphocyte (2).
$A_{131} \Rightarrow$ Hamaker coefficient of uninfected lymphocyte (1), uninfected serum (3) and uninfected lymphocyte (1).
$A_{232} \Rightarrow$ Hamaker coefficient of infected lymphocyte (2), uninfected serum (3) and infected lymphocyte (2).
Superscripts indicate significant difference among parameter levels at 5% significance level
($P < 0.05$). Subscripts indicate significant difference among parameter levels at 5% significance level. ($P < 0.05$). LSD $\Rightarrow$ Least Significant Difference. Columns 2, 3, 4, 5, 6, 7 are the parameter levels = 6. The different superscripts indicate significant difference statistically at ($P < 0.05$). The same superscripts indicate no significant difference statistically at ($P < 0.05$)
The different subscripts indicate significant difference statistically at ($P < 0.05$). The same subscripts indicate no significant difference statistically at ($P < 0.05$)

These results show that the absorbance of HIV negative white blood cells is most significant. Thus, the light absorption capacities of the surfaces of the HIV negative white blood cells are higher than the light absorption capacities of HIV positive WBC having been subjected to antiviral drug treatment.

4.0 Conclusion

The peak absorbance data for various interacting systems were measured. These were used to show that the antiviral drug had the effect of increasing the peak absorbance values of both the uninfected and infected blood components, that is, the drugs were able to increase the light absorption capacity of the blood cells. In current research works, there is a need to achieve a more reliable research result through a synergy between engineers and biological researchers. However, the findings of this work may help in the pharmaceutical industries in the search for more effective antiviral herbal drugs for the treatment of HIV patients.

5.0 Recommendation

Efforts should be made towards using in-vivo experimentation for a better understanding of drugs' mechanism of action as antivirals. The concepts of negative combined Hamaker coefficient A_{132abs} and the increased surface free energy of virus infected systems to determine the efficacy or effectiveness of herbal antiviral drugs in comparison to existing manufactured synthetic antiretroviral drugs for the treatment of HIV, Ebola virus disease (EVD) and Lassa fever, should be pursued. This should involve a synergy or team of medical personnel like pharmacists,

pharmacologists, laboratory scientists, medical doctors, engineers and physicists that would answer various questions about suitability of specified material(s) and toxicity. However, there should be a further study to determine the efficacy or effectiveness of the herbal and synthetic drugs usable for the treatment of other blood-related diseases like malaria using the concept of Hamaker coefficient approach as a thermodynamic modelling tool to study the interaction processes.

Acknowledgement
The Tertiary Education Trust Fund (TetFund) of Nigeria is acknowledged for their Academic Staff Training and Development (AST&D) scholarship that supported the efforts of the research.

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