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HUMAN IMMUNODEFFICIENCY (HIV) – BLOOD INTERACTIONS: APPLICATION OF SESSILE DROP TECHNIQUES TO THE VERIFICATION OF NEGATIVE HAMAKER CONCEPT

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ABSTRACT

An alternative solution to Acquired Immune deficiency Syndrome (AIDS) has been a worldwide concern since the antiretroviral drugs have failed to completely eradicate Human immunodeficiency virus (HIV) from the blood. However the concept of negative Hamaker coefficient has been widely reported as one of the reliable traditional method of separation between particles that interact both in vacuum and in liquid. It was therefore suggested that the concept of the negative Hamaker as a method of separation might provide a solution to interactions between HIV and blood. This novel was applied to interaction between the HIV and lymphocytes that evidently exist as particles. A sessile drop technique was carried out on ten samples of HIV infected and uninfected blood. The CD4+ cell counts of these bloods reveal the extent of immune cell depletion as determined with partec flow meter. Diodomethane was used to determine the contact angles whose results were used to estimate the interfacial energies and absolute values of Hamaker coefficient for infected lymphocytes (HIV), lymphocytes and Serum. A pair – wise summation of geometric means of the absolute Hamaker coefficients yielded a negative value ($-0.145X10^{-19}mJ/m^2$).This implies that the van der Waals attraction (+) between HIV and lymphocytes could be made repulsive(-), hence a good suggestion to the cure of HIV/AIDS.

KEYNOTES: Humanimmunodefficiency virus (HIV), Contact angle, Surface Energy and Hamaker Coefficient.

INTRODUCTION

Human immunodeficiency virus (HIV) gets entry into the cell when its surface molecule, gp120 binds to the surface molecule (CD4+) on the lymphocytes. With the assistance of co-receptors; the CCR5 and CCR4, the HIV is provided a better opportunity to attack the target cells. Since the discovery of HIV as the major causes of AIDS in 1984; Field[1] noted that all the antiretroviral drugs that have been tested for clinical trial have failed to completely eradicate the menace of HIV. However, failure of clinical solutions had left people infected by HIV to rely on managing the sickness rather than curing it. However, to rekindle the hope of people living with HIV, an alternative solution has been suggested by Omenyi et al[2] using engineering surface thermodynamic approach. They started addressing the conditions under which the HIV in the blood could be rendered impotent. The question was that, could there be an additive in form of drugs that could be found which in the presence of the serum as an intervening medium, would render the energy of interaction represented by combined Hamaker coefficient (A_{132}) negative and hence, make the virus and lymphocytes repel each other and prevent attack of HIV.[3] This concept originated from the works of Hamaker[4] who in his classical paper on van der Waals – London interactions stated that a condition could arise under which the sign of the van der Waals interaction between two different uncharged bodies, surrounded by a liquid, might be negative and such bodies would repel each other. With this concepts, many results have been published using polymer particles. Other researchers had their supports to this novel. For instance, Viser[6] in his related research stated that when two materials are immersed in a liquid medium, and the interaction of each of these materials with that of the liquid medium is larger than the interaction between these materials themselves with the liquid; spontaneous separation can occur due to dispersion forces only. Omenyi et al[7] in their experimental deductions using polymer particles concluded that the sign of the net van der Waals interaction between two different solid bodies or between two different dissolved macromolecules in liquid, often is negative;that is, they repel one another, even when they are electrically neutral and when they are immersed in apolar liquid. Fowkes[8] had also demonstrated such a repulsive interaction with poly-(tetraflouroethylene)-glycol-iron oxide. However, this new possibility of changing the attraction between different (even neutral) solids submerged in liquids, or dissolved macromolecules into repulsion is suggested between HIV and lymphocytes which interact in

serum during infection. To evaluate the absolute values of Hamaker constants, sessile drop technique is used to obtain the contact angles on HIV infected lymphocytes (HIV), the lymphocytes and serum. From the measured contact angles, the surface energies (γsv) and corresponding solid-liquid interfacial energy (γ_{SL}) with the known surface tension of probe liquid will be used to determine the combined Hamaker coefficient.

