Thermodynamic Parameters: An Alternative Determinant for Viral Infection Diagnosis

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ORIGINAL RESEARCH

Abstract- Viral particles and its mechanism of interaction in contact with human blood cells were studied. Thermo-energetic concept was employed to analyze the evidences such as viral loads,increase in CD4 countcaused bythe administered drugs interfering in the binding process between the virus and blood cells as adopted in the medical field to establish patient's response to drug treatment. Thermodynamic parameters determining the surface properties of the interacting particles were calculated using the Neumann thermodynamic models. The infected blood samples were inoculated with four conventional antiretroviral drugs after centrifuge and serial dilutions in the laboratory. Glycerin was used as the probe liquid and was dropped at the surface of the prepared slides. It was observed that the contacted angles of infected cells (63.4º) were lowered upon the administration of the antiretroviral drugs. Ritonavir reduced the contact angle to 56.6 ±5.25 º,Lamiduvine lowered it to 56.5±3.3º**,**Didanosine gave a contact angle of 56.8±3.03ºwhile Azidothymidine were able to lower the contact angle of infected blood samples to 56.3±4.32º.The surface energy was decreased owing to viral activities in the blood cells from 44.35±1.90 mJ/m^2 for the uninfected blood sample to33.54±2.31 mJ/m^2 when the sample got infected. The administered drugs were able to increase the surface energies of the treated cells from 38mJ/m^2 to 40 mJ/m^{2}as against the surface energy of infected which was between 31 mJ/m^2 to 39 mJ/m^2.Energy of adhesion was increased by the viral-blood interactionsfrom -12.99±1.75 mJ/m^2 for the uninfected blood sample to -23.22±2.22 mJ/m^2 for the infected sample. The treatments given recorded the energy of adhesion to a range from -18.34 mJ/m^2 to -18.75 mJ/m^{2}which still falls a little lower than that of the infected without treatment(-23.222mJ/m^2**)**. The inability of the drugs administered to revert the negative signs of adhesion energy in the treated samples signifies bonding between the virus and the blood cells. Design expert software was employed to generate mathematical model that accurately predict the variables in the interacting medium for the infected blood sample.

Keywords-Blood Cells, Surface Energy, Adhesion Energy, Contact Angle, CD4 Counts, Antiviral Drugs

1 INTRODUCTION

Recent research has shown viral particle interaction with host cell at the first instance of the virus-host encounter is not reliant on the host cells' hospitality towards the virus which creates an enabling environment for cellular binding(Meertens et al.,2006**)**.. The virus-host interaction is a two-way conversation. The virus uses the host cell's signal transduction system to communicate with the cells (Smith and Helenius, 2004**).** These signals, which are often produced at the cell surface, cause alteration to enable entry, get the cells ready for invasion and disable the host defensive system (Lefevre et al.,2014). The viral particles must undergo a multistep entry mechanism that is strictly regulated in space and time (Blanchard et al.,2006)..Viral attachment to cells, internalization and particle fusion with cellular membranes, secretion of genome to the host cytoplasm (Miller et al.,2015). Antiretroviral drug therapy success rates have previously been measured by lots of indicators, notably CD4 cell counts, viral load, and immunological responses. Clinically, when the viral load is reduced within 24 to 48 weeks of antiviral medication, virological response is assumed to be attained (Iweriolor et al., 2018). Throughout this time, the drugs are consistently administered to the infected blood, and an increase in CD4 cell count establishes the immune response. However, the medications only offer a partial cure with side effects, not completely eliminating the virus from the blood stream (Chukwuneke et al.,2016). Due of the shortcomings and failures of these therapies, this research goes beyond the current practice and is centred on the thermodynamic approach to find alternative indicators for determining antiviral treatment success rate. This can be achieved calculating the net van der Waal forces of attraction or repulsion between the viral particles and the drug-coated lymphocytes. Bonding of the blood cells and virus is indicated by negative van der Waal forces of interaction (Achebe et al.,2012).

