COATING EFFECTIVENESS OF ANTIRETROVIRAL DRUGS IN HIV-BLOOD INTERACTIONS

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ABSTRACT

The coating effectiveness of some popular antiretroviral drugs used for HIV treatment was calculated in this study**.** The coating effectiveness models were derived from absorbance data and from the film thickness of the drugs on the blood cells. Five different antiretroviral drugs (two HAART/FDC and three single drugs) were used together with the blood samples collected from ten HIV positive persons who had not yet commenced treatment with the antiretroviral drugs, ten HIV positive persons who had already commenced treatment with the antiretroviral drugs and ten HIV negative persons. Absorbance measurements were made using a digital Ultraviolet Visible MetaSpecAE1405031Pro Spectrophotometer. The results obtained established the fact that some coating of the drug had really occurred on the surfaces of the lymphocytes. Coating effectiveness was found to be most significant on the surfaces of uninfected lymphocytes and less on surfaces of HIV infected blood cells, showing that HIV has the effect of reducing the surface coating effectiveness of blood cells. The coating effectiveness was found to be highest on drug 2 using both the absorbance and film thickness techniques. This shows, as clinically confirmed, that drug 2 (Tenofovir, Lamivudine & Efavirenz) is the most effective antiretroviral drug. The use of the findings of this work in drug design may be expected to yield good results.

Keywords:
Absorbance, Human immunodeficiency virus, antiretroviral drug, Coating effectiveness, Lymphocyte.

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 because they are DNA-based while the HIV is RNA-based. Hence, the 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 even when the combination therapy is for the HIV patient. There are several classes of drugs, which are usually used in combination, to treat HIV infection. 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 therefore important.

We intend to use the data on absorbance of the interacting systems to determine the extent of the blood cell surface that is coated by a drug film. Achebe and Omenyi [3] have shown

that absorbance is a surface phenomenon. They showed that the peak absorbance of the surface of each blood component varied as the blood cell surface was modified by the application of drug. They also showed that the peak absorbance 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 HIV infected blood component changed by the administration of the anti retroviral drugs?

2. MATHEMATICAL METHODS

Coating Effectiveness from Absorbance Measurements:

The coating effectiveness describes the extent a cell surface that is effectively coated by the film of a drug. When the drug is administered, it dissolves and mixes with the blood plasma. The action of the drug as a blocker is to coat the surface of the cell and therefore act as a blocker to the invading virus.

The following questions arise:

- What is the peak absorbance $(\tilde{a}_o)$ of the cell surface in the absence of the drug and the virus?
- What is the peak absorbance $(\tilde{a}_d)$ of the cell surface treated with antiretroviral drug?
- What is the peak absorbance $(\tilde{a}_h)$ of the cell surface treated with antiretroviral drugs and in the presence of HIV?

The effect of the antiretroviral drug on the surface of a blood cell can be estimated from

$$\eta_d = \frac{\tilde{a}_d - \tilde{a}_o}{\tilde{a}_d} \quad (1)$$

Where η_d is the antiretroviral drug effect.

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 that of the absorbance of the blood component alone is some measure of drug effect. The antiretroviral drug has the capacity to increase the absorbance of a given blood component surface.

The effect of HIV on the surface of a blood component in the presence of the antiretroviral drug, can be estimated from

$$\eta_h = \frac{\tilde{a}_d - \tilde{a}_h}{\tilde{a}_d} \quad (2)$$

Where HIV effect is given by η_h

HIV has the effect of reducing the absorbance of the surface of a given blood component. The amount by which the surface of a blood component surface is decreased by HIV will be given by eq. (2). If HIV fails to reduce the absorbance of a drug film coated blood cell surface, it means that the HIV has not attached itself to the surface, then $\tilde{a}_h = \tilde{a}_d$ and the effect of HIV will be zero.

