

**TITLE PAGE**

**HUMAN IMMUNODEFICIENCY VIRUS (HIV)-BLOOD  
INTERACTIONS: CONTACT ANGLE APPROACH**

**BY**

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## **CERTIFICATION**

This research work on Human immunodeficiency virus (HIV)-blood interactions: Contact Angle approach was originally carried out by Engr Ephraim Maduabuchi Ozoihu of the Department of Mechanical Engineering, School of Post-graduate Studies, Nnamdi Azikiwe University, Awka under the supervision of Prof. Sam Nna Omenyi.

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## APPROVAL

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## **DEDICATION**

This Ph.D. dissertation is dedicated to my parents, **Late Chief & Mrs Ozo- Okeke Nnabuenyi Ozoihu** and to all those who taught it wise to encourage my academic pursuit for the benefit of mankind.

## ACKNOWLEDGMENT

I thank the Almighty GOD in a special way for the courage he gave me before embarking in some risky aspect of this work. He protected me against HIV infection throughout the research experiments and inspired courage in me for further research.

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Ozoihu Ephraim Maduabuchi.

## ABSTRACT

Measurement of contact angle and surface tension provides a better understanding of the interaction between the solids and liquids. Such interaction occurs between the HIV and T lymphocytes (CD4+) in liquid serum during infections. In order to study these interactions; the physiochemical properties such as the surface interfacial energies are explained using van der Waals concept of particle interactions as reported in Hamaker's classical papers on separation of particles suspended in a liquid. The concepts of van der Waals forces are useful in predicting attraction or repulsion between the interacting particles (HIV and T lymphocyte). The surface free energies when determined from contact angle data are used to verify among other physiochemical properties, the negative Hamaker coefficient which confirms the possible repulsion between the virus and lymphocytes. The contact angles are measured on HIV infected bloods and uninfected bloods using the three probe liquids (water, glycerine and diiodomethane). The CD4+ cell counts were also measured using Partec flow Cytometry instrument. It was found that the contact angles measured on infected blood are generally higher than uninfected blood and tends to increase with decrease in CD4+ count for infected blood. From the contact angle data, the change in interfacial free energy of adhesion was found to be  $-23.00 \text{ mJ/m}^2$  indicating that van der force is attractive. This means that attraction occurs between HIV and lymphocyte during HIV infection. The absolute Hamaker coefficients for infected T4 cell  $A_{22}$ , was found to be  $0.227 \times 10^{-16} \text{ mJ/m}^2$  also indicating that attraction occurs between HIV and lymphocytes at a low surface energy of about  $31.81 \text{ mJ/m}^2$ . The absolute Hamaker coefficient for uninfected T4 cell  $A_{11}$ , were also obtained from the contact angle data and found to be  $0.176 \times 10^{-16} \text{ mJ/m}^2$ . The positive value of Hamaker coefficient shows that attraction exist between HIV and T4 cells but lower value of  $A_{11}$  indicated less attraction for uninfected T4 cells and hence suggest a zero or negative concept that made repulsion to be attainable. However, the negative concept of combined Hamaker coefficient  $A_{132}$ , was verified using the pair-wise summation of the geometric mean of the absolute Hamaker coefficients for lymphocytes  $A_{11}$ , HIV  $A_{22}$  and Serum  $A_{33}$ . The result  $(-0.6637 \times 10^{-19} \text{ mJ/m}^2)$  indicated that isolation of virus is attainable. In the resent work of Achebe, a negative value  $(-0.2809 \times 10^{-25} \text{ J})$  was obtained to support the claim that the van der Waal force is repulsive during particle separation. Therefore, finding an agent that will reduce the surface tension of serum (such that  $A_{11} > A_{33} > A_{22}$  or  $A_{11} < A_{33} < A_{22}$ ) to obtain a negative value of Hamaker coefficient will be the next step in formulating a drug to be recommended to the pharmaceutical industries for eradication of HIV infection and this calls for further research.

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SYMBOLS	NOMENCLATURE	UNITS
T	Absolute temperature	K
$\alpha$	Absorption coefficient	Dimensionless
$\Delta G^{AB}$	Acid- Base components of Gibb's free energy.	Joules
$\gamma^{AB}$	Acid- Base components of surface tension	J/m <sup>2</sup>
$\Delta F^{AB}$	Acid- Base components of the interfacial energy	mJ/m <sup>2</sup>
$\gamma^{\phi}$	Acid components of surface tension	J/m <sup>2</sup>
A	Area of the surface	m <sup>2</sup>
$\gamma^{\theta}$	Basic components of surface tension	J/m <sup>2</sup>
K	Boltzman's constant	m <sup>2</sup> KgS <sup>-2</sup> K <sup>-1</sup>
$\Delta F^{adh}$	Change in free energy of adhesion	mJ/m <sup>2</sup>
$\mu$	Chemical potential of the liquid.	Dimensionless
$\theta$	Constant angle	Degree
$\rho_L$	Density of liquid	Kgm <sup>-3</sup>
$\rho_p$	Density of particles	Kgm <sup>-3</sup>
$\epsilon_0$	Dielectric constant	Dimensionless
$\epsilon_1 (i\omega_n)$	Dielectric susceptibility	mV <sup>-1</sup>
U	Dipole moment	D
$\gamma_s^d$	Dispersion interfacial energy of solid	J/m <sup>2</sup>
$\gamma_l^d$	Dispersion surface tension of liquid	J/m <sup>2</sup>
r	Distance between the atoms	Nm
$\omega_e$	Electronic excitation frequency	Hertz
$\alpha_e$	Electronic Polarizability	cm <sup>-2</sup> V <sup>-1</sup>
F	Force	N
R	Gas constant	Nm <sup>-2</sup> mol <sup>-1</sup> K <sup>-1</sup>
A <sub>132</sub>	Hamaker coefficient	Joules
CD4+	Immune cell	Counts/mm <sup>3</sup> of blood
$\gamma_{SL}$	Interfacial energy of solid-liquid.	J/m <sup>2</sup>
$\gamma_{SV}$	Interfacial energy of solid-vapour.	J/m <sup>2</sup>
S <sub>LV</sub>	Interfacial entropies of liquid	KJ/Kg <sup>0</sup> K
S <sub>SL</sub>	Interfacial entropies of solid –liquids	KJ/Kg <sup>0</sup> K