Combination therapy is the standard treatment in use now with low success records (Shiffman et al.,2007). The mechanisms of actions, the interplay of blood and viral particle interactions, and the surface tensiometry of biological fluid properties are not well characterized in the field of medicine (Iweriolor,2019). These problems need to be solved to ascertain the sustained response rate in infected cells. This necessitated the adoption of surface thermodynamic to ascertain the indices of the success rate for antiviral medications with a view of evolving better treatment options. The mechanism of the viral-host interaction is comparable to particles interactions (Achebe et al., 2013). A thorough understanding of how the surface characteristics of the interacting particles are determined would be useful in ascertaining their interaction processes. Collision of these heterogeneous particles develops a connected contact area. Due to this, the work causes a specific portion of each particle to be displaced. Surface energy is work that causes the displacement of the unit area. This energy is determinable as work done against elastic pressures and plastic resistance of areas in

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contact. Since its difficult in calculating the surface energy of solids directly from contact angle, the Young's equation is modified by combining the interfacial tension with the work of adhesion from the Dupre equation at the liquid interface. Surface thermodynamic effects are the cumulative effects on the surface (Okwuchukwu et al.,2015).

Comprehensive identification of material surfaces is fundamental to both industrialist and academic researchers. Interactions involving lymphocytes and biomaterials were studied using surface wettability. $\boldsymbol{\gamma_l}$ must be bigger than $\boldsymbol{\gamma_s}$ to produce a measureable contact angle. The extent of partial wetting of the soiled by the liquid is quantified by the value of θ. The lower value of θ signifies a better wetting ability and conversely, higher value of θ indicates a poorly wet surface. Surface wetting is favourable when contact angle is less than 90^0($\theta<90^0$) and the fluid spreads. Conversely, surface wetting is unfavourable when the contact angle is greater than 90^0($\theta>90^0$), flow restrictions are established on the surface to form compact liquid droplets. More specifically, a contact angle of zero (θ=0) indicates that the droplets have completely complete wetting and have formed a flat puddle (Yuan and Lee,2013).

$$\gamma_{lv}\cos\theta = \gamma_{sv} - \gamma_{sl} \quad (1)$$

γ_{lv} represent the liquid-vapor interfacial tension,γ_{sv} represent the solid-vapor interfacial tension,γ_{sl}represent the solid-liquid interfacial tension. The numerous shortcomings such as viral resistance to drugs, low virological response rate that characterizes the conventional antiretroviral drugs have trigger this study to ascertain an alternative verification of their efficacy and potency. The spread of infections that are viral, HIV is primarily by blood- to- blood contact associated with intravenous drug users, poorly sterilized medical equipment, needle stick injuries, unprotected sex with infected person and blood transfusion. With the recent technology of blood screening before transfusion, the risk from transfusion is less than one per two million (Maheshwari and Thuluvath,2010).

2 Van Der Waal Interactions in Blood Cells

Hamaker established in his classical works that "if two particles are immersed in a fluid and the London van der waal forces between the particle and the fluid are greater than the particles themselves, it might be thought to cause a repulsion rather than attraction" (Hamaker,1936). London forces are specific and as such the resulting force is typically attractive even when the fluid is enveloping the particle When two bodies interact in a liquid medium, dispersion forces will only induce separation if the attraction of the bodies to the liquid is higher compared to the attraction of the bodies towards one another (Van and Giese,2002).. The net van der Waal interaction between two solids or dissolved liquid molecules is negative, regardless of their electrical neutrality or polarity (Omenyi et al.,1980).. The ability to now convert the attraction between solids dissolved in liquids or the repulsion between dissolved molecules significantly impacts separation techniques. For such bodies in a liquid medium, the Hamaker expression describes their free energy as;

$$\Delta F(d) = -\frac{A_{132}}{12\pi d^2} \quad (2)$$

Where ΔF is the change in the energy of adhesion, A_{132} is the Hamaker coefficient, d is the separation distance apart. Assuming a minimum distance d_o,and equation (2) still valid. Hamaker coefficient can now be expressed as;

$$A_{132} = -12\pi d^2\, \Delta F^{adh}(d) \quad (3)$$

From the foregoing, the Hamaker coefficient can be calculated once the free energy of adhesion for such particular system is known (Neumann *et al*,1974).

