BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348–0521, ISSN (E): 2454–4728
Vol. 8, Issue 03, Mar 2020, 35-48

SURFACE FREE ENERGY OF BLOOD INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) FROM CONTACT ANGLE MEASUREMENTS

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ABSTRACT

The surface free energies of HIV infected and uninfected lymphocytes were studied. Lymphocytes in the blood were chosen because HIV normally targets them for destruction. Blood samples were collected from twenty HIV infected patients and twenty uninfected persons. Through centrifugation, the blood components were separated. The plasma and the lymphocytes were retained and prepared by smearing on glass slides and dried in ventilated room for contact angle measurements using water and glycerol. It was found that HIV infection increased the contact angles on the surfaces of the lymphocytes, making them more hydrophobic. If the surface of the lymphocyte is modified to the extent that the contact angle is reduced, in principle the effect of HIV could be reduced. The surface free energies were calculated from contact angle measured for each liquid using the Neumann and Fowkes models. The average values of surface free energies were used to calculate the changes in free energies of adhesion. The results showed that HIV has the capacity to lower the surface energy of the lymphocyte and thereby weakening it and rendering it incapable of resisting the HIV attack. The change in free energy of adhesion which is high for infected cells gives an idea of the strength of the bond between the HIV and the surface of the lymphocyte. A relationship between the surface free energies of the HIV, lymphocytes and the plasma was derived and shown to exhibit an error limit of about 8% in the evaluation of the surface energy of the lymphocyte.

KEYWORDS: HIV, Lymphocytes, Change in Free Energy of Adhesion, Surface Free Energy & Contact Angle

INTRODUCTION

The increasing rate of Human Immunodeficiency Virus **(**HIV) infection globally is well documented (UNAIDS, 2013) and solutions are continuously being sought for its elimination. It has equally been established that the lymphocyte is the target of the virus (Ani, 2015). The mechanism of interaction between the virus and the surface of the lymphocyte continues to be of research interest. The virus actually attaches its CD8+ cells on the wild CCR5 dendrites of the CD4+ T4 cells and if they penetrate the cell core will change the nature of the cells. When the virus attaches itself to the surface of a given lymphocyte, an original area on the surface is destroyed while a new area is created. This change in surface area leads to change in surface energy of the surface. The understanding of the mechanism of interaction may require the determination of the energy associated with the creation of a new surface area. The energy of adhesion of the virus to the surface of the lymphocyte becomes of interest. The term surface energy is used because a change in the surface area of a solid cannot be accomplished without doing work against the elastic forces and plastic resistance of the solid (Good, 1979).

The presence of an interface influences generally the thermodynamic parameters of the interacting systems. Thermodynamically, the surface energy γ (given by $\gamma = \left(\frac{\delta G}{\delta A}\right)_{T,p,c}$) is interpreted as the increase in the Gibbs energy of

the system when the area of the interface under consideration is increased reversibly by an infinitesimal amount dA at constant temperature (T), pressure (P) and composition (c) (Lyklema, 1991).

There is now wealth of spectroscopic and other analytical techniques for probing the surface properties of solid materials (Brady, 1996) which yield a variety of surface properties of those parts of such materials that are situated anywhere between 1.0 and more than 10 nm below their surfaces (Etzler, 2001). Contact angle technique has been reported as being capable of yielding the actual surface or interfacial properties at the precise surfaces of solids that are relevant to their interaction with other condensed phase materials (van Oss and Giese, 2002).

Surfaces can be classified as high or low energy surfaces depending on the types of interactions that hold the substrates together. High energy substrates, which are more easily wet than low energy substrates (de Gennes, 1985; Kern, et. al. 1986) are held together by bonds while low energy substrates are held together by forces. Covalent, ionic, and metallic bonds are much stronger than forces such as van der Waals and hydrogen bonding.

METHODOLOGY

Concept of Contact Angles

Contact angle, θ is a quantitative measure of the wetting of a solid by a liquid. It is defined geometrically as the angle formed by a liquid at the three phase boundary where liquid, air and solid intersect (fig. 1). Low values of θ indicate that the liquid spreads, or wets the surface (hydrophilic), while high values indicate poor wetting (hydrophobic). A zero contact angle represents complete wetting.