$S_{LV}$	Interfacial entropies of solid –vapour	KJ/Kg <sup>0</sup> K
$\Gamma$	Interfacial excess concentration	gdm <sup>-3</sup>
$\gamma^{LW}$	Liftshitz-van der Waal components	J/m <sup>2</sup>
$\Delta G^{LW}$	Liftshitz-van der Waal components of Gibb's free energy	Joules
$\Delta F^{LW}$	Liftshitz-van der Waal components of the interfacial energy	mJ/m <sup>2</sup>
$\beta$	London/van der Waals constant	Dimensionless
$V_A$	Mutual attraction energy	Joules
$q$	Number of atoms	Dimensionless
$h$	Planck's constant	Dimensionless
$\nu$	Poisson's ratio	Dimensionless
$\gamma_s^p$	Polar interfacial energy of solid	J/m <sup>2</sup>
$\gamma_l^p$	Polar surface tension of liquid	J/m <sup>2</sup>
$\alpha(i\omega_n)$	Polarizability	cm <sup>-2</sup> .V <sup>-1</sup>
$P_0$	Pressure (original)	N/m <sup>2</sup>
$F_p$	Pull-of force	N
$R_{CD4+}$	Radius of CD4+	M
$R_{virus}$	Radius of virus particle	M
$\omega_{rot}$	Rotational frequency	Hertz
$C_p$	Specific capacity at constant pressure	JKg <sup>-1</sup> K <sup>-1</sup>
SFE	Surface free energy	mJ/m <sup>2</sup>
$\gamma_{LV}$	Surface tension of liquid –vapour	J/m <sup>2</sup>
$t$	Temperature	K
$P_{vdw}$	Van der waal pressure	N/m <sup>2</sup>
$\lambda$	Wavelength	Hertz
$W^{adh}$	Work of adhesion	Joules
$E$	Young's moduli	N/m <sup>2</sup>
$\xi$	Zeta potential	Milivolt

# CHAPTER ONE

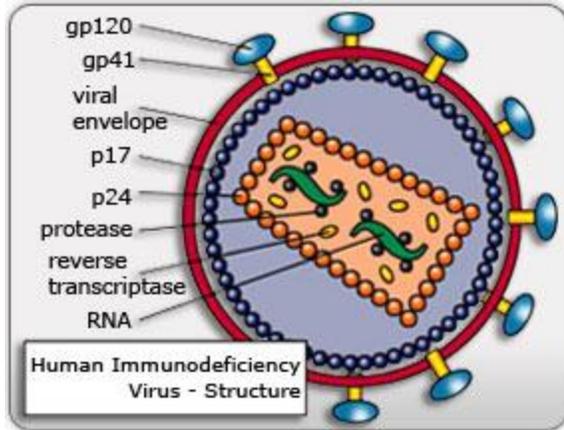
## INTRODUCTION

### 1.1 The Challenges and Rationale:

The early efforts made by the highly active antiretroviral therapy or HAART have reduced the viral burden of those infected with Human immunodeficiency virus (HIV) to very low levels and in many cases delay the progression of HIV diseases for prolong periods. However, antiretroviral regimens have yet to completely and permanently suppress the virus in HIV- infected people. Although, many antiretroviral drugs are being manufactured for the eradication of the HIV infections; but approximately 40,000 new HIV infections occur each year in the United States according to the Joint United Nations Programmes [1]. Recent studies show that, in some chronic HIV infection that over ten billion of new viral particles can be generated each day because of the mutation by RNA during replication. Field [2] concluded that the clinical phase can last for many years, ultimately leading to destruction of the host immune system due to chronic activation or viral replication. It therefore, becomes necessary that alternative measures be taken since the virus even resist attack from certain drugs. In their study of a case realization and the nature of drug resistance encountered by the virus, Omenyi, et al [3] started addressing the conditions under which the HIV in the blood could be rendered impotent. The possible alternative that existed was from the surface thermodynamic point of view. The question was that; could there be an additive in the 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; therefore preventing attack of the lymphocytes by the HIV virus [4].

### 1.2 Background of the study:

HIV exists as a spherical particle (Fig 1.1) called virions [5] with a diameter of about 0.1microns and one seventieth of the diameter of a human CD4+ white blood cells. It is made up of the outer core; the viral envelope (or membrane) and the inner core; the viral core.



**Fig.1 1: Human Immunodeficiency Virus(HIV) Anatomy.**

HIV infects vital cells in the immune system such as helper T- cells(T-lymphocytes) specifically the cell that carries on its surface a special protein molecule called cluster designated 4,CD4+. Other cells in the immune system that are infected are macrophages and dendritic cells. HIV infections lead to low level of CD4+ T-cells through a number of mechanisms including apoptosis of uninfected bystanders cells, direct viral killing of infected cells and killing of infected CD4+ T-cells by CD8 cytotoxic lymphocytes that recognizes infected cells. The HIV protein protrudes through the surface of the viral particles. This protein, known as env consists of a cap made of three molecules called glycoprotein (gp) 120 and the stem consisting of three gp41 molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach and fuse with target cell to initiate the infection cycle. This is done when spikes on the surface of the HIV envelope proteins stick with the CD4+ molecules on the target cells. The interaction that occurs between gp120 and CD4+ and the conformational change in gp120 allows for secondary interaction with a co-receptors, CCR5. This process allows the distal tips of gp41 to be inserted in the cell membrane and forming coiled- coils as a result of conformational change in gp41.This process pulls the viral and cellular membrane together and fuse them. The mechanism can be regarded as a sort of protein-protein interaction and being like molecules, mutual attraction occurs, though with the help of chemokine receptors. The receptors work with the help of co-receptors CCR5 and CCR4 for viral entry into the cell membrane. Thus, the entry of HIV to CD4+ membrane

can be regarded as adsorption of glycoprotein on its surface to receptors on the target cells, followed by fusion of the viral envelope with the cell membrane.

