The Effects Of Antiretroviral Drugs On The Absorbance Characteristics Of Blood Components

O. I. Ani , S. N. Omenyi, C. H. Achebe

Abstract: The effects of antiretroviral drugs on the absorbance characteristics of blood components have been studied**.** The methodology involved the serial dilution of the five different antiretroviral drugs (two HAART/FDC and three single drugs) and the subsequent incubation with the blood samples collected from ten blood samples of HIV negative persons for the absorbance measurement using a digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer. Reflectance, Dielectric constant, etc were derived from the absorbance data. For these drugs to be effective as HIV blockers, they should be able to coat the surfaces of the lymphocytes. The question therefore arises as to what extent these drugs are able to coat the surfaces of the blood cells? This was established using the extent of absorbance change. Models for coating effectiveness were formulated. The coating effectiveness was therefore calculated from peak absorbance values. Red blood cells were shown not to give reliable results. The results obtained however establish the fact that some coating of the drug had really occurred on the surfaces of the lymphocytes. The drug films were determined for lymphocytes and used to explain some observed clinical findings. The use of the findings of this work in drug design may be expected to yield good results.

Index Terms: Absorbance, Antiretroviral drug, coating effectiveness, HAART, Human immunodeficiency virus, Lymphocyte, plasma.

1 INTRODUCTION

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 cells. The extent of the cell surface that is coated is important.We intend to use the concept of absorbance to address this issue. Achebe and Omenyi [1] have shown that absorbance is a surface phenomenon. They showed that the peak absorbance of the surface of each blood component was reduced by the presence of the virus. The question now arises as to what extent the peak absorbance of the surface of a given blood component is changed by the administration of the anti retroviral drugs?

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The estimate of the drug film thickness on the surface of the blood component will equally be presented. This may in principle give us an idea of the coverage of the cell surface by the film of the drug. The concentration of the drug required to give a thick film that will ensure complete blocking of the virus would be an important research area.

2 Methodology

2.1 Materials.

There are several classes of drugs, which are usually used in combination, to treat HIV infection. Use of these drugs in combination is termed anti-retroviral therapy (ART), combination anti-retroviral therapy (cART) or highly active anti-retroviral therapy (HAART). Anti-retroviral (ARV) drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits. Typical combinations include 2 NRTIs (Nucleoside Reverse Transcriptase Inhibitors) + 1 PI (Protease Inhibitor) or 2 NRTIs + 1 NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor) [2].

Fig. 1 *The Tablets of the five different antiretroviral drugs used.*

2.2 Sample Collection

This research work involved the collection of popular and commonly used unexpired Antiretroviral drugs (three single tablets and two HAART as in fig.1), from the University of Nigeria Teaching Hospital (UNTH) APIN CENTRE PEPFAR, Ituku – Ozalla, Enugu State, and the collection of blood samples from ten persons who have not been infected with HIV. The collected blood samples were screened to ensure that they were not infected with HIV. 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 were also used to ensure that the samples did not degenerate [3].

TABLE 1
THE DETAILS OF THE FIVE DIFFERENT ANTIRETROVIRAL DRUGS USED IN THE STUDY

Drug Number	Tablets	Abbreviation	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	E121035 A	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.

2.3 Sample Preparation

The drugs passed through serial dilution at Tahilah Diagnostic Laboratories, Awka, 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^{o}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 start 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 at Tahilah Diagnostic Laboratories, Awka. This helped to obtain such components as White Blood Cells (WBC) also called the Lymphocytes, Red Blood Cells (RBC), and the Plasma or Serum, each sample at a time. Glass slides were prepared and smeared with the samples for absorbance measurements. The slide preparations and sample smearing were done at the same laboratory.