$$\Delta F_{132}^{adh} = \gamma_{sv} - \gamma_{sl} - \gamma_{lv} \quad (4)$$

$\gamma_{sv}$is determined from equation (5), $\gamma_{lv}$is surface tension of the probe liquid

$$\text{Cos}\theta = \frac{(0.015\gamma_{sv} - 2.00)(\gamma_{sv}\gamma_{lv})^{1/2} + \gamma_{lv}}{\gamma_{lv}0.0015(\gamma_{sv}\gamma_{lv})^{1/2} - 1} \quad (5)$$

Having obtained γ_{sv} from eq. (5), the corresponding value of γ_{sl} can be calculated from;

$$\gamma_{sv} - \gamma_{sl} = \gamma_{lv}\text{Cos}\theta \quad (6)$$

Adhesion occurs when Eq. (4) is negative and repulsion is predicted when the free energy of adhesion is positive. Experiments were conducted using Nylon, Polysterene, Teflon, silicon glass and acetal particles in biphenyl and naphthalene to verify this thermodynamic prediction (Chukwuneke, 2015). Dupre introduced the work of adhesion w_a and cohesion w_c after the works of young. The equations given by Dupre are used to derive other parameters from the experimental contact angle and surface tension result (Visser,1981).. To obtain a measurable contact angle, γ_{lv} must be greater than γ_{sv}, therefore when $\gamma_{lv}<\gamma_{sv}$ the liquid forms no contact angle on the solid but spread and wet it completely. However, when the liquid-vapor and solid surfaces are in equilibrium, the reduction of the surface free energy of the solid to the vapor adsorption is termed the equilibrium spreading pressure (Good, 1992). Then Eq. (6) becomes:

$$\gamma_{lv}\cos\theta = \gamma_{sv} - \gamma_{sl} + \pi e \quad (7)$$

The spreading pressure S depends on the amount of free energy that is reduced due to adsorption of liquid vapor. The spreading coefficient calculates the variation in surface energy between the dry solid and moist solid covered by microscopic film of liquid (Israelachvili, 2011).

$$S = \gamma_s - (\gamma_{sl} + \gamma_{lv}) \quad (8)$$

$$\text{The spreading work } w_s = \gamma_{lv}(\cos\theta - 1) \quad (9)$$

$$\text{Wetting tension, } T = F/P = \gamma_{lv}\cos\theta \quad (10)$$

3 Materials and Methods

The conventional antiretroviral drugs used for this study are prevalent ones used for HIV treatment obtained from Anambra state University Teaching Hospital, Awka (DHHS,2018). They are Azidothymidine (AZT), Lamivudine, Didanosine (DDI) and Ritonavir (RTV).

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Other materials include ten samples (10ml each) of HIV infected blood, disposable syringes and needles, micropipette, HIV test kits, masking tapes, glycerin solution, test tubes, test tube racks, Prepared slides of infected blood samples as test surface, 5.0μl syringe. The equipment are refrigerator, incubator, autoclave machine, centrifuge machine, Blood roll mixer and Nikkon digital camera, partec cyflow counter machine (NMC,2022).

3.1Clinical Laboratory Procedures

The Ethical clearance used was obtained from the Chukwuemeka Odimegwu Ojukwu University Teaching Hospital. Standards and universal precautions followed in course of this research were in accordance with the Blood borne pathogen standards enacted by the Centre for Disease Control (CDC,2018). Also, standards were equally observed in the collection, transportation, preparation, storage and preservations of the blood specimen used for the experiment. Personal protective equipment (PPE) was equally used while performing the experiment (Forma and Valsamakis,2018). Experiments concerning the blood component separation, inoculation and smearing of the human blood samples were carried out at the laboratory Unit of the Anambra State University Teaching Hospital, Amaku. The Partec Cyflow Counter was cleaned before the experiment by introducing 1600µl each of following solution individually, the cleaning solution, decontamination solution, and health fluid.850μl of count check beads green were used on the machine for quality control.