This attraction could be associated with van der Waals forces and can be explained in terms of London/van der Waals interaction energy. To predict this interactions, the surface properties of the interacting particles must be known. Thus, the major driving force behind the protein adsorption is the surface energy. The surface free energy of a liquid is a measure of its surface tension while the surface free energy of solid can be revealed by contact angle measurement and because the interaction involves two surfaces (HIV and CD4+(lymphocytes); it can be viewed as surface effect. From a purely surface thermodynamic consideration; experimental conditions have been predicted under which the effective Hamaker coefficient  $A_{132}$  (of a system comprising two different materials 1 and 2, immersed in a liquid 3) acquire a negative value. This realization has opened up a number of novelty separation techniques. It has been long surmised, as a theoretical curiosity, that conditions could arise under which the signs of the van der Waals interaction between two different bodies, surrounded by a liquid might be negative, that is such bodies would repel each other. Therefore, isolation of HIV from lymphocytes is synonymous to this techniques since HIV interacts with lymphocytes and suspended in serum as the intervening medium. This means that separation can be made possible if the combined Hamaker coefficient of HIV- blood (lymphocytes) interaction in serum is found to be negative, so that a van der Waals repulsion prevails. To verify this, various surface thermodynamic treatments of particles engulfment and rejection studies at solid interfaces have been published. A negative Hamaker coefficient  $A_{132}$  for the interaction of two polymer 1 and 2 in the solvent 3 implies repulsion between the two polymer molecules while  $A_{131}$  and  $A_{232}$  are always positive since like molecules will attract each other. Thus, a negative Hamaker coefficient  $A_{132}$ , favours phase separation. Understanding this phenomena has been recently enhance by the application of Lifshitz theory which Achebe[4] in 2010 used to verify the concept of negative Hamaker coefficient by integration of macroscopic observations of HIV infected and uninfected blood samples.

Another approach yet to be considered in this work is the contact angle approach. That is measuring the contact angle at the interface where HIV and T-cells interact in a liquid (serum). Then applying the results to Young's equation enables the determination of surface interfacial free energies of adhesion and hence the Hamaker coefficient  $A_{132}$  of the two particles (HIV and lymphocytes) in serum.

### **1.3 Immune System cell loss in HIV infection:**

Recent data suggest that billions of CD4+ T cell may be destroyed every day, eventually overwhelming the immune system's regenerative capacity. This can occur in the following ways:

**Direct cell killing:** Infected CD4 + T Cells may be killed directly when large amounts of virus are produced and bud off from the cell surface, disrupting the cell membrane, or when viral proteins and nucleic acids collect inside the cell, interfering with cellular machinery.

**Apoptosis:** Infected CD4 + T cell may be killed when the regulation of cell function is destroyed by HIV proteins, probably leading to cell suicide by a process known as programmed cell death or apoptosis. Recent reports indicate that apoptosis occurs to a large extent in HIV – infected individuals, both in the blood stream and lymph nodes. Uninfected cells also may undergo apoptosis when the HIV envelope bound to antibodies and sends an inappropriate signal to CD4 + T cells causing them to undergo apoptosis, even if not infected by HIV.

**Innocent bystanders:** Here, HIV particles may bind to the cell surface, giving them the appearance of an infected cell and making them for destruction by killer T cells after antibody attaches to the viral particle on the cell. This process is called antibody dependent Cytotoxicity. Killer T cells also may mistakenly destroy uninfected cells that have consumed the HIV particles and display HIV fragments on their surfaces.

**Damage to precursor Cells:** Studies suggest that HIV also destroys precursor cells that mature to have special immune functions, as well as the microenvironment of the bone marrow and the thymus needed for the development of such cells. These organs probably lose the ability to regenerate, further compounding the suppression of the immune system.

**Anergy:** The CD4+ T cells can be turned off by activation signals from HIV that leaves them unable to respond to further immune stimulation.

#### **1.4 Role of Immune Activation in HIV Disease.**

Unlike adaptive immunity, innate immunity does not recognize every possible antigen. Instead, it is designed to recognize molecules shared by groups of related microbes that are essential for the survival of those organisms and are not found associated with mammalian cells. Most body defense cells have pattern-recognition receptors for these common PAMPS and so there is an immediate response against the invading microorganism. Pathogen-associated molecular patterns can also be recognized by a series of soluble pattern-recognition receptors in the blood that function as opsonins and initiate the complement pathways. In all, the innate immune system is thought to recognize approximately  $10^3$  of these microbial molecular patterns.

**The innate immune responses involve the following:**

- phagocytic cells (neutrophils, monocytes, and macrophages);
- cells that release inflammatory mediators (basophils, mast cells, and eosinophils);
- natural killer cells (NK cells); and
- molecules such as complement proteins, acute phase proteins, and cytokines.

#### **1.5 Mechanism of Phagocytosis**

After infection the number of white blood cells increase at the initial phase. This stage, they are phagocytic in nature. By ingesting microbial pathogens, phagocytic leucocytes accomplish two immune functions. Firstly, they initiate a microbial death pathway. They target ingested pathogen to degradative organelles, such as lysosomes and to vesicles containing components of the phagocyte oxidase complex. Secondly, phagocytic leucocytes, particularly dendritic cells (DCs), utilize phagocytosis to direct antigens to both MHC I and II compartments. Thus, phagocytosis serves a dual role as an effector of innate and an initiator of acquired immunity. The steps involved in phagocytosis are,

## **Activation of the Phagocyte**

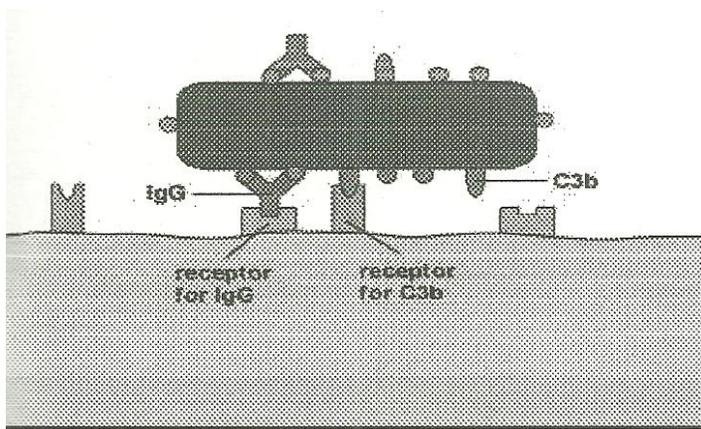
Resting phagocytes are activated by inflammatory mediators such as bacterial products, complement proteins, inflammatory cytokines and prostaglandins. As a result, the circulating phagocytes produce surface glycoprotein receptors that increase their ability to adhere to the inner surface of capillary walls, enabling them to squeeze out of the capillary and be attracted to the site of infection. In addition, they produce endocytic pattern-recognition receptors that recognize and bind to pathogen-associated molecular patterns or PAMPs - components of common microbial molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, and mannose that are not found in human cells - to attach the microbe to the phagocyte for what is called attachment (Fig 1.2). They also exhibit increased metabolic and microbicidal activity by increasing their production of ATPs, lysosomal enzymes, lethal oxidants, etc.