2.4 Measurements

The CD4 cells count of the blood samples collected were obtained using a digital CD4 count machine which is known as Cytoflowmeter or Flow cytometry instrument. This in a sense is an indicator of the level and progression of the HIV infection process in the subjects; the values ranged from 400 to 950 cells/mm^3, which indicate that they are HIV-free. Absorbance measurements were done on all the different components of all the ten blood samples. A digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer was used at the laboratory of the Department of Mechanical Engineering, Nnamdi Azikiwe University, Awka 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 [3].

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 fig. 2.

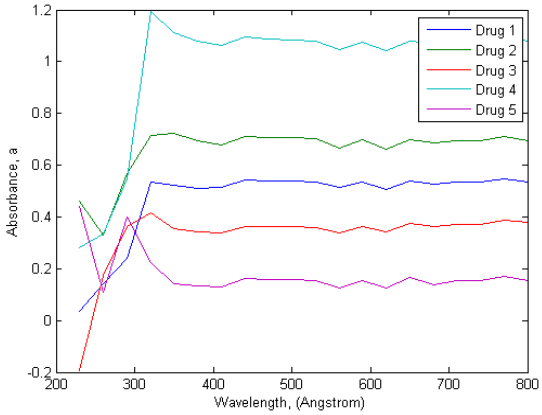

Fig. 2 *The relationship between Absorbance á and Wavelength, λ for the antiretroviral drugs alone*

The absorbance was found to exhibit a peak at 320 for the first four drugs and at 290 for the fifth drug. The absorbance ranged from 0.398 for drug five to 1.2 for drug 4 (see table 2)

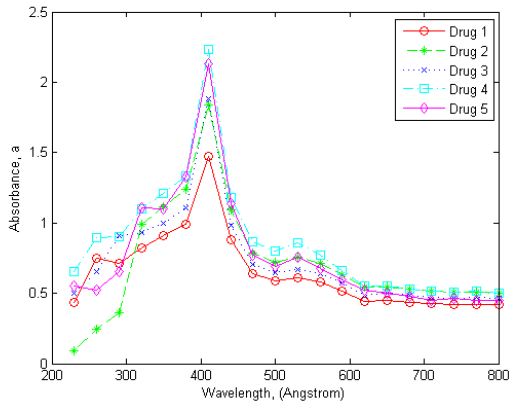

Fig. 3 *The relationship between Absorbance, á and Wavelength, λ for five different antiretroviral drugs on ten samples of Whole blood*

Fig 3 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 as given in table 2.

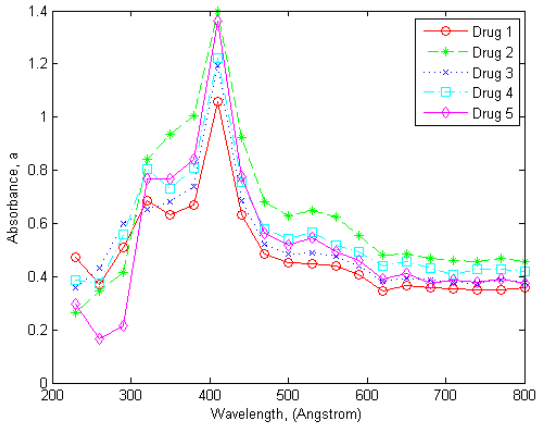

Fig. 4 *Variation between Absorbance, á and Wavelength, λ for five different antiretroviral drugs on ten samples of Red blood cells*

Fig 4 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 (see table 2).

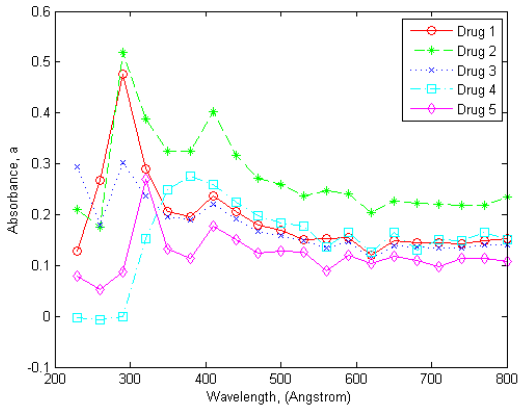

Fig. 5 *Variation of Absorbance, á with Wavelength, λ for five different antiretroviral drugs on ten samples of Lymphocytes*

Fig 5 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 (see table 2).