A sample tube was filled with 20μl of cell differential monoclonal antibody and 20μl of whole blood, which were then mixed together. The mixture was incubated in a dark area at room temperature for 15minutes. 800μl of buffer solution is added to the mixture, which is then gently mixed before the program is started. This was done to identify the CD4+ counts on the infected blood samples. The ten infected blood samples kept below room temperature in an EDTA container was separated into its components using a centrifuge machine. This separated the blood samples in form of plasma and red blood cell at the bottom. 1000 µl of each blood components from their boundary layer were pipette to smear the slides respectively using a spreader bent at 45^o to achieve an even distribution on the slides. The blood samples in the container were placed in a blood roll mixer to unify the components and another 1000 µl whole blood withdrawn to smear the slide. A total of four slides were obtained from each sample and the slides were allowed to dry naturally at room temperature.

3.2 Contact Angle Experimental Procedure

The glycerin used as probe liquid for the study was dropped on the prepared slides surfaces using a microliter syringe. The droplet volume was minute enough to disregard effects of gravity. A camera was used to photograph the propagation, and images were printed. The angle of contact was measured carefully at the solid – vapour, solid- liquid and liquid interface. This was done on all the 400 slides prepared.

3.3 Data Analysis

Matlab computational tool was used to compute for the viral-blood interactions using the angle of contact experimental data, Different energies of interaction was calculated using equation (5). Glycerin was the liquid used with a known surface tension of 63.4 dyne/cm at 20^{o}C(100%glycerol). The energies of adhesion were calculated by employing the Neumann thermodynamic models in equation (4).

4Results and Discussions

4.1 Cd4 Counts and Angles of Contact

Quantity of CD4+ cells reveal the infection severity and the patient's immune depletion rate. Infected samples usually have low CD4 cell numbers. Also observed is a scenario arises where an infected sample can have high value of CD4 count as in table 1, indicating that CD4 counts cannot be a true marker for virological clearance and treatment response as against reports by many researchers in the medical sciences who opined that CD4 counts indicates the infection rate or viral proliferation. This approach has revealed that a patient with an increasing CD4 counts does not mean a reduction in viral replication. There are instances where a patient can have a high CD4 count and still have a higher contact angle as seen in blood sample 10 in table 1. This showcased the relevance of this thermodynamic perspective.

The average angle of contact measured on samples infected (63.4±3.20^o) is greater compared to that of the treated samples, as can be seen by carefully examining table 1. In tadem with the works of Ozoihu(2014) where he reported a value of 63.75±4.09^o for contact angle of infected lymphocytes. A value of 67.3±5^o was reported on the application of the state equation to calculate the contact angle of infected lymphocytes (Adlier et al.,2008). This is because the viral loads act as barriers, encourage surface roughness and heterogeneity, and therefore slow down the spreading capacity of probe liquid. Higher contact angle readings indicate hydrophobicity (Yuan and Lee,2013). In all the treatments administered, a common trend can be observed. They all have the potency required to reduce the infected angles of contact (63.4°). Ritonavir reduced to 56.6° for white blood samples, Lamivudine was able reduce it to 56.5°^{o}, Didanosine to 56.8^{o}and Azidothymidine to 56.3°(Ani,2016).

4.2Energy of Surface Interaction

The work done due to viral-blood interactions is measured by a quantity called surface energy. According to academic data, uninfected cells have surface energies of 40 mJ/m^2 and above while infected cells have surface energies between 31 and39 mJ/m^2. This corresponds with the works of Shang et al(2008) which reported that solids exhibiting lower surface energy have high contact angles as it is in this infected component. From the aforementioned, it follows that the viral presence on the samples causes a reduction on the surface energy. This impairs the body's immune capacity as reported in the works of (ASM, 1980). Eq. (5) was used to compute for Table 2 using MATLAB.