## **Chemotaxis of Phagocytes:**

It is a phenomenon of chemical attraction of phagocytes to microorganism. The chemotaxis chemicals which attract the phagocytes are the components of bacterial factors (bacterial proteins, capsules, LPS, peptidoglycan, teichoic acids, etc.), complement proteins (C5a), chemokines (chemotactic cytokines such as interleukin-8 secreted by various cells), fibrin split products, kinins, and phospholipids released by injured host cells. Some microbes, such as the influenza A viruses, Mycobacterium tuberculosis, blood invasive strains of Neisseria gonorrhoeae, and Bordetella pertussis have been shown to block chemotaxis.

## **Attachment of the Phagocyte to the Microbe or Cell**

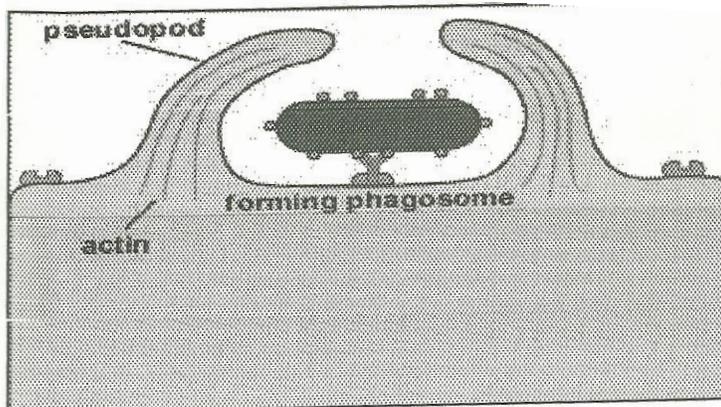
Attachment of microorganisms is necessary for ingestion. The plasma membrane of the phagocyte gets attached to the surface of a microbe. If the cell wall of the microorganisms is coated with certain plasma protein promoting the attachment of microbe to the phagocyte, only then they can phagocytize. The coat proteins are called opsonins and the process is called opsonization.



**Fig1. 2: Attachment of phagocyte to microbe.**

### **Ingestion of the Microbe or Cell by the Phagocyte**

After attachment, the plasma membrane of phagocytes extends short projections known as pseudopods which engulf the microorganisms or foreign materials. The process is known as ingestion (fig1.3).



**Fig1.3 Ingestion and phagosome formation**

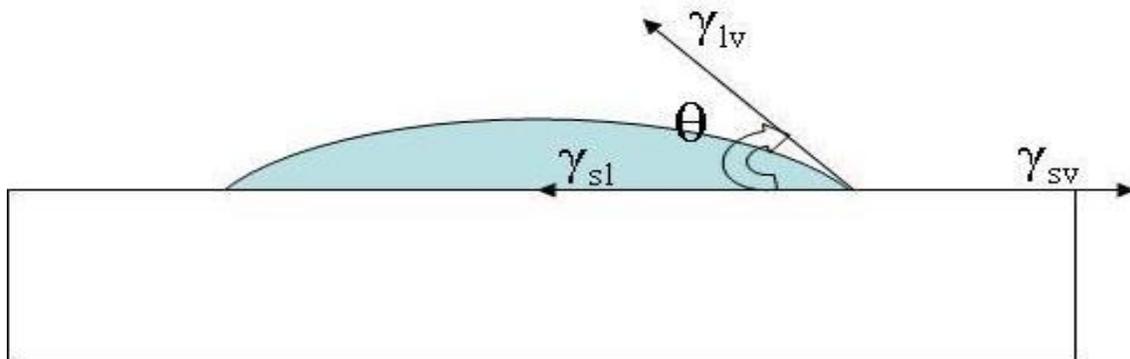
During this process, an electron pump brings protons ( $H^+$ ) into the phagosome. This lowers the pH within the phagosome so that when a lysosome fuses with the phagosome, the pH is correct for the acid hydrolases to effectively break down cellular proteins.

## Digestion.

After engulfment, phagocyte comes in the contact of lysosome that contains the digestive enzymes and bactericidal chemicals. After making contact, the membrane of the phagosome and lysosome gets fused and a single layered structure is formed which is called phagolysosome. Within 10-30 minutes the contents of phagolysosomes degrade the microorganism or foreign materials.

### 1.6: Concept of Contact Angle

Sequel to the interactions that occur between HIV and lymphocytes; the interaction energies expressed as Hamaker coefficient is quantified through the contact angle approach. Contact angle,  $\theta$  is a quantitative measure used to study the energetic of the surfaces; significantly 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 a liquid, gas and solid intersect. Low values of  $\theta$  indicate that the liquid spreads, or wets well, while high values indicate poor wetting. A zero contact angle represents complete wetting indicating that the surface has high energy. A drop with a large contact angle is hydrophobic and a very low surface energy.



**Fig 1.4: Contact Angle Profile Using Probe Liquid.**

Contact angle can also be considered in terms of the thermodynamics of the materials involved. This analysis involves the interfacial free energies between the three phases and is given by Young's equation.

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cos\theta \quad (1.1)$$

Where  $\gamma_{LV}$ ,  $\gamma_{SV}$  and  $\gamma_{SL}$  refer to the interfacial energies of the liquid/vapour, solid/vapour and solid/liquid interfaces.

For any given solid/ liquid interaction there exists a range of contact angles which may be found. The value of static contact angles are found to depend on the recent history of the interaction. When the drop has recently expanded the angle is said to represent the ‘advanced’ contact angle. When the drop has recently contracted the angle is said to represent the ‘receded’ contact angle. These angles fall within a range with advanced angles approaching a maximum value and receded angles approaching a minimum value.

If the three phase (liquid/solid/vapour) boundary is in actual motion the angles produced are called Dynamic Contact Angles and are referred to as ‘advancing’ and ‘receding’ angles. The difference between the maximum (advanced/advancing) and minimum (receded/receding) contact angle values is called the contact angle hysteresis. A great deal of research has gone into analysis of the significance of hysteresis. It has been used to help characterize surface heterogeneity, roughness and mobility. For situations in which surface roughness generates hysteresis the actual microscopic variations of slope in the surface create the barriers which pin the motion of the contact line and alter the macroscopic contact angles. There has been a great deal of research investigating the significance of hysteresis and utilization of contact angle data.