The combined effect η_{dh} of HIV and administered antiretroviral drug on the surface of the blood cell is calculated from eqs. (1) and (2), and given by eq.(3). This gives an idea of the effect of both the HIV and the antiretroviral drug acting together on the blood and its components.

$$\eta_{dh} = \eta_d \eta_h \quad (3)$$

2.2 Coating Effectiveness from Film Thickness Measures

It is believed that the drug forms some thin film around each blood component. To determine the film thickness from absorption coefficient data, certain optical data must be established [6]. The absorbance, transmittance and reflectance are related by the expression.

$$\acute{a} + T + R = 1 \quad (4)$$

where $\acute{a}$ is absorbance, T is transmittance and R is reflectance.

The transmittance and absorbance are related by

$$T = 10^{-\acute{a}} \quad (5)$$

Reflectance could be easily derived by substituting the values of absorbance and transmittance in equation (4). The values of refractive indices n was calculated by employing the mathematical relation

$$n = \left[\frac{1-R^{1/2}}{1+R^{1/2}}\right] \quad (6)$$

A value for extinction coefficient, k is obtained as

$$k = \left[\frac{\alpha\lambda\times 10^{-9}}{4\pi}\right] \quad (7)$$

where, α is the absorption coefficient defined as follows

$$\alpha = \left[\frac{\acute{a}}{\lambda\times 10^{-9}}\right] \quad (8)$$

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

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

$$n = n(w)^{\cdot} + \imath k(w) \quad (9)$$

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

$$n = \left(\frac{1+R}{1-R}\right) + \sqrt{\frac{4R}{(1+R)^2} - k^2} \quad (10)$$

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

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

$$\alpha = \frac{1}{d} In\left[\frac{(1-R)^4}{2T} + \sqrt{\frac{(1-R)^4}{4T^2} + R^2}\right] \quad (11)$$

But $\alpha = \frac{\acute{a}}{\lambda}$

Therefore

$$d = \frac{\lambda}{\acute{a}} In\left[\frac{(1-R)^4}{2T} + \sqrt{\frac{(1-R)^4}{4T^2} + R^2}\right] \quad (12)$$

Where d is the film thickness and $\acute{a}$ is the Absorbance

Odey [7] used eq. (12) 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.(12) therefore and relevant optical data, the film thicknesses could be calculated.

Normally, it is the drug treated blood cell cast on a glass slide that is used for film thickness measurement. Realizing that the blood cell has a thickness, say t_b, then if the film thickness of the drug and cell is t_{db}, the effective thickness of the film becomes

$$t_f = t_{db} - t_b \quad (13)$$

Where t_f is effective drug film thickness.

If t_d is the film thickness of the drug alone on the glass slide, then film thickness effect can be defined as by the coating effectiveness, σ_t

$$\sigma_t = \frac{t_{db} - t_b}{t_d - t_b} \quad (14)$$

Eq.(14) 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 less than that of the cell is considered. If the effective thickness of the film on the surface of the blood cell $t_{db} - t_b$ is equal to that of the drug film alone less than that of the blood cell, then the coating effectiveness, 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 effective thickness of the film on blood cell surface is less than that of drug film alone which is less than that of the blood cell, then σ_t will be less than 1.

3. DETERMINATION OF ABSORBANCE

3.1. Materials:

Five antiretroviral drugs, commonly in use to treat HIV, as listed on table 1 were used. These drugs (three single tablets and two HAART)were collected from the University of Nigeria Teaching Hospital (UNTH) APIN CENTRE PEPFAR, Ituku – Ozalla, Enugu State. 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.

Blood samples were collected from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and Anambra State Teaching Hospital, Amaku. as follows:

A. from ten HIV positive persons without antiretroviral treatment,
B. from ten HIV positive persons with antiretroviral treatment and
C. from ten HIV negative persons.

Altogether, a total of thirty samples from different individuals were collected and screened to determine the infection status as shown in figure 1, and stored in anticoagulant test tubes and ice packs to ensure the freshness. The samples were thereafter stored in a refrigerator for proper preservation.