Resolution of forces in fig.1in the horizontal direction gives an expression in eq. (1) referred to as Young's equation.

$$\gamma_{sv} = \gamma_{sL} + \gamma_{Lv} Cos\theta \quad (1)$$

Where γ_{sv} is the surface free energy of the surface with respect to air, γ_{Lv} is the liquid surface free energy, γ_{sL} is the energy between the surface and the liquid while θ is the angle between the solid surface and tangent to the liquid surface at the liquid/solid/air interface. Thus, through the contact angles which are easily measured, the surface free energy of the surface can be calculated. Eq. (1) which is widely used to characterize surface properties from the measured contact angles with liquids, whose surface free energies γ_{Lv} have already been determined, is not easy to use. The problem is that γ_{sL} is not known and it is not easy to measure.

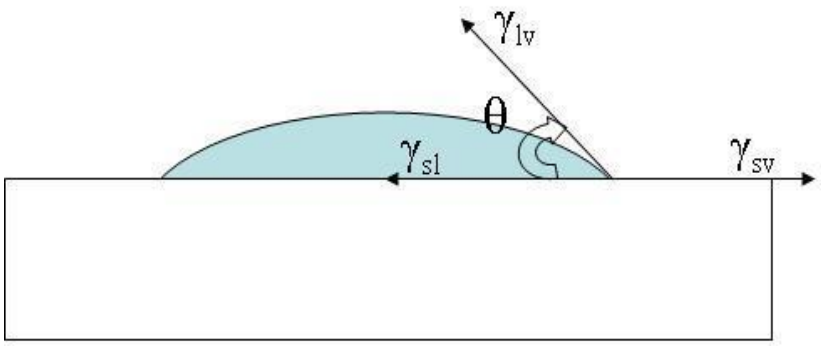

Figure 1: The Profile of a Drop on a Flat Surface.

The major simplification of eqn.(1) was possible as a result of Dupre's (1869) equation that combined work of adhesion at the solid – liquid interface with the surface and interfacial tensions of the solid – vapour, solid – liquid and liquid –vapour interfaces that led to

$$\gamma_{LV}(1+\cos\theta)=W_{SL} \tag{2}$$

The next simplification of eqn.(2) was due to Good and Girifalco (1957) who, analogous to the Berthelot (1898) combining rule of intermolecular interaction, showed that the work of adhesion can be expressed as a geometric mean of the surface tension of the pure components γ_{SV} and that of the liquid γ_{Lv}, i.e.,

$$W_{SL} = 2\Phi\left(\gamma_{SV}\gamma_{LV}\right)^{0.5} \tag{3}$$

Combination of the Good – Girifalco equation and the Young-Dupre equation results in a fundamental equation (eq. (4)) of wettability that allows estimation of the surface free energy of the solid.

$$\gamma_{SV}(1+\cos\theta)=2\Phi\left(\gamma_{SV}\gamma_{LV}\right)^{0.5} \tag{4}$$

When the primary forces constituting the cohesive and adhesive interactions are of the dispersive type, Φ =1. Then Eq.(4) reduces to Eq. (5)

$$\gamma_{SV}=0.25\gamma_{LV}(1+\cos\theta)^2 \tag{5}$$

The fact that γ_{SL} in eq.(1) acts like a force is evident in the capillary rise of liquids or the movement of liquids on a solid surface under the influence of the surface energy gradient of the solid. To estimate such quantity requires another set of equations based on surface characteristics. Various models that have been applied were able to split solid surface energy into components. However, the idea of the partition of the surface free energy into individual components is based on the assumption that the quantity γ_{SL} is determined by various interfacial interactions that depend on the properties of both the measuring liquid and the solid studied. Fowkes (1968) applied surface component approach to determine the surface free energy of non-polar solid, that is the solid for which $\gamma_{SV} = \gamma_{SV}^{d}$ is valid. If the measuring liquid is a dispersive one, $\gamma_{LV}=\gamma_{LV}^{d}$ and

$$\gamma_{SV}=\gamma_{SV}^{d}=0.25\gamma_{LV}(1+\cos\theta)^2 \tag{6}$$

$$\gamma_{SL}=\gamma_{SV}+\gamma_{LV}-2\left(\gamma_{SV}^{d}\gamma_{LV}^{d}\right)^{0.5} \tag{7}$$