Theory

Hamaker[4] stated that when two identical particles or molecules, are separated by a short distance in vacuum, the dispersion interaction is given (Eq.1)

$$A_{ii} = \pi^2 q_i^2 \beta_{ii} \quad (1)$$

$$\beta_{ii} = -\frac{3h\nu\alpha^2}{4(4\pi\varepsilon_o)^2} \quad (2)$$

Where, h is the Planck's constant, ε_o = the characteristic frequency of the molecule, α = the polarizability of the molecule.

It follows from Eqn.(2) that:

$$w(r)London = -\frac{\beta_{ii}}{r^6} \quad (3)$$

r = their separation

The macroscopic quantities in eqn. (2) have been measured using a spectral analysis by Achebe[3] The extended interactions involving different particles *i* and j can be estimated according to Berthlot's principle of geometric means[4]

$$\beta_{ij} = \sqrt{\beta_{ii}}\,\beta_{jj} \quad (4)$$

It then followed that the Hamaker constant for two different materials is given thus;

$$A_{ij} = \sqrt{A_{ii}}\,A_{jj} \quad (5)$$

Eqn.(5) is the geometric combining rule widely used in calculating dispersion energies of interaction between two different materials. This is applicable to the interaction that occurs between HIV and Lymphocytes.

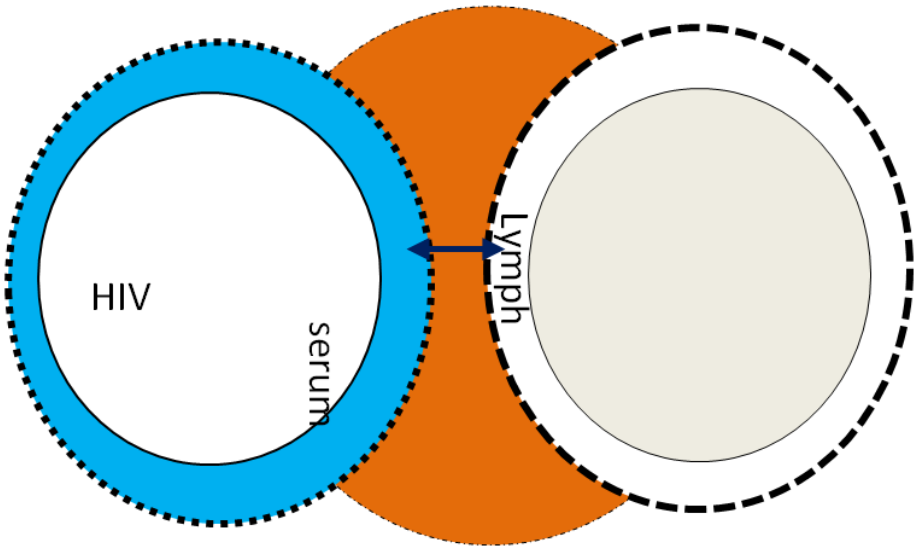

Fig 1: Interaction of two particles bodies, 1and 2 at a Separation, d.

For two particles of the same materials 1(lymphocytes) or 2 (HIV) in medium 3(serum), the combining rule states, thus;

For lymphocyte,1 in serum 3.

$$A_{131} = A_{11} + A_{33} - 2A_{13} \quad (7a)$$

or

$$A_{131} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)^2 \quad (7b)$$

For HIV, 2 in serum 3.

$$A_{232} = A_{22} + A_{33} - A_{23} \quad (8a)$$

$$A_{232} = \left(\sqrt{A_{22}} - \sqrt{A_{33}}\right)^2 \quad (8b)$$

For two different particles (1and 2) in medium 3, the Hamaker combining rule states thus,

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad (9a)$$

$$A_{132} = \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)\left(\sqrt{A_{22}} - \sqrt{A_{33}}\right) \quad (9b)$$

Eq.7 and Eq.8 suggest that the Hamaker constants A_{131} and A_{232} are always positive although they can be zero. For two different materials, the Hamaker constant, A_{132} given in Eq. (9a and 9b) can be negative when

$\sqrt{A_{11}} > \sqrt{A_{33}}$ and $\sqrt{A_{33}} > \sqrt{A_{22}}$ (10a)