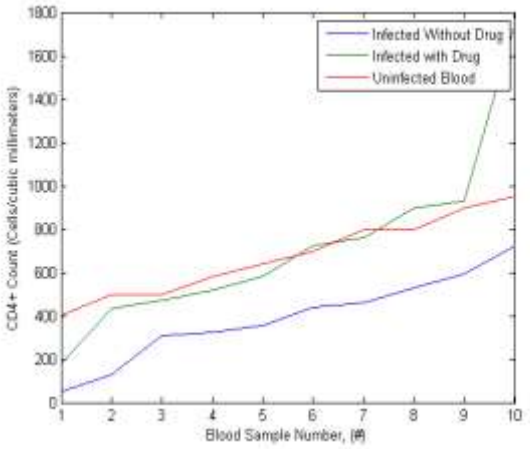

Figure 1. *CD4 counts versus Blood sample numbers for different antiretroviral drugs in both infected blood and uninfected blood.*

Figure 1 shows the degree of HIV infection in the infected blood samples. The CD4 counts of various blood samples collected were initially determined using the CD4 counter - Partec Flow Cytoflometry instrument in order to know the degree of infection of each blood sample with HIV. The CD4 counts of the first two samples of HIV positive patients who have not commenced treatment are below 200cells/mm^3 indicating high degree of HIV infection compared to the blood samples of the HIV positive patients who have commenced treatment, while the blood samples of the uninfected patients have higher CD4 counts above 200cells/mm^3. This indicates that the antiretroviral treatment increases the CD4 counts of the HIV infected systems lowered initially by viral presence in the blood.

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/200 mg/300mg	7220929	01/2016	Strides Arcolab Limited
2	Tenofovir, Lamivudine & Efavirenz	TDF + 3TC + EFV	300mg/300 mg/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.

3.2. 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. 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. About 600 slides were successfully prepared in the laboratory.

3.3. Measurements

Absorbance measurements were done on all the different components of thirty samples (HIV infected blood of ones that had not started ARV treatment, 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 800 Hertz alongside with their corresponding transmittance values.

4. RESULTS AND ANALYSIS

4.1. HIV Positive Persons with Antiretroviral Treatment

The absorbance values measured for each of the ten samples were averaged; it was the average values for each blood component incubated in each antiretroviral drug for those that had previously started drug treatment that were plotted as a function of the wavelength. A sample plot is given in figure 3 for drug 1. Plots for other drugs follow the same pattern.

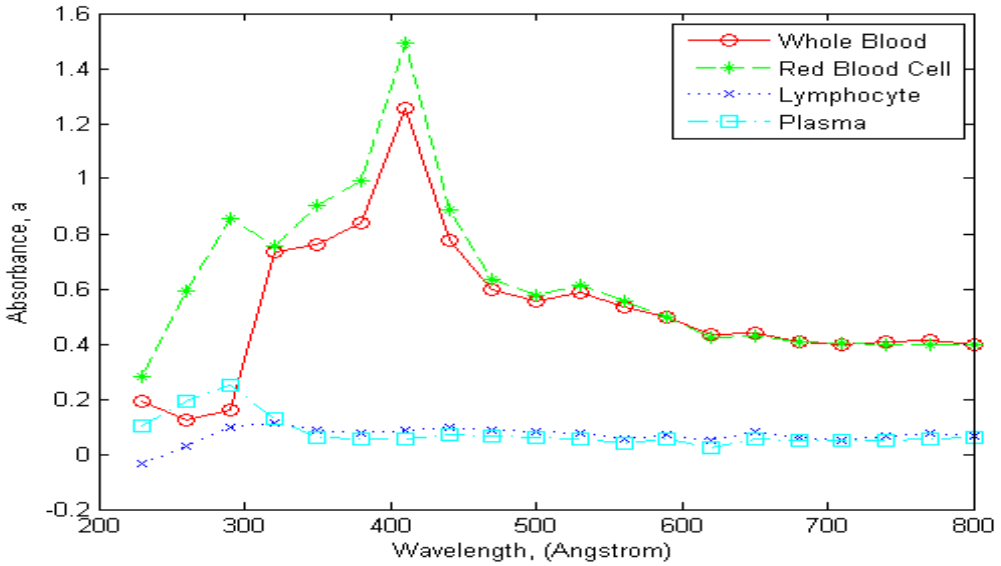

Figure 3. *Absorbance, á versus Wavelength, λ for HIV Positive Blood of patients that had commenced antiretroviral drug treatment before this study – for drug 1.*