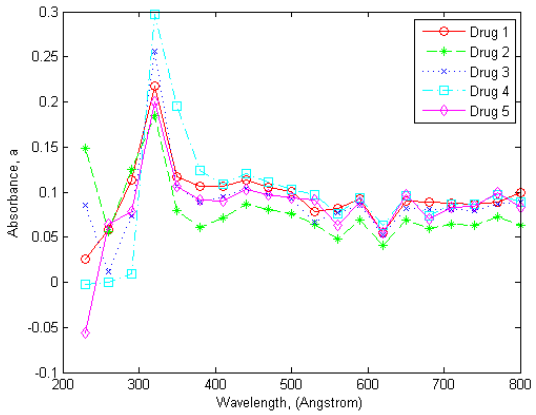

Fig. 6 *Variation of Absorbance, á with Wavelength, λ for five different antiretroviral drugs on ten samples of Plasma*

Fig 6 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 waveleghts 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 (see table 2)

TABLE 2
PRESENTATION OF PEAK ABSORBANCE (α) AND WAVELENGTH (Λ) AT WHICH IT OCCURS

S/N	Drug	No blood		Whole blood		Red blood cells		Lymphocytes		Plasma	
		á (peak)	λ, Å	*á* (peak)	λ, Å	(peak)	λ, Å	*á* (peak)	λ, Å	*á* (peak)	λ, Å
1	No drug[A]	-	-	-	-	1.7439	410	0.0882	290	0.0741	290
2	Drug 1	0.525	320	1.05	410	1.05	410	0.48	320	0.22	320
3	Drug 2	0.722	320	1.40	410	1.40	410	0.52	320	0.18	320
4	Drug 3	0.400	320	1.20	410	1.20	410	0.30	320	0.26	320
5	Drug 4	1.200	320	1.24	410	1.24	410	0.28	320	0.30	320
6	Drug 5	0.398	290	1.38	410	1.38	410	0.27	320	0.20	320

[A]Achebe [4]. Table 2 shows that the peak absorbance values of the blood components with antiretroviral drugs are higher than the peak absorbance values of the blood components without antiretroviral drugs. This indicates that the antiretroviral drug has the effect of increasing the peak absorbance values of the blood components, i.e., the drugs are made able to increase the light absorption capacity of the blood cells. Previous researches Achebe [4] have shown that the virus reduces the peak absorbance values of the blood components. This work compared the peak absorbance values of HIV positive blood and HIV negative blood and reported that the absorbance values of uninfected blood components were higher than those of infected blood components. The absorbance values of HIV positive samples are generally decreased by a significant factor. The apparent 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. However, the restorative action of antiretroviral drugs is a positive sign to the reduction of the virus effect. However, this work is devoted to showing that the surface property of a blood component can be significantly changed by the antiretroviral drugs.

3.2 Coating effectiveness from peak absorbance measures

The question arises as to how we can establish that some coating of the drug has really occurred on the surfaces of the blood cells. Table 2 actually points out this fact as the presence of the drug has caused the absorbance of each blood component to increase. For these drugs to be effective as HIV blockers, they should be able to coat the surfaces of the lymphocytes. To establish the extent of absorbance change and hence coating effectiveness, we propose an expression as given in eq. (1).

$$\eta_a = \frac{\tilde{\alpha}_{bd} - \tilde{\alpha}_b}{\tilde{\alpha}_d - \tilde{\alpha}_b} \qquad (1)$$