Neumann et al (1975; 1983) applied equation of state to obtain the energy at the solid – liquid interface as:

$$\gamma_{SL} = \frac{\left\{(\gamma_{SV})^{\frac{1}{2}} - (\gamma_{LV})^{\frac{1}{2}}\right\}}{\left\{1 - 0.015(\gamma_{SV}\gamma_{LV})^{\frac{1}{2}}\right\}} \quad (8)$$

Eq. (8) is used in conjunction with eq. (1) to determine the surface free energy of a surface. This technique has been used successfully to determine the surface tensions of several polymer particles, including biological cells and bacteria.

Some minor difference may exist in the results of these models, however the model that would give results comparable with the literature values would be more acceptable.

Once the surface free energy of the surface is determined, then the thermodynamic free energy of adhesion of a particle P on a solid S in a liquid L can be calculated from the Duprè equation;

$$\Delta F_{PLS}^{adh}(d_O) = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \quad (9)$$

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.

The force of cohesion, which is the interaction of a material with itself is described by;

$$\Delta F_{ii}^{coh}(d_o) = -2\gamma_{iv} \quad (10)$$

If the contact angle is known, γ_{sL}, solid-liquid interfacial tension can be calculated by any model and by the use of Young's equation or any other appropriate equation, the surface free energy of the solid material can be determined.

Materials Used

Test Liquids: Two liquids (called probe liquids) were used for the experiments. These liquids are, water and glycerol. Some of the properties of these are presented in table 1. The superscript d stands for the dispersive component,

Table 1: Properties of the Liquids

Liquids	Molecular Formula	Density (g/cm^3)	Boiling Point (^0C)	Dipole Moment (D)	Surface Tension (mJ/m^2)
Water	H_2O	1	100 at 1atm	1.8546	γ^d = 21.8 γ^- = 25.5 γ^+ = 25.5
Diiodomethane	CH_2I_2	3.32	181 at 1 atm	1.08	γ^d =50.8 γ^- = 0 γ^+ = 0
Glycerol	$C_2H_80_3$	1.261	290 at 1 atm		γ^d =34 γ^- = 3.92 γ^+ = 57.4

HIV Infected Blood and Uninfected Blood

Sample collection: Twenty samples of HIV infected blood and twenty samples of uninfected blood were sourced from Nnamdi Azikiwe teaching hospital, Nnewi. The samples were treated with anti-coagulant (0.5M Ethylene-diamine-tetra-acetic acid-EDTA) to ensure that the blood does not coagulate before the experiment (Ozoihu, 2014). Also, the samples were maintained below the room temperature in the refrigerator (Haier Thermocool) to ensure the blood does not denature.

Lymphocytes Cell Count: The CD4 counts of blood samples were determined using Partec Flow Digital counter. The essence was to ascertain the degree of infection of each blood sample with HIV. Normally, the blood samples of the uninfected patients have higher CD4 counts above 500cells/mm^3. It is noted that details of length of exposure of each patient to HIV were not available. It was difficult to ascertain whether any of the patients had started antiretroviral drug treatment and if so, the type of drug taken.

Sample Isolation: Each sample of both infected and uninfected blood was separated into the components by centrifugation. A swinging head (four- bucket type) centrifuge was used and operated at a speed of 1500rpm for 30minutes. Three distinct layers appeared with the plasma at the top, white blood cells (lymphocytes) called the buffy coats appeared at the middle while the red blood cells appeared at the bottom of the plastic test tube containing the blood. Each layer was drawn off and stored in a marked test tube.