Or

$\sqrt{A_{11}} < \sqrt{A_{33}}$ and $\sqrt{A_{33}} < \sqrt{A_{22}}$ (10b)

Lymphocytes (uninfected blood) are represented as 1, HIV represented 2 (infected blood) and infected serum represented 3. A_{11}, A_{22} and A_{33} are always positive (+) if van der Waal forces are attractive and this represent the absolute values of Hamaker coefficient. However, in case the combined values of A_{132} becomes negative (-); the repulsive van der forces are predicted. Such bodies will repel each other Eq. 9.$(A_{11} \neq A_{22})$.The change in free energy of adhesion due to van der Waals attractive forces is given in Eqn.(11).

$$\Delta F^{adh} = \left[-\frac{A_{132}}{12\pi d_0^2} \right] \quad (11)$$

d_o is the smallest distance between particles (1.6 X10^{-10})m (Hough and White)[9]

$$\Delta F^{adh} = \gamma_{SV} - \gamma_{SL} - \gamma_{LV} \quad (12)$$

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cos\theta \quad (13)$$

Where $\gamma_{SV}, \gamma_{SL}, \gamma_{LV}$ are the energies of solid – vapour, solid – liquid and liquid – vapour at the interface respectively. Eq.(13) relates these energies to the contact angle. The major simplification of Eq.(13) was possible as a result of Dupre's[10] equation that combined work of adhesion at the interface with interfacial tensions.

$$W_{SL} = \gamma_{SV} + \gamma_{LV} - \gamma_{SL} \quad (14)$$

The combination of Eq. (13) and Eqn.(14) results in Eq.(15)

$$\gamma_{LV}(1+\cos\theta) = W_{SL} \quad (15)$$

Eq.(15) can be expressed as geometric mean of the surface tension, Good and Girifalco[11]

$$W_{SL} = 2\Phi(\gamma_{SV}\gamma_{LV})^{0.5} \quad (16)$$

Combining Eqn.(13) and (16) results in a fundamental equation (Eqn.(17)) of wettability that allows estimation of the surface free energy of the solid particles.

$$\gamma_{SV}(1+\cos\theta) = 2\Phi(\gamma_{SV}\gamma_{LV})^{0.5} \quad (17)$$

When the primary forces constituting the cohesive and adhesive interactions are of the dispersive type, Φ =1. Then Eq.(17) reduces to Eq. (18)

$$\gamma_{SV} = 0.25\gamma_{LV}(1+\cos\theta)^2 \quad (18)$$

Neumann et al[12] applied equation of state to obtain the energy at the solid – liquid interface.

$$\gamma_{SL} = \frac{\left\{(\gamma_{SV})^{\frac{1}{2}} - (\gamma_{LV})^{\frac{1}{2}}\right\}}{\left\{1 - 0.015(\gamma_{SV}\gamma_{LV})^{\frac{1}{2}}\right\}} \quad (19)$$

Although, 25% difference in the results of each model are acceptable, but the model that would provide less attraction and results comparable with the literature would be chosen as a better option.

METHODOLOGY

Sessile Drop technique for the determination of Contact Angles on HIV infected Surfaces.

The sessile drop technique was considered in this work among other techniques for contact angle measurements. This is due to the fact that:

- When HIV infected blood or uninfected blood was smeared on a glass slide and allowed to dry for contact angle measurement; the best approach would be by sessile drop technique since the contact angle can be measured by any known method.
- The surfaces so formed are not expected to be smooth since the blood cells cannot dissolve to form solutions.
- In absence of a powerful photomicroscope with optical graticule, the best approach to contact angle determination would be the use of high resolution camera to capture the drop profile, making it amenable to use protractors.
- Another approach would require the use of dimensions of the sessile drop profile in a software. This technique was advanced by Neuman and Smith[12]
- The liquid used must not spread on the cast surface of blood component.

Materials used for the experiment.

(a). Test Liquids. Diiodomethane was used to measure the contact angles.

(b)Samples of HIV infected blood and Uninfected blood

(i) Sample collection. Ten samples of HIV infected blood and uninfected bloods were obtained 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. Also, the samples were maintained below the room temperature in the refrigerator (Haier Thermocool) to ensure the survival of the virus and other living components of the blood before the experiment.