Figure 3 gives the absorbance results for drug 1 (all the graphs are not shown) in the HIV positive blood components with prior treatment with antiretroviral drug. The absorbance of the interacting systems significantly increased as the wavelenghts increased until a peak absorbance was reached at 320 λ Åfor the Lymphocytes and Plasma, and 410Å for the Red blood cells and the Whole blood. Further increase in the wavelength gave rise to a decrease in the absorbance values which became almost constant between wavelenghts 600 and 800 Å. The peak values fall within the visible range of the ultraviolet radiation. The peak absorbance values are presented in table 2 together with the corresponding wavelengths.

Table 2. Data for absorbance for blood components of patients on drug treatment before this test

Drugs No.	**Whole blood**		**RBC**		**WBC**		**Plasma**	
	λ, Å	α (peak)	λ, Å	(peak)	λ, Å	(peak)	λ, Å	α(peak)
1	410	1.25	410	1.50	320	0.14	320	0.20
2	410	1.60	410	1.20	320	0.10	320	0.08
3	410	1.20	410	1.58	320	0.08	320	0.12
4	410	1.36	410	1.20	320	0.17	320	0.18
5	410	1.17	410	0.80	320	0.18	320	0.16

The peak absorbance values for the five antiretroviral drugs on Lymphocytes range from 0.08 to 0.18, on plasma they range from 0.08 to 0.20, on Red blood cells they range from 0.80 to 1.58, and on whole blood they range from 1.17 to 1.60.

4.2. HIV positive persons without antiretroviral treatment

The absorbance values measured for each of the ten samples were averaged; it was the average values for each blood component incubated in each antiretroviral drug for those that had not previously started drug treatment that were plotted as a function of the wavelength. A sample plot is given on fig 4 for drug 1. All other graphs follow the same pattern.

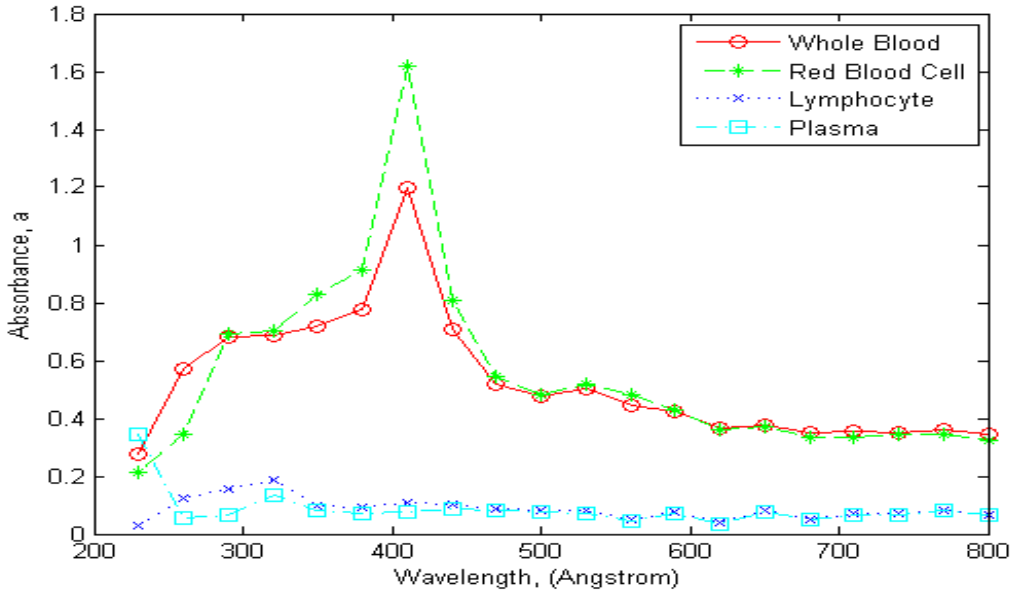

Figure 4. *Absorbance, á versus Wavelength, λ for for HIV positive blood components of patients that had not previously commenced antiretroviral drug treatment – for drug 1.*