Slide Preparation: The microscopic slide of 25.4 mm x 76.2 x1.2 mm was used for the preparation of test surfaces. A dropper was used to draw each of the blood components and smeared carefully on a glass slide to ensure even distribution of the blood samples on the slide surfaces. Three slides were prepared for each of the twenty samples for different blood components since three liquids were used for each sample. The samples were allowed to dry naturally in room temperature because exposing the prepared slides to the sun is likely to cause oxidation and the surface energy might be increased unconditionally. All the well prepared and dried surfaces were covered with microscopic cover slips, ready for the experiment *(Ozoihu, 2014).*

Contact Angle Measurement: The contact angles were measured *(Ozoihu, 2014)* by the sessile drop technique which involved the measurement of the angles on the captured drop profile using a protractor. Each of the test liquids was dropped on the surface of the prepared microscopic slides (25.4mm x 76.2 x1.2mm) of the separated blood components (HIV infected and uninfected Plasma and lymphocytes) using a microliter syringe of 5.0µl capacity. The tip of the syringe was positioned a few micro meters away from the surface of the solid surface (slide) to eliminate impact effect when the drop was released. The droplet volume was selected to be small enough so that gravity effect is negligible. The spreading process was captured with a digital camera (CANON ZOOM LENS 3.4X) of 6.3- 21.6mm and 3.0- 5.8mm lens. Other basic elements of an optical tensiometry include the light source, sample stage and the image capture. The images were cropped and printed on paper (A4). The contact angles were carefully measured using protractor at the solid- vapour, solid – liquid and liquid interface (Fig 2).

Some issues in the procedure above include the following:

- The surfaces so formed were not expected to be smooth since the blood cells were not dissolved in a solvent to form solutions. The use of this technique despite this pitfall stemmed from successful reports by other authors.
- There could be other precipitates in the blood such as the bicarbonate ions or particles of opportunistic infections

that may affect the results,

- In absence of a powerful photomicroscope with optical graticule, the best approach to contact angle determination will be the high resolution camera to capture the drop profile, making it amenable to use with protractors, as stated above.
- Another good approach to contact angle measurement would consider the `use of high resolution camera to capture the drop profile and with an appropriate software together with drop dimensions calculate the contact angles as advanced by Hoorfar and Neumann (2006) and Zuo, et. al. (2004)
- The liquid used must not spread on the cast surface of blood components**.**

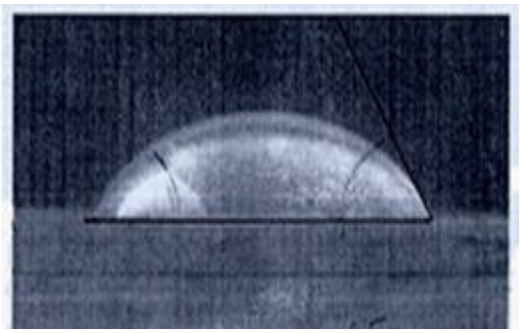

Figure 2: Contact Angle Measurement on Blood Components *(Ozoihu, 2014)*.

RESULTS AND DISCUSSIONS

Analyses of Contact Angle Data

The results of the contact angle and CD4+ counts measurements are listed in table 1. The infected lymphocytes are used to represent the virus because there is currently no known means of isolating the virus. The assumption here is that the infected lymphocyte is an approximation of the actual virus owing to the manner of the infection. The virus actually attaches its CD8+ cells on the wild CCR5 dendrites of the blood CD4+ T4 cells but the extent of the coverage of the surface of the lymphocyte by the virus at a given time is however not known.

Table 2: Contact angles and CD4+ Cell counts measured on HIV and Lymphocytes

HIV (Infected lymphocytes)			Lymphocytes (Uninfected lymphocytes)		
CD4+ Counts/mm^3 of blood	Contact Angles(θ^o)		CD4+ Counts/mm^3 of blood	Contact Angles(θ^o)	
	Water	Glycerol		Water	Glycerol
438	80	58	4500	59	45
278	78	59	6000	55	51
282	76	66	8000	50	47
682	64	67	4900	57	48
606	70	68	5000	63	48
20	85	59	4500	64	50
613	67	68	4000	60	48
468	76	73	6200	63	51
853	74	69	4900	58	55
356	75	65	4800	60	50
268	64	61	4000	64	50
625	75	60	5000	60	61
230	69	63	4000	63	50
246	70	60	4000	64	53

339	71	65	6000	55	48
316	66	63	4400	65	51
220	73	60	4700	58	52
374	69	66	4800	59	46
593	65	60	4300	58	50
372	78	65	6000	58	49

Table 3

Serum (HIV infected)		
CD4+Counts/mm^3	**Contact Angles(θ^o)**	
	Water	**Glycerol**
438	69	59
278	68	58
282	75	55
682	72	65
606	70	62
20	72	60
613	60	70
468	60	56
853	59	54
356	70	58
268	65	50
625	69	63
230	70	63
246	57	50
339	66	58
316	60	59
220	65	54
374	50	49
593	60	58
372	70	63