Where $\tilde{\alpha}_d$ is peak absorbance for drug film only, $\tilde{\alpha}_b$is peak absorbance for blood component only, and $\tilde{\alpha}_{bd}$ is peak absorbance for drug film coated given blood component Eq. (1) is actually saying that, from absorbance concept, the difference the drug film makes in the absorbance of a blood component when compared with the difference in the absence of the drug, can give us an idea of the effectiveness of the coating. The difference between the absorbance of the drug film alone and that of the blood component alone, is given by $\tilde{\alpha}_d - \tilde{\alpha}_b$. If the blood component is now coated completely by the drug film, one would expect that absorbance to be equal to that of the drug. When the coating is not complete, one would expect $\tilde{\alpha}_{bd} - \tilde{\alpha}_b$ to be less than $\tilde{\alpha}_d - \tilde{\alpha}_b$. If there is no coating of the blood component by the drug film at all, η_a will be zero. If the surface of the blood component is completely covered by the drug film, one would in principle η_a to be 100%. The computed values of η_a for the three blood components using the absorbance values of table 2 are reported on table 3.

TABLE 3
EFFECTIVENESS OF COATING, η_a

Drug	Red blood cells	White blood cells	Plasma
1	1.1142	0.8970	0.3236
2	-6.2146	0.6813	0.1635
3	-0.1162	0.6793	0.5704

4	-0.9857	0.1725	0.2006
5	-0.3240	0.5868	0.3887

Table 3 shows the effectiveness of coating of the blood components with the antiretroviral drugs. The effectiveness of coating of the antiretroviral drugs on Red blood cells gave varied results, one positive and the rest negative values. These suggest that the drugs do not have any effect on the red blood cells surfaces. Note that the drugs were specifically designed to affect the surfaces of white blood cells which are normally targeted by HIV. So, no relevant and reliable effect was actually expected [5]. The values of coating effectiveness for the white blood cells and plasma gave positive for five different antiretroviral drugs. Note also that the drugs are in solution in the plasma and so are bound to affect its property. Drug 1 gave the highest value of effectiveness of coating for the White blood cells, followed by drug 2, while drug 4 gave the lowest value. The values for drugs 1 and 2 are higher than that of drugs 3, 4 and 5. Drugs 1 and 2 are HAART while drugs 3, 4 and 5 are single drugs. Some research findings of the biological researchers show that HAART (a regimen that contains three antiretroviral drugs from two different classes of antiretroviral drugs) are clinically more effective. However, from the above concept, it appears that drugs 3 and 5, though single drug appear to be effective as antiretroviral drugs in HIV treatment.

3.3 Determination of film thickness

We believe that the drug forms some thin film around each blood component though for RBC, this film may not be of regular nature as found above. To determine the film thickness from absorbance data, certain optical data must be established [6]. The absorbance, transmittance and reflectance are related by the expression.

ά + T+ R= 1 (2)

where ά is absorbance, T is transmittance and R is reflectance. The transmittance and absorbance are related by

$$T = 10^{-ά} \quad (3)$$

Reflectance could be easily derived by substituting the values of absorbance and transmittance in equation (2).The values of refractive indices n was calculated by employing the mathematical relation

$$n = \left[\frac{1-R^{\frac{1}{2}}}{1+R^{\frac{1}{2}}}\right] \quad (4)$$

A value for extinction coefficient, k is obtained as

$$k = \left[\frac{\alpha\lambda\times10^{-9}}{4\pi}\right] \quad (5)$$

where, α is the absorption coefficient defined as follows

$$\alpha = \left[\frac{ά}{\lambda\times10^{-9}}\right] \quad (6)$$

This implies that $k = \frac{ά}{4\pi}$

Dorranian and Dorranian [7] reported structural and optical characterization of PMMA surface treated in low power Nitrogen and oxygen RF plasmas. From this work, the samples were treated in a plane parallel capacitive couple RF discharge at 13.56MHz frequency and 25W power for different times. The modified surfaces were characterized by Fourier transform infrared spectrometer (ATR- FTIR) and atomic force microscope (AFM) micrographs. The optical properties of the samples were characterized by the complex refractive index expressed as

$$n = n(w) + \imath k(w) \quad (7)$$

Where n is the real part and k is the imaginary part. n can be obtained from the following equation as in Dorranian and Dorranian [7].