Figure 4 gives the results for drug 1 (others follow the sam pattern) in the HIV positive blood components of patients that had not commenced antiretroviral treatment before this study. As observed before, the absorbance values significantly increased as the wavelenghts increased until a peak absorbance was attained. Further increase in the wavelength gave sharp decrease in the absorbance values which remained almost constant between wavelenghts 600 and 800Å. The peak absorbance values for the five antiretroviral drugs on Lymphocytes range from 0.18 to 0.30, on the Plasma they range from 0.08 to 0.28, on Red blood cells they range from 0.60 to 2.10, on Whole blood they range from 1.00 to 1.40, falling within the visible range of the ultraviolet radiation which is 300 – 600Å; these values are presented in table 3.

Table 3. Absorbance data for blood components of patients not on drug treatment before this test

Drugs No.	**Whole blood**		**RBC**		**WBC**		**Plasma**	
	λ, Å	**α (peak)**	**λ, Å**	**(peak)**	**λ, Å**	**α (peak)**	**λ, Å**	**α (peak)**
1	410	1.20	410	1.60	320	0.18	320	0.16
2	410	1.36	410	1.76	320	0.26	320	0.08
3	410	1.00	410	2.10	320	0.20	320	0.19
4	410	1.40	410	2.08	320	0.30	320	0.28
5	410	1.36	410	1.70	320	0.19	320	0.20

4.3 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 antiretroviral drug for HIV negative patients that were plotted as a function of the wavelength as given by [4]. The peak absorbance values from the work of Ani [4] are listed in table 4.

Table 4. Data for absorbance for blood components of patients that were HIV negative

Drugs No.	**hole blood**		**BC**		**BC**		**lasma**	
	λ, Å	α (peak)	λ, Å	α (peak)	α, Å	α (peak)	α, Å	α (peak)
1	410	0.05	410	0.50	320	0.48	320	0.22
2	410	0.40	410	0.88	320	0.52	320	0.18
3	410	0.20	410	0.90	320	0.30	320	0.26
4	410	0.24	410	0.28	320	0.28	320	0.30
5	410	0.38	410	0.18	320	0.27	320	0.20

Table 4 gives the data for absorbance for blood components of patients that were HIV negative. 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 when table 4 is compared with tables 2 and 3. 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, 2010) 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.

4.4. Results

Using the relevant data together with the proposed expressions, the effects of drug films on blood cells were calculated as presented in tables 6 to 8.

Table 5. Effects of drug film coating on blood component

Drugs No.	Uninfected Blood Components		
	WBC	RBC	lasma
1	0.8163	-0.1626	0.6632
2	0.8304	0.0724	0.5883
3	0.7060	0.0822	0.7150
4	0.6850	0.2351	0.7530
5	0.6733	0.2000	0.6295

Table 5 shows the effects of coating on uninfected blood components with the antiretroviral drugs. The effects on Red blood cells gave inconsistent results, one negative and the rest are positive values which are comparatively small. These suggest that the drugs do not have any effect on the red blood cell 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 coating effects for the white blood cells and plasma are 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 2 gave the highest effect for the White blood cells, followed by drug 1, while drug 5 gave the lowest value. Efavirenz is usually administered once daily; it may most likely deposit a thick layer of its film on the Lymphocytes. We can infer that the regimen of drug 2 that contains Efavirenz in combination would possibly be the most effective when compared with the other antiretroviral drugs if thick drug film contributes to effective blocking of HIV. 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. The effect values for drugs 1 and 2 are higher than those 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. This is in agreement with results of table 6 that show them to have the highest effect.