Table 4: Contact angle summary

	Water	**Glycerol**	**Average CD4 count**
HIV (infected lymph.)	74.5±6.1	65.2±4.9	409±239.9
Lymphocytes	60.9±4.2	49.3±2.7	5280±1165
Serum	67.5±5.7	59.7±4.8	

Table 1 shows the contact angle results on lymphocytes (HIV infected and uninfected) and the infected serum using different liquids; water and glycerol. The sessile drop technique was used to establish the contact angle on the substrates. The CD4+ cell count measured for each sample was used to describe the degree of immune cell depletion during infection. Below 200 counts/mm^3 of blood, the system results to Acquired Immunodeficiency Syndrome (AIDS). At a very low CD4+counts (20counts/mm^3 of blood) the viral load becomes very high.

Statistical analysis was done with data of table 1 to show whether any discernible relationship exists between the CD4 cell counts and the contact angles. For HIV, the Pearson moment correlation coefficients between the CD4 cell counts and the contact angles between CD4 count and contact angle with water and glycerol, in view of the above, appears significant. This notwithstanding, the contact angles for water give a negative correlation with CD4 counts (contact angle appears to increase with decrease in CD4 count), glycerol gives a positive correlation (contact angle appears to increase with increase in CD4 count).

Analysis of the results for the lymphocytes (uninfected lymphocytes) gives a reverse variation – whereas Pearson correlation coefficients are positive with water, they are negative with glycerol (0.558 and -0.022 respectively). These results are significant with water but very low with glycerol and as discussed above, one can state that there is no discernible or consistent relationship between the CD4 count and the contact angles for uninfected lymphocytes.

There is the need to determine the real relationship between HIV (infected lymphocytes) and lymphocytes (uninfected lymphocytes). The contact angles of the virus are compared with the contact angles for the lymphocytes to ascertain the trends. The average contact angles with water and glycerol are 74.5 and 65 for the virus (average CD4 count = 459.6) and 61 and 49 for the lymphocytes (average CD4 count = 5280), respectively. It is clear that the presence of the virus leads to an increase in the average contact angles of lymphocytes, i.e., the virus makes the surface of the lymphocyte more hydrophobic.

When the surface of the lymphocyte is infected, the changes that are observed are likely due to the presence of the virus. When there is no infection, the blood cells can behave in a way consistent with the health of the donor. The summary is that the virus normally would change the character of the surface of the lymphocyte as it adheres to it. This is expected since the virus actually causes the damage or death of the cell.

From table 2, CD4 count gives the idea of the level of infection in a patient: high contact angles are associated with virus infected cells while low contact angles are associated high CD4 counts – condition of no infection. Thus, in principle a measure of the contact angle can give an idea of the presence of the virus in the blood.

The contact angle data for the infected plasma are also given in table 1. The correlation coefficients between the CD4 counts and each set of the contact angles are – 0.55 and 0.19 respectively. The results appear inconsistent with the measuring liquids, hence no special conclusion can be drawn from them. However, the average values of the contact angles are seen to be between those of the virus and the lymphocytes for each liquid.

The non - consistent values of the correlation coefficients may be attributable to the fact that the donors of the blood used could not all possibly be in the same state of health. The general trend given by the average values of the contact angles is accepted in this work to give a true situation, i.e., HIV tends to increase the contact angle on the lymphocyte thereby making it hydrophobic.

Determination of Surface Free Energies

The surface free energies (listed in table 3) were calculated using each model using the following symbols:

γ_{pv}= Surface free energy of HIV (infected lymphocytes)

γ_{sv} =Surface free energy of lymphocytes (uninfected lymphocytes)

γ_{lv}=Surface free energy of serum (infected)

Where γ_{ps}, γ_{pl}, γ_{sl} are obtained by geometric means of eq. (11) and are used to determine the thermodynamic free energies of adhesion ΔF^{adh} given by eq. (9).

$$\gamma_{ij} = \sqrt{\gamma_{iv}\gamma_{ij}} \tag{11}$$

Table 5: Surface Free Energy (mJ/m^2) data using Neumann Model (Equation of states)