$$n = \left(\frac{1+R}{1-R}\right) + \sqrt{\frac{4R}{(1+R)^2} - k^2} \quad (8)$$

Where $k = \frac{\alpha\lambda}{4\pi}$

Transmission and refraction spectra of the samples can be converted to the absorption coefficient using the following relation

$$\alpha = \frac{1}{d}\, In\left[\frac{(1-R)^4}{2T} + \sqrt{\frac{(1-R)^4}{4T^2} + R^2}\right] \quad (9)$$

But $\alpha = \frac{ά}{\lambda}$

Therefore

$$d = \frac{\lambda}{ά}\, In\left[\frac{(1-R)^4}{2T} + \sqrt{\frac{(1-R)^4}{4T^2} + R^2}\right] \quad (10)$$

Where d is the film thickness and ά is the Absorbance Odey [6] used eq. (10) successfully to determine the thicknesses of polyvinyl alcohol (PVA), Polyethylene glycol (PEG), Polyacrylamide (PAM) and polyvinyl acetate (PVAC) films deposited on glass slides. We have therefore assumed that the same equation will, in principle, be applicable to thin films of blood components on glass slides. With eq.(10) therefore and relevant optical data, the film thicknesses were calculated and listed in table 4.

3.4 Film thickness ratio

Having obtained the film thicknesses, we estimate the film thickness ratio. The idea is to determine how far the thickness of the drug film around a blood component approximates to that of the drug thickness in the absence of the blood component. Eq. (11) is proposed to convey this idea, and the film thickness ratio becomes:

$$\sigma_t = \frac{\tilde{t}_{db}}{\tilde{t}_d} \quad (11)$$

Where $\tilde{t}_d$ is film thickness for drug film alone, $\tilde{t}_{db}$ is film thickness for drug film coated given blood component As stated, eq.(11) gives an idea of how far the thickness of the film on the blood component approximates to the thickness of the film if the drug alone is considered. If the thickness of the film on the surface of the blood cell is equal to that of the drug film alone, then the thickness ratio σ_t is 1. This does not guarantee that the whole surface of the blood cell is covered since complete coverage does not necessarily depend only on film thickness. If the thickness of the film on blood cell surface is less than that of drug film alone, σ_t will be less than 1. In this consideration, only the lymphocytes will be considered. This is because, as stated above, the antiretroviral drugs are not designed to affect the surfaces of red blood cells, and so no valuable information can be obtained considering red blood cells in this section.

TABLE 4
FILM THICKNESS RATIO

Drug	Film thickness $\tilde{t}_d$ for Drug film alone (nm)	Lymphocytes	
		Film thickness, $\tilde{t}_{db}$ (nm)	σ_t
Drug 1	420.35	88.24	0.2099
Drug 2	550.86	301.53	0.5474
Drug 3	280.81	38.42	0.1368
Drug 4	867.55	306.83	0.3537
Drug 5	346.23	78.39	0.2264

Table 4 shows the film thicknesses and thickness ratios of the blood components. The film thicknesses of the Lymphocytes varied with different antiretroviral drugs. This indicates that the antiretroviral drug actually affects the surface properties of the Lymphocytes in different ways. The film thickness ratio of the Lymphocytes with drug 2 gave the highest value while that of drug 3 gave the lowest value. It is also worth noting that drugs 2 and 4 have Efavirenz. Both of them gave higher values of film thicknesses than the other antiretroviral drugs used in the study. This indicates that the Efavirenz which is usually administered once daily, may most likely deposit a thick layer of its film on the Lymphocytes. We can conclude with the findings that the regimen with Efavirenz combination would possibly be the most effective when compared with the other antiretroviral drugs if thick drug film contributes to effective blocking of HIV. This is in affirmation with the findings of the biological researchers. The recent clinical report shows that Efavirenz is preferred to other antiretroviral drugs used in HIV treatment by larger or general population of HIV patients. The preference of HAART or regimen with Efavirenz combination is because of its fast viral suppression, easy compliance because of the dosage (i.e taken once a day), it is best for HIV patients with co-infections like HIV-TB, HIV-Malaria, HIV-Hepatitis, and the pregnant HIV patients are now treated with it because it is not harmful to the foetus.