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Table 1. CD4 Counts and contact angles

Samples	$CD4^+$ count (cells/mm³)	Contact Angles Uninfected θ(°C)	Infected θ(°C)	Ritonavir θ(°C)	Lamivudine θ(°C)	Didanosine DDIθ(°C)	Azidothymidine (AZT)θ(°C)
1	428	47	65	54	57	51	56
2	600	46	61	49	55	54	46
3	625	48	63	50	54	57	55
4	312	52	64	60	60	59	60
5	464	50	67	58	58	56	58
6	247	51	66	62	62	58	61
7	852	45	58	57	51	53	57
8	115	49	68	65	59	54	58
9	704	44	52	52	53	53	59
10	798	51	60	59	56	55	53
AVE	514.5	48.5	63.4	56.6	56.5	56.8	56.3
SD	243.1059	2.7508	3.2045	5.253	3.374743	3.02765	4.32178

*AVE is the average *SD is the standard deviation

Table 2. Surface energies (infected and treated cells)

Blood Samples S/N	Uninfected γ_{sv}(mJ/m²)	Infected γ_{sv}(mJ/m²)	Ritonovir Treated γ_{sv}(mJ/m²)	Lamuvidine Treated γ_{sv}(mJ/m²)	Didanosine Treated γ_{sv}(mJ/m²)	Azidothymidine Treated γ_{sv}(mJ/m²)
1	45.27	32.38	40.34	38.18	42.48	38.90
2	45.95	35.27	43.88	39.62	41.77	45.95
3	44.58	33.83	43.18	40.34	3.18	39.62
4	41.77	33.10	36.00	36.00	36.73	36.00
5	43.18	30.95	37.45	37.45	38.90	37.45
6	42.48	31.66	34.55	34.55	37.45	35.28
7	46.63	37.45	38.18	42.48	43.18	38.18
8	43.88	30.23	32.38	36.73	40.34	37.45
9	47.30	34.55	41.77	41.05	41.05	36.73
10	42.48	36.00	36.73	38.90	39.62	41.05
AVE	44.35	33.54	38.45	38.53	39.87	38.66
SD	1.903218	2.313597	3.779195	2.434114	2.28393	3.07714

*AVE is the average *SD is the standard deviation

From Table 2, it is evident that the surface energy is increased upon the administration of drugs. This signifies the viral ability of decreasing energies of interaction. On average consideration, ritanovir treatment increased it from 33.54±2.31mJ/m²to 38.45±3.78mJ/m², Lamiduvine surface energy from 33.54±2.31mJ/m² to 38.53±2.43mJ/m², Didanosine gave value of 39.87±2.28mJ/m² and 38.66±3.08mJ/m² is the value obtained when the infected blood sample was treated with Azidothymidine. Though each treatment has their respective antiviral efficacies and potencies, one would expect the drugs to meet up with the values obtained for the uninfected cells.

4.3 Van der Waal Forces of Adhesion

When the change in adhesion (ΔF^{adh}) is negative, adhesion to cell surfaces is favourable from a thermodynamic perspective. The interaction energy that exists between the interacting particles is the adhesion energy. After interacting with blood cells, virus attaches to the lymphocytes, creating a thermodynamic relationship that may be examined using Van der Waal forces. Thermodynamically, engulfment between the particles is predicated by a sign convention. When a viral particle adheres to lymphocytes, it destabilizes host cell's defensive mechanism thereby increasing adhesion energy. Table 3 was computed using equation (4). Using Neumann model, the adhesion energy of uninfected cells ranges from -10 to -14 mJ/m², while that of infected samples ranges from -22 mJ/m² and above (Ozoihu,2014; Ani,2016; Iweriolor,2020). This is suggestive of the fact the presence of the viral infection causes an increase of energy of adhesion (Iweriolor et al,2018; Okwuchukwu et al,2015; Omenyi et al, 1980; Ani, 2016). In Table 3, the adhesion energy of the infected samples(-23.222mJ/m²) is greater compared to that obtained for the uninfected (-10 to -14 mJ/m²). The 78% increase in adhesion energy is due to the viral presences which causes the immune system to be depleted resulting in low value of CD4 count. The various treatments administered in their respective capacities tried to lower the adhesion energy but still retained the negative sign.