(ii) 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 the top, white blood cell, 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.

(**iii) 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 from the boundary layers and smeared carefully on a slide to ensure even distribution of the blood samples on the slides. A slide was prepared for each of the ten samples on different blood components since a liquid (diodomethane) is needed for a test. The samples were allowed to dry naturally in a ventilated room 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 slip, ready for the experiment.

(c) Equipment used in contact angle measurement

Microlitre syringe of 5.0µl capacity: A drop of diodomethane was allowed on the prepared slide using micro liter syringe of 5.0µ capacity.

Microscopic Slide: The microscopic slide of 25.4mm x 76.2 x1.2mm was used for the preparation of test surfaces for both infected and uninfected blood.

Digital Camera (CANON ZOOM LENS 3.4X): 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.

Contact Angle Measurement on Blood Components

The liquid (diiodomethane) was dropped on the surface of the prepared slides of the HIV infected leucocytes, infected Plasma and uninfected Leucocytes 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. 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).

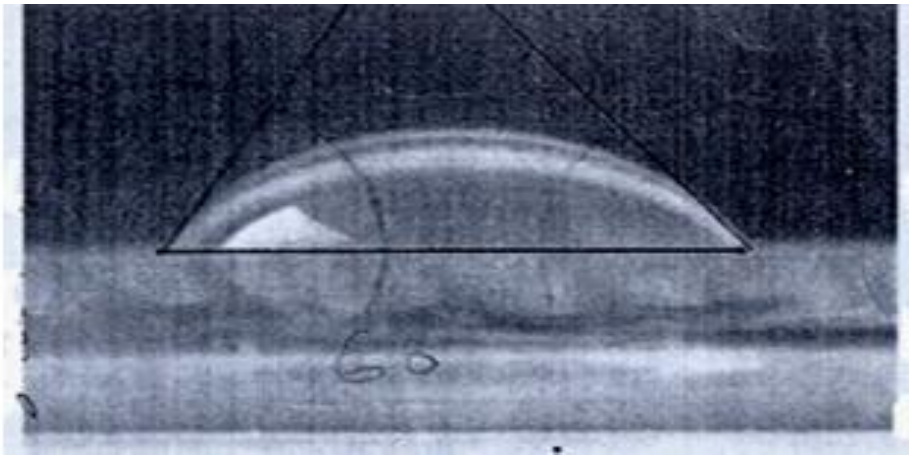

Fig 2: Contact Angle Measurement on Blood Components.

RESULTS AND DISCUSSION

Analyses of CD4 cell count and sessile drop result: The sessile drop method gave the values of the angles θ formed between the HIV and liquid drop whose surface tension is known. The angles with the corresponding CD4+ cell count are listed in table 2. The infected lymphocytes are used to represent the virus. The number of CD4+ count is a measure of immune cells. When the number of counts is very low, there is increase in immune cell depletion due to virus attack. The contact angle is usually high at lower CD4+ for a hydrophobic surface due to poor wetting. In many cases, there is no good relationship between the CD4+ count and contact angle, because other opportunistic infections could as well increase the contact angle. To buttress this point, person moment correlation factor using infected lymphocyte and measured contact angle in table 2a gave 0.114789. This shows that there is no good relationship between the dependent and independent variables. For uninfected lymphocytes in table 2b, the person correlation moment factor statistically gave -0.84475, indicating a very good relationship with low contact angle and increased CD4+ cell count.

Table 2a: CD4+ Cell Count, Contact Angle and Corresponding Hamaker Coefficient.