The primary focus of contact angle studies is in assessing the wetting characteristics of solid/liquid interactions. Other experimental parameters may be derived directly from contact angle and surface tension results. These includes;

**Work of Adhesion.** This is the work required to separate the liquid and solid phases, or the negative free energy associated with the adhesion of the solid and liquid phases. It is given by the Young – Dupre equation as;

$$W_a = \gamma_{LV} (1 + \cos\theta) \quad (1.2)$$

**Work of cohesion:** This is the work required to separate a liquid into two parts. It is a measure of the strength of molecular interactions within the liquid. It is given by.

$$W_c = 2\gamma \quad (1.3)$$

**Work of Spreading:** This is the negative free energy associated with spreading liquid over solid surface. This is also referred to as spreading coefficient and is given as

$$W_s = \gamma_{LV}(\cos\theta - 1) \quad (1.4)$$

**Wetting Tension:** This is a measurement of force/length defined as

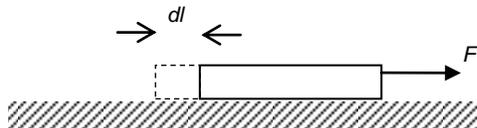
$$T = F_w/P = \gamma_{LV}(\cos\theta) \quad (1.5)$$

The value, the wetting force normalized for length, also represents the product of the cosine of the contact angle and the surface tension. It is also referred to as Adhesion or Work of Wetting.

### 1.7: Concept Surface Energy

Contact angles measured above are the most practical way to characterize surface energies of solids. The surface energy across an interface or the surface tension at the interface is a measure of the energy required to form a unit area of new surface at the interface, and the intermolecular bonds or cohesive forces between the molecules of the liquid.

$$dw = \gamma dl = \gamma dA. \quad (1.6)$$



**Fig 1.5: Schematic Diagram Showing Application of a Force on a Surface**

The surface energy across a liquid-vapour interface is called the surface tension and written as  $\gamma_{LV}$ . This is actually well defined and measured as found in many literature. Similar term for solid –vapour can be defined and written as,  $\gamma_{SV}$  (Fig 1.5). However,  $\gamma_{SV}$  cannot be measured. The physical reason for this is that a solid will not deform the way a liquid will; more specifically, a solid will support a shear stress. Young’s equation (Eq. 1.1) describes the balance forces at the liquid-vapour-solid three –phase line (the “edge”). Now  $\theta$  can be measured  $\gamma_{LV}$  can be looked up for. The difference,  $\gamma_{SV} - \gamma_{SL}$  can be calculated from what is measured on the left hand side of Eqn (1.1). In

essence,  $\gamma_{SV}$  hides behind  $\gamma_{SL}$  which is the interfacial tension between the liquid and solid. Since, in general, the individual values of  $\gamma_{SV}$  and  $\gamma_{SL}$  need to be known; another equation is needed. There are models which provide approximate answers by giving another equation with which to separate  $\gamma_{SV}$  and  $\gamma_{SL}$ . It is very important to understand that these are, indeed, very approximate. The models are based on independent knowledge of how liquids adhere to one another. For a well-known, well-characterized surfaces, there can a 25% difference in the answers provided by each model.

The numerous interfacial tension equations that are found in the literature are divided into three groups; the empirical approach (zisman model), equation of state approach (Good & Girifalco model), surface tension components approach (Fowkes, Owens & Wendt, Wu model).

### 1.7.1: Empirical Approach

#### Zisman Model:

This method is used to determine the so-called critical surface free energy  $\gamma_c$  that differs from the quantity  $\gamma_S$ . The value of  $\gamma_c$  of a solid is equal to the value of  $\gamma_{LV}$ , of the liquid being in contact with the solid for which the contact angle is zero. From contact angle measurement of homologous series of simple molecular liquids on low-energy solid surfaces, Zisman gave a phenomenological relation,

$$\cos \theta = 1 + b(\gamma_c - \gamma_L) \quad (\text{for wetting region}), \quad (1.7)$$

where  $b$  and  $\gamma_c$  are constants depending on the solid and liquid series. Combining Eq(1.7) with Eq(1.26) and making relevant transformations, one may obtain a relationship between  $\gamma_c$ ,  $\gamma_S$  of the studied solid.

$$\gamma_S = (b\gamma_c + 1)^2/4b \quad (1.8)$$

The way of deriving Eq.( 1.7) indicates that another solution is possible:

$\gamma_S = \gamma_c$  and Experimental studies by Zisman have shown that the  $\gamma_c$  cosine of the angle  $\theta$  of a simple molecular solid surface which satisfied the condition,  $\cos\theta = 1$ , ie

$$F(\gamma_c) = \cos\theta = 1. (\theta = 0) \quad (1.9)$$

### 1.7.2: Equations of States

The existence of an equation of state was demonstrated with the assumption that solid – liquid tension is the parameter whose value depends on the properties of the solid and the measuring liquid (Table 1.2). This is reflected in the so- called equation of state:

$$F(\gamma_{SV} \gamma_{LV} \gamma_{SL})=0 \quad (1.10a)$$

Or

$$\gamma_{SL} = f(\gamma_{SV} \gamma_{LV})$$

(1.10b)

Berthelot initiated this direction of studies and assumed that the interfacial adhesion work ( $W_{SL}$ ) was equal to the geometric mean of the cohesion work of a solid ( $W_{SS}$ ) and the cohesion work of a measuring liquid  $W_{ll}$

$$W_{SL} = (W_{SS} W_{ll})^{0.5} \quad (1.11)$$

Then, using the relation

$$W_{SS} = 2\gamma_{SV}, W_{ll} = 2\gamma_{LV}$$

And the Dupre equation:

$$W_{SL} = \gamma_{SV} + \gamma_{LV} - \gamma_{SL} \quad (1.12)$$

Berthelot formulated a hypothesis in the form of the following equation

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV} \gamma_{LV})^{0.5} \quad (1.13)$$

Independently of Berthelot, Antonow attempted to determine  $\gamma_S$  and presented the following formula;

$$\gamma_{SL} = | \gamma_L - \gamma_S | \quad (1.14)$$

### L.A .Girifalco and R.J.Good Model

This model is the earliest combining rule and the most used in manufacturing. The combining rule is an equation that tells more about the interfacial tension across the interface in terms of the original substance-vapour surface tensions of the materials forming the interface. The combining rule equation is related to Young's equation with the following formula,

$$(1 + \cos\theta)\gamma_{LV} = 2(\gamma_{SV}\gamma_{LV})^{0.5} - \pi_e \quad (1.15)$$

$\pi_e$  is the vapour pressure ( $\approx 0$ ).