Ritonovir reduced the adhesion energy by 20%, Lamuvidine reduced by 21%, Didanosine gave 19% reduction while Azidothymidine also show a decrease in the adhesion energy by 21%. The negative sign retained after treatment is an indication of the fact that the virus still binding and cannot guarantee virological and immunological clearance. The clinical method will show an increase inCD4count due to medications disrupting the virus's ability to bind to the cells. This energetic concept has been able to establish that virological clearance may not be achieved by virtue of CD4 increments. The negative sign in the energy of adhesion has to change to positive to ensure total repulsion and separation for virological clearance to be established.

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Table 3. Energy of adhesion

Blood Samples S/N	Uninfected F^{adh}(mJ/m²)	Infected F^{adh} (mJ/m²)	Ritonovir Treated F^{adh} (mJ/m²)	Lamuvidine Treated F^{adh} (mJ/m²)	Didanosine Treated F^{adh} (mJ/m²)	Azidothymidine Treated F^{adh} (mJ/m²)
1	-24.34	-24.34	-16.73	-18.78	-14.72	-18.09
2	-21.55	-21.55	-13.41	-17.41	-15.39	-11.50
3	-22.95	-22.95	-14.06	-16.73	-18.78	-17.41
4	-23.64	-23.64	-20.86	-20.86	-20.16	-20.86
5	-25.72	-25.72	-19.47	-19.47	-18.09	-19.47
6	-25.03	-25.03	-22.25	-22.25	-19.47	-21.55
7	-19.47	-19.47	-18.78	-14.72	-14.06	-18.78
8	-26.41	-26.41	-24.34	-20.16	-16.73	-19.47
9	-22.25	-22.25	-15.39	-16.06	-16.06	-20.16
10	-20.86	-20.86	-20.16	-18.09	-17.41	-16.06
AVE	-23.222	-23.222	-18.55	-18.45	-18.75	-18.34
SD	2.225303	2.225303	3.588857	2.31219	2.05280	2.89876

*AVE is the average *SD is standard deviation

4.4 Response Surface for Infected Cells

By design expert, the coefficient of determination (R^2), lack of fit, response plots, and analysis of variance (ANOVA), adhesion energy of interaction obtained using MATLAB computation for the infected cells were examined in order to establish the statistical significance level and generate model equation that would express the relationship between the predicted response and independent variables in coded values.

From Table 4, the quadratic model is suggested and focus is on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared. There is reasonable agreement between the predicted R square value (0.9026)and the adjusted R squared value(0.7836) since their differences is less than 0.2. Hence the quadratic model can be used to navigate the design space and indicates a good measure that outcomes are likely to be predicted.The significance of the model equation representing the energy of adhesion was evaluated by the analysis of variance as can be seen in Table 5. It can be observed that the model F-value of 23.25 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. ANOVA results showed that the model result is statistically significant with p values of < 0.001 as evident in Table 5.

Table 6 shows the coefficients of the quadratic model equation in terms of actual factors which can be used to make predictions about the response for given levels of each variable. Model terms B, AB, A^2 and B^2 are significant model terms since their values are not greater than 0.1000. This means that the model is a good one for predicting surface energy of adhesion in relation with contact angle and interfacial surface energy due to the viral infection. Also from table 6, as a rule, VIFs less than 10 is tolerable. It can be seen that the maximum VIF obtained for all model terms is 1.02.The surface plot 1n Fig.1 is a 3D surface plot which also reveals graphically the trend of interaction between the independent variable and the response variables by visualizing with the color coding. An increase in contact angle causes a decrease in surface energy of interaction leading to a corresponding increase in the surface energy of adhesion.