	HIV (infected lymphocytes)				Serum			
CD4	Water	Gly	Diiodomethane	Averages	Water	Gly	Diiodomethane	Averages
	γsv	γsv	γsv	AVE-γsv	γsv	γsv	γsv	AVE-γsv
438	25.07	37.45	25.70	29.40	33.58	36.72	32.01	34.10
278	26.55	36.72	25.70	29.66	33.58	37.45	28.57	33.20
282	28.07	31.66	34.27	31.33	28.84	39.61	27.42	31.96
682	37.65	30.94	26.84	31.81	31.18	32.38	28.57	30.71
606	32.77	30.23	26.84	29.95	32.77	34.54	32.01	33.10
20	21.52	36.72	28.57	28.94	31.18	36.00	35.38	34.18
613	31.98	30.23	28.57	30.26	40.95	28.81	35.92	35.22
468	28.07	26.72	34.27	29.68	40.95	38.89	34.83	38.22
853	29.62	29.52	32.01	30.38	41.77	40.33	34.27	38.79
356	28.84	32.38	28.58	29.93	32.77	37.45	25.70	31.97
268	37.65	35.27	33.15	35.35	36.83	43.18	34.27	38.09
625	28.84	36.00	39.10	34.64	33.58	33.83	30.30	32.57
230	33.58	33.83	38.59	35.33	32.78	33.83	26.70	31.10
246	32.78	36.00	30.30	33.02	43.42	43.18	30.87	39.15
339	31.98	32.38	26.85	30.40	36.02	37.45	34.27	35.91
316	36.02	33.83	34.27	34.70	40.96	36.73	39.73	39.14
220	30.40	36.00	36.47	34.29	36.83	40.34	39.10	38.75
374	33.58	31.66	34.27	33.17	49.12	43.88	35.38	42.79
593	36.83	36.00	33.15	35.32	40.95	37.45	38.59	38.99
372	26.55	32.38	31.45	30.12	32.78	33.83	37.54	34.71

Table 6

	Lymphocytes (unfected lymphocytes)			
CD4	Water	Gly	Diiodomethane	Averages
	γsv	γsv	γsv	AVE-γsv
4500	41.77	46.62	38.58	42.32
6000	26.55	42.47	25.70	31.57
8000	49.11	45.26	43.37	45.91
4900	43.42	44.57	38.06	42.02
5000	38.47	44.57	32.58	38.54
4500	37.65	43.18	34.27	38.36
4000	40.95	44.57	38.06	41.19
6200	38.47	42.47	35.38	38.77
4900	42.62	39.61	32.01	38.07
4800	40.95	43.18	35.92	40.01
5000	37.65	43.18	35.38	38.73
4000	40.95	35.27	39.61	38.61
4000	38.48	43.18	42.03	41.23
4000	37.65	41.05	34.83	37.84
6000	45.07	44.58	35.38	41.67
4400	36.83	42.47	37.01	38.77
4700	42.60	41.77	35.93	40.10
4800	41.78	45.95	40.60	42.77
4300	42.60	43.18	37.01	40.93
6000	40.95	43.88	39.61	41.48

Table 7: Surface Free Energy (mJ/m2) Summary

	HIV	Lymphocyte	Serum
Neumann Model	31.89±2.33	39.94±2.83	35.45±3.31
Fowkes Model	29.60±1.44	39.95±2.82	35.09±3.76
Average CD4 count	409±199	5000±999	

The average values of the surface free energies obtained using each test liquid and calculated using Neumann's equation are listed in table 3 for HIV (HIV infected lymphocyte), lymphocyte (uninfected lymphocyte) and serum (infected). The averages of the ten average values of the surface free energies are summarized in table 4. The surface free energies were recalculated from the contact angle data using the Fowkes model and the final average values are also listed in table 4 together with the average CD4 counts. The surface free energy of the lymphocytes is 39.94 mJ/m^2 whereas that of infected lymphocytes is 31.89 mJ/m^2 (Ozoihu, 2014), with HIV causing a reduction in surface free energy by about 20% using the Neumann's model and by about 26% using Fowkes model. It is clear that HIV has a lower surface energy than the lymphocyte and when it attaches itself to the surface of the lymphocyte, has the capacity to reduce the surface energy of the lymphocyte considerably, by more than 20%. The implication of this is that the infection weakens the lymphocyte surface making it very susceptible to destruction by HIV itself or by the opportunistic diseases. There was no difference between the free energies of the lymphocytes using the Neumann and Fowkes models but for the HIV, value obtained using the Neumann model is higher than that obtained using the Fowkes model by about 7%. For the plasma, the difference in the use of the two models is about 1%.