Girifalco and Good attempted to formulate the equation of state well. They introduced the parameter  $\Phi$ , characterizing the interfacial energy as follows:

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2\Phi(\gamma_{SV}\gamma_{LV})^{0.5} \quad (1.16)$$

In case of an interfacial system, in both of which interactions of the same type occur,  $\Phi = 1$ , was assumed

### **A.W. Neumann model:**

The combining rule for Neumann's geometric mean to Young's equation are stated thus;

$$(1 + \cos\theta)\gamma_{LV} = 2\sqrt{\gamma_{SV}\gamma_{LV}} \exp\left(-\beta(\gamma_{LV} - \gamma_{SV}^2)\right) \quad (1.17)$$

Neumann et al [6] derived three other form of the equation of state; the first one was obtained from the fundamental thermodynamic relations concerning the intermolecular interactions:

$$\gamma_{SL} = \frac{\{(\gamma_{SV})^{0.5} - (\gamma_{LV})^{0.5}\}}{\{1 - 0.015(\gamma_{SV}\gamma_{LV})^{0.5}\}} \quad (1.18)$$

The second one was a modified Berthelot hypothesis expressed by,

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV}\gamma_{LV})^{0.5} \exp\{1 - \beta_1(\gamma_{LV}\gamma_{SV})\} \quad (1.17)$$

The third one was a further modification of the Berthelot hypothesis

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV}\gamma_{LV})^{0.5} \exp\{1 - \beta_2(\gamma_{LV} - \gamma_{SV})^2\} \quad (1.18)$$

The coefficients  $\beta_1 = 0.0001247$  and  $\beta_2 = 0.0001057$  have been determined experimentally. They measured the contact angle for several standard solids (fluorocarbon – covered mica, fluorinated copolymer of polyethylene, poly (ethylene terephthalate) and for various measuring liquids. Then, some iterative procedures, adjusted to the automated axisymmetric drop shape analysis (ADSA) were used.

### 1.7.3: Surface tension components approach:

Fowkes was a pioneer of such an approach. He assumed that the surface free energy of a solid (and of a liquid) is a sum of independent components of surface tensions associated with specific interactions:

$$\gamma_s = \gamma_s^d + \gamma_s^p + \gamma_s^h + \gamma_s^i + \gamma_s^{ab} + \gamma_s^0 \quad (1.19)$$

where,  $\gamma_s^d, \gamma_s^p, \gamma_s^h, \gamma_s^i$  and  $\gamma_s^{ab}$  are the dispersion, polar, hydrogen (related to hydrogen bonds), induction, and acid-base components respectively, while  $\gamma_s^0$  refers to the remaining interactions.

Fowkes investigated mainly two- phase system containing a substance (solid or liquid) with the dispersion interaction only.

$$\gamma_{SL} = \gamma_s + \gamma_L - 2\left(\gamma_s^d \gamma_L^d\right)^{0.5} \quad (1.20)$$

Eq.(3.20) is of the form of Berthelot hypothesis limited to the interfacial London interactions.

Zettlemoyer modified Eq.(3.20) by replacing the geometric mean of the interfacial interaction with the arithmetic one and obtained the following relationship:

$$\gamma_{SL} = \gamma_s + \gamma_L - 2\left(\gamma_s^d + \gamma_L^d\right) \quad (1.21)$$

Owens and Wendt significantly changed the Fowkes idea while the sum of all the components occurring on the right hand side of Eq.(1.19) except  $\gamma_s^d$ , can be considered as associated with the polar interactions  $\gamma_s^p$ . Consequently, the following equation was obtained;

$$\gamma_{SL} = \gamma_s + \gamma_L - 2\left(\gamma_s^d \gamma_L^d\right)^{0.5} - 2\left(\gamma_s^p \gamma_L^p\right)^{0.5} \quad (1.22)$$

#### S.Wu Model:

**Wu** accepted the idea by Owens and Wendt to divide the surface into two parts, but used the harmonic means of the interfacial interactions instead of the geometric ones (table 1.1) in Eq (1.22) and derived Eq.(1.23)

$$\gamma_{SL} = \gamma_{sv} + \gamma_{lv} - 4\left\{\gamma_s^d \gamma_L^d / \left(\gamma_s^d + \gamma_L^d\right) + \gamma_s^p \gamma_L^p / \left(\gamma_s^p + \gamma_L^p\right)\right\} \quad (1.23)$$

In spite of only slight differences between the values of  $\gamma_{SL}$  calculated with Eq. (1.22) and Eq.(1.23) the approach by Wu has not been widely used in the studies on the wettability and surface of polymeric materials.

The latest idea came from van Oss, Chaudhury, and Good. They divided  $\gamma_{sl}$  into two components: the Lifshitz-van der Waals components  $\gamma^{LW}$  and acid-base component  $\gamma^{AB}$ . The later components is considered to be equal  $2(\gamma^+\gamma^-)^{0.5}$  where  $\gamma^+$  and  $\gamma^-$  mean the acidic and basic constituents. As a result Eq(1.24) was formulated.

$$\gamma_{sl} = \left\{ \left( \gamma_S^{LW} \right)^{0.5} - \left( \gamma_L^{LW} \right)^{0.5} \right\}^2 + 2 \left\{ \left( \gamma_S^+ \right)^{0.5} - \left( \gamma_L^+ \right)^{0.5} \right\} \left\{ \left( \gamma_S^- \right)^{0.5} - \left( \gamma_L^- \right)^{0.5} \right\} \quad (1.24)$$

### Surface Free Energy Determination Based on Partitioned Surface Tension.

The methods used by different models stem from the modified Young's equation.

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos\theta \quad (1.25)$$

Or

$$\gamma_{SL} = \gamma_{SV} - \gamma_{LV} \cos\theta \quad (1.26)$$

Where  $\gamma_S$  is the SFE of a solid,  $\gamma_{SL}$  is the SFE corresponding to the solid-liquid interface,  $\gamma_L$  is the SFE of a measuring liquid, and  $\theta$  is the contact angle between the solid and the measuring liquid. For notational compactness,  $\gamma_{SV} = \gamma_S$  and  $\gamma_{LV} = \gamma_L$ .