Table 6. Effect of HIV on drug coated blood component

Drugs No.	**HIV infected without previous drug treatment**			**HIV infected with previous drug treatment**			**Uninfected Blood Components**		
	WBC	**RBC**	**Plasma**	**WBC**	**RBC**	**Plasma**	**WBC**	**RBC**	**Plasma**
1	0.6250	-0.0667	0.2727	0.7083	0.0000	0.0909	0.8163	-0.1626	0.6632
2	0.5000	0.0638	0.5556	0.8077	0.3617	0.5556	0.8304	0.0724	0.5883
3	0.3333	0.1053	0.3333	0.7333	0.1684	0.5385	0.7060	0.0822	0.7150
4	0.0357	0.0877	0.0357	0.3929	0.4737	0.4000	0.6850	0.2351	0.7530
5	0.2963	0.2202	0.2963	0.3333	0.6330	0.4074	0.6733	0.2000	0.6295

Table 6 shows the effects of coating of the infected blood components with the antiretroviral drugs. The effectiveness of coating of the antiretroviral drugs on Red blood cells gave varied results, two negative and the rest are very low positive values. These suggest, as stated before, that the drugs do not have any effect on the red blood cells surfaces. Note that the drugs were specifically designed to block HIV from attacking the white blood cells. So, no relevant and reliable effect was actually expected [5]. The values of coating effectiveness for the white blood cells and plasma are 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, as stated before.

The values for drugs 1 and 2 are higher than that of drugs 3, 4 and 5 as also noted in table 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. Note also that the effect is more pronounced for the cases of patients that were in antiretroviral drug treatment before the samples were taken (81%) when compared with those that had not commenced any treatment (50%), for drug 2 cases. In this case, increase in absorbance was about 38%, and varies with each drug. (We wish to inform that the type of drug administered to patients before this study, was not known).

Table 7. Combined effect of coating

Drug No.	HIV infected without previous drug treatment			HIV infected with previous drug treatment			Uninfected Blood Components		
	WBC	RBC	Plasma	WBC	RBC	Plasma	WBC	RBC	Plasma
1	0.5102	0.0108	0.1809	0.5782	0.0000	0.0535	0.8163	0.1626	0.6632
2	0.4152	0.0005	0.3269	0.6707	0.0262	0.3269	0.8304	0.0724	0.5883
3	0.2353	0.0009	0.1925	0.5177	0.0138	0.3850	0.7060	0.0822	0.7150
4	0.0245	0.0206	0.0502	0.2691	0.1114	0.3012	0.6850	0.2351	0.7530
5	0.1995	0.0440	0.1632	0.2244	0.1266	0.2565	0.6733	0.2000	0.6295

Table 7 shows the combined effect of coating of the infected blood components with the antiretroviral drugs. The effectiveness of coating of the antiretroviral drugs on Red blood cells gave varied results, as previously stated. Here, they are so small that it is clear that the antiretroviral drugs do not have any discernible effect on the red blood cells. The values of coating effectiveness for the white blood cells and plasma are all positive for five different antiretroviral drugs with those for white blood cells being generally higher.

The values for drugs 1 and 2 are consistently higher than those of drugs 3, 4 and 5, confirming earlier conclusions. For drug 2, the combined effect was about 67% for blood of patients who were undergoing treatment and 42% for those that had not commenced treatment. he pharmaceutical companies that produced these drugs have tested them and were specific on what each drug can do. The report presented here is not aimed at informing these companies about their drugs, but our main aim is to show that from a different approach, it is possible to study the effectiveness of the drugs.

4.5. Test of Significance

It is important to study the data on combined coating effectiveness for the three scenarios for the lymphocytes which are targets for HIV. The relevant data are presented in table 9.

Table 8.: Analysis of combined drug coating effectiveness for lymphocytes at the different scenarios

The scenarios		**Drug 1**	**Drug 2**	**Drug 3**	**Drug 4**	**Drug 5**
	HIV +ve with drug pretreatment	0.5782	0.6707	0.5177	0.2691	0.2244
	HIV +ve without drug pretreatment	0.5102	0.4152	0.2353	0.0245	0.1995
	HIV –ve	0.8163	0.8304	0.7060	0.6850	0.6733