Change in Free Energies of Adhesion

The changes in free energy of adhesion (ΔF^{adh}) were calculated using the interfacial free energies as listed in table 3 together with the relevance of eqn. (9) and (11). These data were plotted as shown in fig. 3 as a plot of the changes in free energy of adhesion against the surface free energy of the lymphocyte.

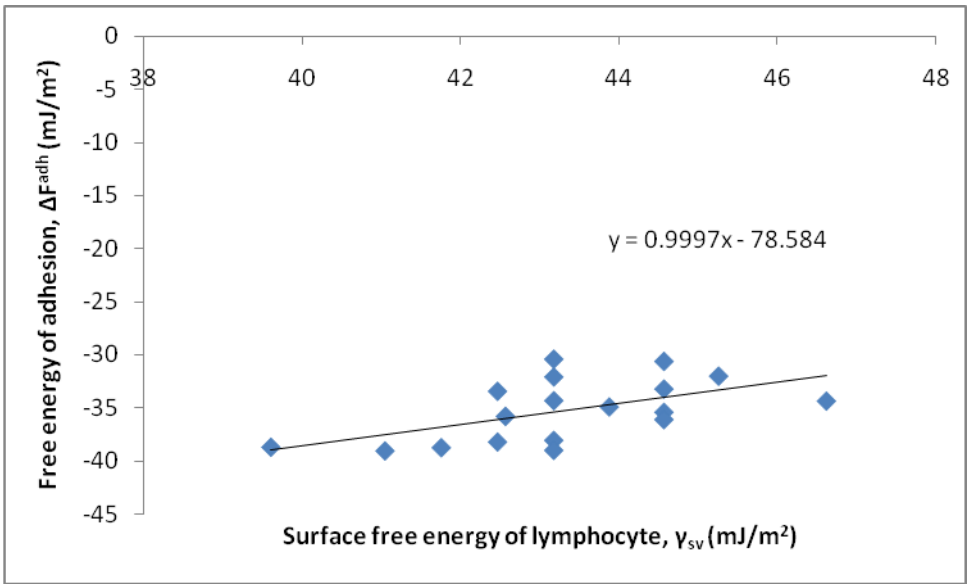

Figure 3: The Relationship between the Change in Free Energy of Adhesion with the Surface Free Energy of the Lymphocyte.

The changes in free energies of adhesion (ΔF^{adh}) are all found to be negative indicating that the net van der Waals forces are attractive. Figure 3 shows that reduction in the surface free energy that lead to increased value of negative free energy of adhesion; ΔF^{adh} is actually a measure of the strength of adhesion or bonding of the virus on the surface of the lymphocyte. The result indicates that this strength of adhesion or bonding is strongest if surface energy of lymphocyte is low. Thus, to reduce the adhesive energy of HIV on the surface of the lymphocyte, a means that will lead to an increase in the surface energy should be sought. It is believed therefore that if the adhesive energy or adhesive bond is low, the attraction between the virus and the lymphocyte will be reduced thus reducing the risk of HIV action on the surface of the

lymphocyte (Achebe, 2010; Ani, 2015).

From this relationship between the adhesive energy and surface energy of the lymphocyte, we sought a functional relationship between them. From fig. 3, the trend line is given by eqn. (12) obtained by fitting the data to a straight line.

$$\Delta F^{adh} = 0.9997\gamma_{sv} - 78.58 \tag{12}$$

Using eq. (9), eq. (12) can be written as.