	HIV			Serum	
Samples S/No	**CD4+ Count (Count/mm^3 of blood)**	**Contact Angle(^{o}C)**	**A132 (mJ/m^2)**	**Con tact Angle(^{o}C)**	**A132 (mJ/m^2)**
1	268	64	2.31E-17	65	2.41E-17
2	625	75	3.27E-17	69	2.76E-17
3	230	69	2.76E-17	70	2.85E-17
4	246	70	2.85E-17	57	1.64E-17
5	339	71	2.93E-17	66	2.50E-17
6	316	66	2.50E-17	60	1.94E-17
7	220	73	3.10E-17	65	2.41E-17
8	374	69	2.76E-17	50	8.58E-18
9	593	65	2.41E-17	60	1.94E-17
10	372	78	3.51E-17	70	2.85E-17
AVERAGE			2.84E-17		2.22E-17

Table 2b: CD4+ Cell Count, Contact Angle and Corresponding Hamaker Coefficient

Lymphocytes		
CD4+ Count (Count/mm^3 of blood)	**Contact Angle θ(^{o}C)**	**A132 (mJ/m^2)**
4000	64	2.31E-17
5000	60	1.94E-17
4000	63	2.22E-17
4000	64	2.31E-17
6000	55	1.43E-17
4400	65	2.41E-17
4700	58	1.74E-17
4800	59	1.84E-17
4300	58	1.74E-17
6000	55	1.43E-17
AVERAGE		1.99E-17

Contact angle, Surface energy, Hamaker coefficient

The energies of interaction represented by absolute Hamaker coefficient are obtained from contact angle data. Tables 2a and 2b show that Hamaker coefficients increases as contact angle increases for infected blood (HIV). The contact angles however decrease for uninfected blood. At high contact angle marked by HIV infection, the surface energy of blood is reduced (Ozoihu, 2014).The reduction in work done on the surfaces in contact (surface energy) is a consequence of HIV attack. Once the surface energy is low with the presence of HIV; the contact angles and the energies of interaction represented by Hamaker coefficient tends to increase (tables 2a and 2b).This result was validated from the work of Achebe[3] who reported that Hamaker coefficients are always positive when HIV attacks the blood cells and penetrate

them. He carried out a spectra analyses of macroscopic properties in Lifshitz theory on HIV infected blood. After integrating within the limits, Hamaker coefficients were found to be higher for HIV infected blood samples when compared to uninfected blood. Increase in Hamaker coefficient is due to HIV infection (table 2a) while decrease in Hamaker coefficient is due to lymphocytes free of HIV infection.

Statically, the mean values of absolute Hamaker constants for ten different samples of HIV infected lymphocytes, infected serum and uninfected lymphocytes are $2.22x10^{-17}mJ/m^2$,$1.94x10^{-17}mJ/m^2$ and $1.99x10^{-17}mJ/m^2$ respectively. The results indicate that absolute Hamaker constant increases with HIV infection. It is evident that energies interact more in non - wetting or hydrophobic surfaces due to decrease in surface area caused by the virus. However, Hamaker constants tends to increase with decrease in surface energy as result of virus attack (fig 3) and table 3 shows that surface energy decreases with increase in contact angle. The persons correlation moment factor between HIV infected blood and surface energy (fig 3) is -0.42 indicating a fairly good relationship. The summary of Absolute values of Hamaker coefficient are summarized as follows.

LYMPHOCYTES: $A_{11} = 0.199 \times 10^{-16}\ mJ/m^2$

HIV: (HIV infected lymphocytes) = $A_{22} = 0.284 \times 10^{-16} mJ/m^2$

SERUM: $A_{33} = 0.222 \times 10^{-16}\ mJ/m^2$.

The absolute values are all positive indicating that attraction occurs between HIV and lymphocytes in serum. Therefore, when the HIV interactions with the blood is positive, there is attraction.

Table 3: Surface energy of HIV infected blood.

Samples S/No	CD4+ Count (Count/mm^3 of blood)	Contact Angle(^{o}C)	Surface energy(mJ/m2)
1	268	64	37.65
2	625	75	28.84
3	230	69	33.58
4	246	70	32.78
5	339	71	31.98
6	316	66	36.02
7	220	73	30.40
8	374	69	33.58
9	593	65	36.83
10	372	78	26.55