### F.M. Fowkes Model

**Fowkes** relates the Young's equation to Eq.(1.21) for the calculation of SFE of non - polar solid ie, the solid for which  $\gamma_S = \gamma_S^d$  is valid.

$$\gamma_{sv} = \gamma_s^d = \gamma_S^2 (1 + \cos\theta)^2 / (4\gamma_L^d) \quad (1.27)$$

If the measuring liquid is characterized by the dispersion interaction only, then,

$\gamma_L = \gamma_L^d$  and Eq.(1.21) yields,

$$\gamma_{sv} = \gamma_s^d = 0.25 \gamma_{LV} (1 + \cos\theta)^2 \quad (1.28)$$

In general, Eq.(1.28) is widely accepted for the calculation of interfacial free energy of surfaces.

After introducing this value of  $\gamma_S^d$  to Eq.( 1.28) the relation can be transformed into,

$$\gamma_L^d = \gamma_L^2 (1 + \cos \theta)^2 / 72 \quad (1.29)$$

$\gamma_{LV}$  varies from  $-\gamma_{LV}$  (say, -72) for non-wetting surfaces to  $+\gamma_{LV}$  (+72) for full wetting surfaces.

Fowkes method can also be applied to determine  $\gamma_S$  of any solid. In this case the quantities  $\gamma_S = \gamma_S^d + \gamma_S^p$  and  $\gamma_L = \gamma_L^d + \gamma_L^p$  are assumed to be valid using Eq(1.21, 1.27 and 1.29).

Using equation 1.23 and the determined values of  $\gamma_S^d$  and  $\theta_p$ , the quantity  $\gamma_S^p$  can be determined from the following formula:

$$\gamma_S^p = \frac{\left\{ 0.5\gamma_{LV}(1 + \cos \theta) - (\gamma_S^d \gamma_L^d)^{0.5} \right\}}{\gamma_L^p} \quad (1.30)$$

When applying the Fowkes method, it is recommended to use water and diiodomethane as the measuring liquids:

### **M.J. Owens -- R.C.Wendt Model**

In the **Owens- Wendt** method, an assumption similar to those in the Fowkes method is made. The two methods, being identical in the mathematical aspect, but differ slightly in the way of calculating the SFE. The combination of Eq.(1.23& 1.27) leads to the following relationship;

$$\left( \gamma_S^d \gamma_L^d \right)^{0.5} + \left( \gamma_S^p \gamma_L^p \right)^{0.5} = 0.5\gamma_{LV}(1 + \cos \theta) \quad (1.31)$$

There are two unknowns in Eq(1.31) which are insufficient to determine the SFE of a material. Thus, the contact angle has to be measured using two measuring liquids which would yield two equations of the form,

$$x + ay = b(1 + \cos \theta_1) \quad (1.32)$$

$$x + cy = d(1 + \cos \theta_2) \quad (1.33)$$

where  $x = (\gamma_S^d)^{0.5}$ ,  $y = (\gamma_S^p)^{0.5}$ ,  $\theta_1$  and  $\theta_2$  are the contact angle values for the two measuring liquids, and  $a, b, c, d$  are the coefficients dependent on the kinds of these liquids.

**Van Oss – Chaudhury –Good model:**

Taking into account that the component  $\gamma^{AB}$  is equal  $2(\gamma^+\gamma^-)^{0.5}$  and combining Eq.(1.12 and 1.14),van Oss,Chaudhury, and Good obtained the following relationship;

$$\left(\gamma_{SV}^{LW} \gamma_{LV}^{LW}\right)^{0.5} + \left(\gamma_S^+ \gamma_L^-\right)^{0.5} + \left(\gamma_S^- \gamma_L^+\right)^{0.5} = 0.5(1 + \cos\theta) \quad (1.34).$$

Since three unknowns,  $\gamma_S^{LW}, \gamma_S^+, \gamma_S^-$  appeared in Eq.(1.34), the solution of a system of three independent linear equations is needed to determine these quantities. The van Oss-Chaudhury- Good method is undoubtedly one of the recent achievements in the studies on the SFE of polymeric materials. The free energy of adhesion using the surface tension component approach, can be written as

$$\Delta F^{adh} = \gamma_{PS}^{LW} - \gamma_{PL}^{LW} - \gamma_{SL}^{LW} + \gamma_{SL}^{SR} - \gamma_{PL}^{SR} - \gamma_{SL}^{SR} \quad (1.35)$$

or by grouping the interactions of the different components.

$$\Delta F^{adh} = \Delta F^{LW} + \Delta F^{SR} \quad (1.36)$$

where the superscript  $SR$  can be interchangeable with the superscript  $AB$ . To calculate the total particle /liquid and particle/solid interfacial tensions, as required in the free energy of adhesion equation, for  $\gamma_S^{total} = \gamma_S^{LW}$  and  $\gamma_L^{total} = \gamma_L^{LW}$

it can be seen that the polar contributions to the particle surface tension play no role in determining these interfacial tensions. For situations where the matrix material is purely dispersive, Eq.(1.36) reduces simple to

$$\Delta F^{adh} = \Delta F^{LW} \quad (1.37)$$

From the above equation, the total free energy of adhesion given by the dispersion interactions can be obtained from Lifshitz theory considerations,

$$\Delta F_{132}^{adh} = \left[ -\frac{A_{132}}{12\pi L^2} \right] \quad (1.38)$$

Where A is the Hamaker constant of the system.

Isrealachvili introduced the cut- off distance parameter ,  $d_0$ , which represent the closest distance that two surfaces can approach. The parameter,  $d_0$  eliminates the divergence in Lifshitz theory. The free energy of adhesion, using the concept of  $d_0$ , is related, thus;

$$\Delta F_{132}^{adh} = \left[ -\frac{A_{132}}{12\pi d_0^2} \right] \quad (1.39)$$

Hough and White [7] found that the value of  $1.6 \times 10^{-10}$  m for  $d_0$  gave satisfactory estimates of surface tension of liquid. It can be seen from Eq. (1.39) that, if Hamaker constant is known for particle/ liquid/solid, then it is possible to estimate the total dispersion, or van der Waals interaction.