4 Conclusion

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 results in this research work validate that there is effective surface coating or binding of the antiretroviral drugs on the surface of the lymphocytes. This facilitates the blocking of the invading virus. We conclude that the antiretroviral drugs affect the surface properties of the white blood cells as they increased the peak absorbance values of the blood components to the extent that they could easily block or repel HIV. This work also shows that the drug films on surfaces of the blood cells can be determined and may provide valuable information on drug effectiveness.

References

[1] Achebe, C.H., Omenyi, S.N., (2013): The effects of human immunodeficiency virus (HIV) infections on the absorbance characteristics of different blood components. Int. J. Sci. Invent. 2(5), 53-61 www.ijesi.org

[2] Peter K. Quashie (2013): "HIV Drug Resistance and the Advent of Integrase Inhibitors". *Current Infectious Disease Reports* **15** (1): 85–100

[3] Ani, O.I., (2015): Surface Energetics Study of the Interactions between HIV and Blood Cells Treated with Antiretroviral Drugs, Ph.D. Dissertation, Department of Mechanical Engineering, Nnamdi Azikiwe University, Awka, Nigeria

[4] Achebe, C.H., (2010): Human Immunodefficiency Virus (HIV)-Blood Interactions: Surface Thermodynamics Approach, PhD. Dissertation, Nnamdi Azikiwe University, Awka, Nigeria

[5] Ozoihu, E.M., (2014): Human Immunodefficiency Virus (HIV)-Blood Interactions: Contact Angle Approach, PhD. Dissertation, Nnamdi Azikiwe University, Awka, Nigeria

[6] Odey, C. P. (2015): Surface free energies of polymer surfaces from spectrophotometric data, M. Eng. Thesis, Department of Mechanical Engineering, Nnamdi Azikiwe University, Awka, Nigeria.

[7] Dorranian, D.and M. Dorranian, optical characteristic of solid, 1st edn (springer, Berlin, (2002) Dzyaloshinskii, I.E., Lifshitz et al., (1961): The general theory of van der Waals forces. Adv. Phys. 10, 165

[8] Achebe, C.H., Omenyi, S.N., (2014): The Application of Negative Hamaker Concept to the human immunodeficiency virus (HIV)-blood interactions mechanism. Transactions on Engineering Technologies, pp 282-289

[9] Hamaker, H.C., (1937): Physica, Vol.4, p.1058

[10] Langbein, D., (1969): Journal of Adhesion, Vol.1, p.237

[11] London, F., (1930): Z. Physics, Vol.63, p.245

[12] Omenyi, S.N., (1978): Attraction and Repulsion of Particles by Solidifying Melts, Ph.D Thesis, University of Toronto, pp. 23, 33, 34

[13] Omenyi, S.N., (2005): The Concept of Negative Hamaker Coefficients: Nnamdi Azikiwe University, Awka, Inaugural Lecture Series No.8.1, p.23

[14] Van der Waals, J.D., (1873): Thesis, Leiden

[15] Visser, J., (1981): Advances in Interface Science, Elsevier Scientific Publishing Company, Amsterdam, Vol.15, pp.157-169

[16] Achebe, C.H., and Omenyi, S.N., 'Mathematical Determination of the Critical Absolute Hamaker Constant of the Serum (as an Intervening Medium) Which Favours Repulsion in the Human Immunodeficiency Virus (HIV)-Blood Interactions Mechanism', Lecture Notes in Engineering and Computer Science: Proceedings of The World Congress on Engineering 2013, WCE 2013, 3-5 July, 2013, London, U.K., pp1380-1384