Table 8a:. Summary Table of ANOVA

Source of Variance	**SS**	**Df**	**MS**	**SD(SqrtMS$_w$)**	**F**	**Level of Significance**
Between groups (True)	0.55221	2	0.027610	0.163529814	10.3347	0.05
Within groups (Error)	0.32091	12	0.02674			
Total	0.87312	14				

Table 8b.: F Comparison for Coating Effectiveness Groups

Ratio	**Groups Comparison**			
	A, B and C	**A and B**	**A and C**	**B and C**
Computed F	10.33	2.87	7.87	20.2
Critical F	3.88	4.75	4.75	4.75

The analysis of variance (ANOVA) for the three sets of data in table 9 gives the computed F ratio as 10.33 which is higher than the critical F ratio of 3.88 for 0.05 level of significance. Thus, there are significant differences between the means of the data for scenarios, A, B and C. To identify the scenario that is most significant, analyses of the means of these data become necessary. Between scenarios A and B, the computed F ratio is 2.87 which is less than the critical F ratio of 4.75. This shows that there is no significant difference between scenarios A and B. Analyses of A and C (computed and critical F ratios are 7.87 and 4.75 respectively), and of B and C (computed and critical F ratios are 20.2 and 4.75, respectively) show that there are significant differences between the means of these pairs of data as presented in tables 9a and 9b. These results show therefore that scenario C, the combined drug coating effectiveness of HIV negative white blood cells, is most significant. Thus, the coverage of the surfaces of the HIV negative white blood cells by drug films is more effective than the coverage of HIV positive WBC cell surfaces whether or not they had earlier been subjected to antiretroviral drug treatment or not. The drug films adhere more effectively on surfaces of blood cells not infected by HIV. This suggests that HIV has the effect of reducing the surface area of blood cells, which has previously been reported [1,3,4]

Table 9. Coating effectiveness

Drug	Film thickness t_d for Drug film alone (nm)	Lymphocytes		
		Film thickness (film + Cell), t_{db} (nm)	Cell thickness, t_b (nm)	Coating Effectiveness, σ_t
Drug 1	420.35	88.24	80	0.0242
Drug 2	550.86	301.53	80	0.4705
Drug 3	280.81	38.42	80	-0.2071
Drug 4	867.55	306.83	80	0.2870
Drug 5	346.23	78.39	80	-0.0061

The thickness of the lymphocyte is estimated as follows: A typical red blood cell has the thickest point to be between 200 – 250nm while the minimum thickness is 80 – 100nm. It is stated that this is smaller than most other cells [19]. Hochmuth et al (20) reported a membrane thickness of 78nm. We can comfortably assume that the average thickness of a lymphocyte cell, for our purpose, is 80nm as the lowest limit.

Table 10 shows the film thickness effect of the lymphocytes with which the coating effectiveness is estimated. The coating effectiveness using absorbance data differs from the coating effectiveness using film thickness. It must be noted that the lymphocytes, when cast on a glass will not lead to a smooth surface. Thus, in some cases, the drug film will have to fill the rugosities before appearing significant on the surface of the lymphocyte. As a result, the data using film thickness may not be expected to be as accurate as those using absorbance data.

The above notwithstanding, the coating effectiveness of the Lymphocytes are seen to vary with different antiretroviral drugs. The antiretroviral drug actually affects the surface properties of the Lymphocytes in different ways. The film thickness effect 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.

5. CONCLUSION

In contemporary research works, there may be a great need to achieve a more reliable research result through a synergy between engineers and biological researchers. The peak absorbance data for various interacting systems were measured. These were used to calculate the coating effectiveness of antiretroviral drugs in HIV-blood interactions. The coating effectiveness of antiretroviral drugs in the presence and absence of HIV was calculated using absorbance data and film thickness of drugs on blood cells. The coating effectiveness was found to be highest on drug 2 using both the absorbance and film thickness techniques. This shows that drug 2 (Tenofovir, Lamivudine & Efavirenz) is the most effective antiretroviral drug used in the study, which also had been clinically confirmed. The use of the findings of this work by pharmaceutical industries may help in the search for more effective antiretroviral drugs for the treatment of HIV patients.

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