$$\gamma_{ps} - \gamma_{pL} - \gamma_{sL} = 0.9997\gamma_{sv} - 78.58$$

$$0.9997\gamma_{sv} - \gamma_{ps} + \gamma_{pL} + \gamma_{sL} - 78.58 = 0$$

The above equation can be approximated to:

$$\gamma_{sv} - \gamma_{ps} + \gamma_{pL} + \gamma_{sL} - 78.58 = 0 \tag{13}$$

Using eq. (11), eq. (13) can be written as:

$$\gamma_{sv} - \sqrt{\gamma_{pv}\gamma_{sv}} + \sqrt{\gamma_{pv}\gamma_{Lv}} + \sqrt{\gamma_{sv}\gamma_{Lv}} - 78.58 = 0$$

$$\gamma_{sv} - \sqrt{\gamma_{sv}}\left(\sqrt{\gamma_{pv}} - \sqrt{\gamma_{Lv}}\right) + \sqrt{\gamma_{pv}\gamma_{Lv}} - 78.58 = 0 \tag{14}$$

Applying the quadratic formula to eq. (14), we obtain an expression for the surface energy of the lymphocyte if surface energies of the virus and the plasma are known.

$$\sqrt{\gamma_{sv}} = ½\left(\sqrt{\gamma_{pv}} - \sqrt{\gamma_{Lv}}\right) \pm ½\sqrt{\left(\sqrt{\gamma_{pv}} - \sqrt{\gamma_{Lv}}\right)^2 - 4\left(\sqrt{\gamma_{pv}\gamma_{Lv}} - 78.58\right)} \tag{15}$$

Eq. (15) was used to recalculate the surface energy of lymphocyte. Using the positive sign gave an average value of surface energy of lymphocytes as 43.06±3.30 mJ/m^2 while the negative sign gave 47.12±2.60 mJ/m^2. The average value of the surface free energy as measured was 39.94±2.83 mJ/m^2. We can see that eq. (15) used with the positive sign gives value of surface energy that is about 7.8% different from the measured values, whereas the use of negative sign in eq. (15) gives a difference of about 18%.

Based on the above discussion, eq. (15) now reduces to:

$$\sqrt{\gamma_{sv}} = ½\left(\sqrt{\gamma_{pv}} - \sqrt{\gamma_{Lv}}\right) + ½\sqrt{\left(\sqrt{\gamma_{pv}} - \sqrt{\gamma_{Lv}}\right)^2 - 4\left(\sqrt{\gamma_{pv}\gamma_{Lv}} - 78.58\right)} \tag{16}$$

It should be understood that the data used to establish eq. (16) were based on contact angle measurements on blood obtained from ten HIV patients and ten persons that were not suffering from HIV. It was not ascertained whether any of the ten HIV patients had started taking antiretroviral drugs or not, nor were the nature of opportunistic diseases known. It is believed that these conditions will affect the results obtained. It is not surprising therefore that large discrepancies are reported in some areas of these results.

CONCLUSIONS

We sought to determine the surface free energies of HIV infected and uninfected lymphocytes from contact angle data. Lymphocytes were studied because the HIV normally targets the lymphocytes for destruction. Blood samples were collected from twenty HIV infected patients and twenty uninfected persons. Through centrifugation, the blood components were separated. The plasma and the lymphocytes were prepared by smearing on glass slides and drying in ventilated room for contact angle measurements using water and glycerol.

It was found that HIV infection increased the contact angles on the surfaces of the lymphocytes, making them more hydrophobic. Since HIV lowers the $CD^{+}4$ count, it is simple to summarize that one can measure the contact angle and if it increases, one would suspect HIV. Thus, if it is possible to modify the surface of the lymphocyte to the extent that the contact angle is reduced, in principle the effect of HIV could be reduced.

The surface free energies were calculated for each liquid using the Neumann and Fowkes models. However, it was the average values of surface free energies that were used to calculate the changes in free energies of adhesion. The results showed that HIV has the capacity to lower the surface energy of the lymphocyte and thereby weakening it and rendering it incapable of resisting the HIV attack. The change in free energy of adhesion gives an idea of the strength of the bond between the HIV and the surface of the lymphocyte. It was shown that for low surface energies of the lymphocytes, the adhesive energy is large and in that case the lymphocyte would be an easy target for destruction by the virus.

A functional relationship between the surface free energies of the HIV, lymphocytes and the plasma was derived. It was shown that with it, within an error limit of about 8%, it could be used to evaluate the surface energy of the lymphocyte.

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