Energy of adhesion model equation for infected cells is given as

$$F^{adh}=-21.15+0.28A-0.72B-1.07AB-2.15A^2+0.046B^2 \quad (11)$$

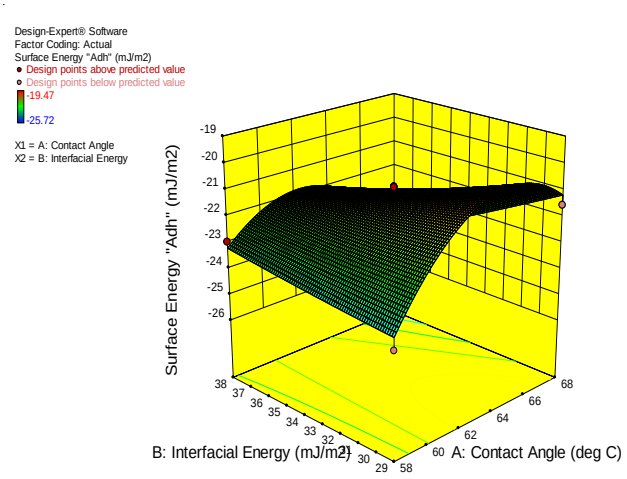

Fig. 1: 3D Surface plot for infected cells

Table 4. Response summary

	Sequential	Lack of Fit	Adjusted	Predicted
Source	p-value	p-value	R-Squared	R-Squared
Linear	0.5637	0.0082	-0.0700	-0.7404
2FI	0.3084	0.0077	-0.0526	-0.7842
Quadratic	< 0.0001	0.5120	0.9026	0.7836
Cubic	0.2903	0.7677	0.9169	0.8932

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Table 5. Analysis of Variance (ANOVA)

Source	Sum of Squares	D*f*	Mean Square	F- Value	p-value (Prob> F)	
Model	42.24	5	8.45	23.25	0.0003	Significant
A-Contact Angle	0.65	1	0.65	1.78	0.2237	
B-Interfacial Energy	4.20	1	4.20	11.57	0.0114	
AB	4.58	1	4.58	12.60	0.0093	
A^2	32.06	1	32.06	88.24	< 0.0001	
B^2	0.014	1	0.014	0.040	0.8474	
Residual	2.54	7	0.36			
Lack of Fit	1.03	3	0.34	0.91	0.5120	not significant
Pure Error	1.51	4	0.38			
Cor Total	44.79	12				

*D*f* is the degree of freedom

Table 6.Adhesion energy model equation

Factor	CoefficientEstimate	D*f*	StandardError	95% CI Low	95% CIHigh	VIF
Intercept	-21.15	1	0.27	-21.79	-20.51	
A-Contact Angle	0.28	1	0.21	-0.22	0.79	1.00
B-Surface Energy	-0.72	1	0.21	-1.23	-0.22	1.00
AB	-1.07	1	0.30	-1.78	-0.36	1.00
A^2	-2.15	1	0.23	-2.69	-1.61	1.02
B^2	0.046	1	0.23	-0.49	0.59	1.02

***A** is contact angle,*B is surface energy,*Df is degree of freedom,*CI is the clearance interval,*VIF is the variance inflation factor.

5 Conclusion

Establishing synergy among engineers and medical professionals for the aim of analysing and utilizing this research's findings has unquestionably been highlighted by this study. This research has demonstrated that some antiviral medications are only effective in boosting the immune systems of infected patients by altering the energies of interaction but they are unable to completely isolate the virus from the host (lymphocytes).

Medically, increasing CD4 count signifies the drugs response to infection. This research has been able to utilize the energetic concepts to establish the fact that immunological response to infection as reveals by the medical evidences is not enough to guarantee virological clearance. The energies of interaction (adhesion) are increased by the viral presences in blood samples. The energy of adhesion of the infected cells (-23.22±2.22mJ/m^2) was observed to be higher than that of the uninfected cells obtained from literature (-12.99mJ/m^2). The retention of the negative value of the treated samples of the infected cells signifies a bonding between the virus causing infection and the blood cells. Thermodynamic analysis stands as a better alternative to immunological and virological marker for determining efficacy and efficiency of antiviral drugs used by infected patients.

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