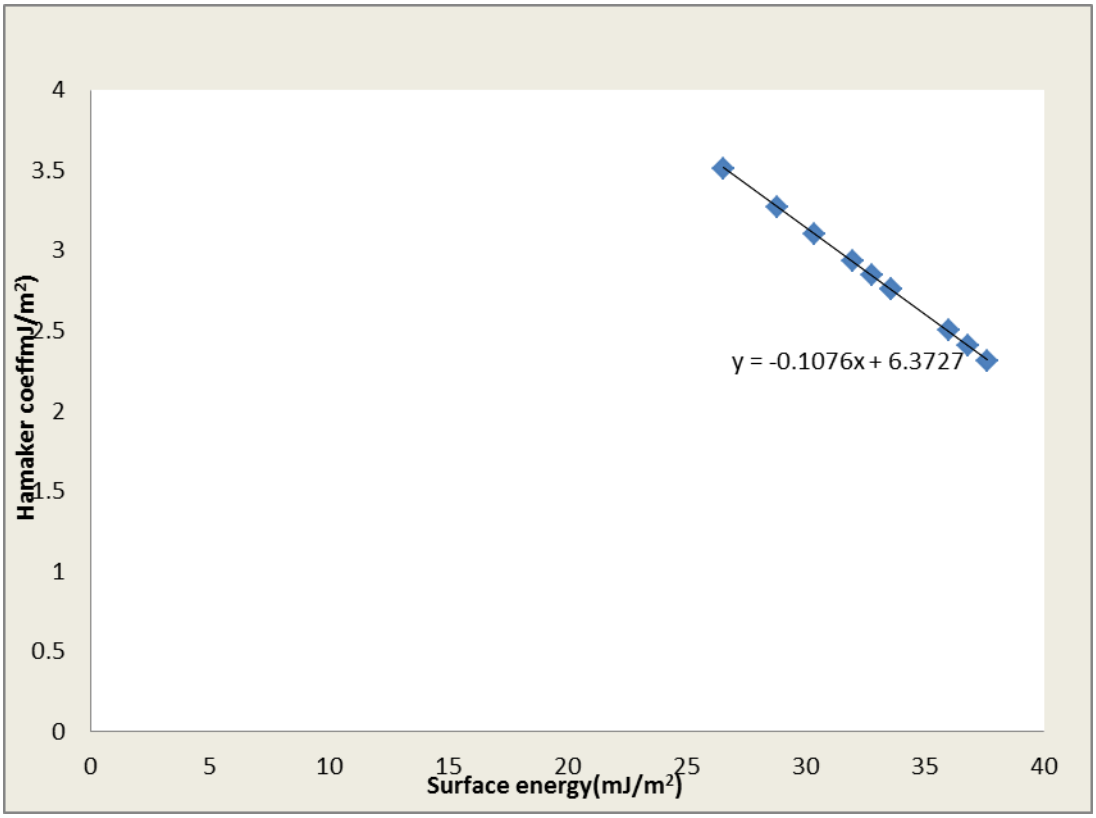

Fig 3: Graph of Hamaker coefficient Versus Surface energy.

The equation of the line

$$y = -0.1076x + 6.3727$$

Hamaker constant (A_{132}) is given

$$A_{132} = 12\pi d_o^2(\Delta F^{adh}) \quad (20)$$

$$\Delta F^{adh} = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \quad (21)$$

$$d_0 = 1.6nm$$

Substituting for y in equation of the line

$$0.96 \times 10^{-18}(\gamma_{PS} - \gamma_{PL} - \gamma_{SL}) = -0.1 \times 10^{-17}\gamma_{SV} + 6.3 \quad (22)$$

$$0.96(\gamma_{PS} - \gamma_{PL} - \gamma_{SL}) = \gamma_{SV} + 6.3 \quad (23)$$

Approximating the value 0.96 to unity, then

$$\gamma_{PS} - \gamma_{PL} - \gamma_{SL} = \gamma_{SV} + 6.3 \quad (24)$$

Recall the principle of geometric mean and collecting like terms

$$\gamma_{ij} = \sqrt{\gamma_{iv}\gamma_{ij}} \quad (26)$$

$$\gamma_{SV} - \sqrt{\gamma_{PV}\gamma_{SV}} + \sqrt{\gamma_{PV}\gamma_{LV}} + \sqrt{\gamma_{SV}\gamma_{LV}} + 6.3 = 0 \quad (27)$$

$$\gamma_{SV} - \sqrt{\gamma}_{SV}(\sqrt{\gamma}_{PV} - \sqrt{\gamma}_{LV}) + \sqrt{\gamma_{PV}\gamma_{LV}} + 6.3 = 0 \quad (28)$$

Simplifying to a quadratic equation and applying the formular;

$$\sqrt{\gamma}_{SV} = \tfrac{1}{2}(\sqrt{\gamma_{PV}} - \sqrt{\gamma}_{LV}) \pm \tfrac{1}{2}\sqrt{(\sqrt{\gamma_{PV}} - \sqrt{\gamma_{LV}})^2 - 4(\sqrt{\gamma_{PV}\gamma_{LV}}) + 6.3} \quad (29)$$

$$\sqrt{\gamma}_{SV} = \tfrac{1}{2}(\sqrt{\gamma_{PV}} - \sqrt{\gamma}_{LV}) + \sqrt{(\sqrt{\gamma_{PV}\gamma_{LV}})^2 - 4(\sqrt{\gamma_{PV}\gamma_{LV}})) + 6.3} \quad (30)$$

Eqn. (29) is a developed model to show the relationships of surface energy with other interfacial energies that constitute the Hamaker concept. However,the model can be used to calculate the surface energy of virus from the Hamaker relation.

Combined Hamaker coefficient and Implication to HIV cure

The absolute Hamaker coefficients for the average of ten samples of HIV infected and uninfected blood are shown in tables 2a and 2b on average scale. A pair-wise summation of the combined absolute values (eqn.9b) is used to predict van der Waals repulsion if the combined value is negative. When attractive van der Waals occur between similar particles interacting in serum; the result is always positive. For instance eqn.7b is used to confirm that the interaction between lymphocytes and serum is positive (A_{131} = 0.625 x 10^{-19} mJ/m^2) while eqn.8b also provides that the interaction between the HIV and serum is attractive (A_{232} = 0.336 x10^{-18} mJ/m^2). The possibility of changing the attractive van der waals to repulsion is the implication to HIV cure and this can be extended to different categories of viral infections. However, for the rentivirus under discussion, eqn.9b is used to predict the possibility of eliminating the virus from the blood since the interactions between the HIV and lymphocytes in serum are found to be negative; $A_{132} = -0.145 \times 10^{-18} mJ/m^2$.The concept of the negative Hamaker Coefficient is an indication that isolation of HIV from the blood is possible (given that $A_{11} < A_{33} < A_{22}$ or $A_{11} > A_{33} > A_{22}$.).This suggests that any additive that will elute or lower the surface energy of the medium A_{33}, such that the value falls in between A_{11} and A_{22} could create a barrier between the virus and lymphocyte.

CONCLUSION

The Hamaker coefficients for HIV infected and uninfected lymphocytes were obtained using the sessile drop techniques. Blood samples were collected from ten HIV infected patients and ten uninfected persons. With four bucket centrifuge, the blood samples were separated into different components. Infected lymphocytes (HIV), plasma and the lymphocytes were

prepared by smearing on glass slides and dried in ventilated room. Diiodomethane was used for sessile drop measurement for contact angle determination.

It was found that HIV infection increased the absolute Hamaker coefficient. The results of positive values obtained for absolute Hamaker Coefficient for HIV (A_{22}) is 0.284 x 10^{-16}mJ/m^2 indicating that the blood samples are infected with the virus. Therefore, attractive van der Waals force prevail during infection. The negative Hamaker coefficient for HIV and lymphocytes interacting in serum A_{132} (-0.145x10^{-18}mJ/m^2) obtained by combining rule indicate that changing van der Waals attraction to repulsion is possible and can be regarded as a traditional means of particle separation. The combining rule was also applied to similar particles interacting in serum. Thus the lymphocytes interacting with serum only A_{131} (0.625 x 10^{-19}mJ/m2) indicates that attractive van der Waals occur between them. Similarly, interaction between HIV and serum A_{232} (0.336 x10^{-19}mJ/m2) is positive indicating attraction.

A functional relationship between the surface energy and the Hamaker coefficient of HIV, lymphocytes and the plasma was derived. This can be used to quantify the degree of HIV infections from the surface constitution.

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