**Table 1.1: Competing theories for surface energy determination.**

Theory	Liquids	Rule	•	$\gamma^{LW}$	$\gamma^{AB}$	$\gamma^A$	$\gamma^B$
Girifalco,et al	1	Geometric	•				
Wu	2	Harmonic	•	•	•		
Owens-Wendt	2	Geometric	•	•	•		
Lewis AB	3	Geometric	•	•		•	•

**Table 1.2. Typical Test Liquids;**

Liquids	$\gamma$	$\gamma^{LW}$	$\gamma^{AB}$	$\gamma^A$	$\gamma^B$
Water	72.8	21.8	51	25.5	25.5
Glycerol	64	34	30	3.92	57.6
Formamide	58	39	19	2.28	39.6
Methylene Iodide	50.8	50.8	0	0	0
Ethylene Glycol	48	29	19	1.92	47

### 1.8 Concept of Interfacial Free Energy of Adhesion

Neumann [8,] stated that thermodynamic models based on interfacial tensions have successfully been used to explain many phenomena such as cell adhesion. It is possible to use interfacial energy balance of all the interfaces to predict whether adhesion will be thermodynamically favorable. When HIV attaches itself to the surface of the lymphocytes in a liquid suspension; three interfaces are involved, Cell- Substrum-Liquid. Each of these will have its own interfacial energy and a balance of these energies are called the interfacial free energy of adhesion. Surface energy is certainly an important determinant in adhesion of HIV to the surface of lymphocytes and any approach which allows the

determination of interfacial tension involving solid phase can be used to calculate the free energy of adhesion. The behavior of particles at the solidification fronts is another method that evaluates approaches for estimating interfacial tensions.

In his previous papers, Neumann show that Fowkes' approach did not correctly predict particle behavior at the solidification fronts. This is because lack of agreement between theoretical predictions and experimental observations indicates that Fowkes' approach for estimating interfacial tension is deficient. However, Lifshitz theory was considered effective for calculating the free energy of adhesion, because bulk properties are used to determine the total dispersion interactions. The most common approach that has not been used for estimating these interfacial tensions on biological materials involve interpretation of contact angle data. These data would be of great importance for estimating change in free energy of adhesion on HIV-blood interface.

When HIV particle is embedded in blood serum, the interaction can be predicted by the change in free energy of adhesion,  $\Delta F^{adh}$ , which is obtained from Eq. (1.40).

$$\Delta F^{adh} = \gamma_{SV} - \gamma_{SL} - \gamma_{LV} \quad (1.40)$$

If  $\Delta F^{adh}$  is negative, the process of adhesion is thermodynamically favorable and adhesion would lead to engulfment of HIV by lymphocyte (attachment). But if the  $\Delta F^{adh}$  is positive, the process of adhesion is thermodynamically unfavorable and adhesion would lead in principle to HIV-lymphocyte separation (detachment). Different model were applied to Eq(1.40) because  $\gamma_{SV}$  and  $\gamma_{SL}$  cannot be measured directly.

### 1.9 Concept of Hamaker Coefficient

Hamaker's 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, ie that such bodies would repel each other. This means that, if two particles are embedded in a fluid and the London-van der Waals force between particles and fluid is greater than between the particles themselves, it might be thought that the resultant action will be a repulsion rather than attraction. Some authors like Dr J.H. de Boer was not convinced with this

hypothesis until Viser [9] found 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 [10] had shown theoretically and experimentally 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 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 can be regarded as a traditional separation method.

Based on this novelty; there is a possibility that HIV can be isolated from the blood (lymphocytes) if their interactions are known. The basic assumption here is that the interactive term  $A_{132}$  must be negative to achieve this objective when,

$$\sqrt{A_{11}} > \sqrt{A_{33}} \text{ and } \sqrt{A_{33}} < \sqrt{A_{22}} \quad (1.41)$$

Or

$$\sqrt{A_{11}} < \sqrt{A_{33}} \text{ and } \sqrt{A_{33}} > \sqrt{A_{22}} \quad (1.42)$$

where uninfected T4 lymphocytes are represented as 1, HIV infected T4 lymphocytes represented 2 and serum represented 3. The absolute values of the interactive terms obtained with different models are expressed as Hamaker coefficient, using equation 1.39

$$\Delta F^{adh} = \left[ \frac{-A_{132}}{12\pi d_0^2} \right] \quad (1.43)$$

### 1.10: Hamaker's Approach: Separation of Particles interacting in a Liquid

When two identical atoms or molecules,  $i$  are separated by a short distance in vacuum; the dispersion interaction can be expressed as;

$$A_{ii} = \pi^2 q_i^2 \beta_{ii} \quad (1.44)$$

$$\beta_{ii} = -\frac{3h\nu\alpha^2}{4(4\pi\epsilon_0)^2} \quad (1.45)$$

$$W(r)_{London} = -\frac{\beta_{ii}}{r^6} \quad (1.46)$$

According to the Berthlot's principle, the dispersion interaction constant between dissimilar molecules of different materials can be estimated as the geometric mean of the interaction constants of individual materials. Thus, the extended interaction to two different atoms i and j becomes.

$$\beta_{ij} = \sqrt{\beta_{ii} \beta_{jj}} \quad (1.47)$$

It follows then that the Hamaker constant for two different atoms is given by;

$$A_{ij} = \sqrt{A_{ii} A_{jj}} \quad (1.48)$$

Which is known as geometric combining rule, and is widely used for calculating dispersion energies of interaction between dissimilar materials.

Hamaker, first calculated the dispersion (van der Waals- London) interaction energy for larger bodies by a pair-wise summation of the properties of the individual molecules (assuming all these properties are additive and non-retarded). Using this macroscopic approximation, the total dispersion energy for two semi- infinite flat parallel bodies (of material i), separated by a distance, r in air or in vacuum, becomes (for, r greater than a few atomic diameters)

$$W(r)_{London} = -\frac{A_{ii}}{12\pi r^2} \quad (1.49)$$

Where  $A_{ii}$  is the Hamaker constant for material  $i$ . Hamaker pair-wise summation procedure can also be used to calculate the combined Hamaker constant of two macroscopic identical or different particles interacting in a third medium.

For two atoms of the same material 1 in medium 3 (eg two individual clay particles in an aqueous suspension) the combining rule of Eq (1.48) gives,

$$A_{131} = \left( \sqrt{A_{11}} - \sqrt{A_{33}} \right)^2 \quad (1.50)$$

Or

$$A_{131} = \left( A_{11} + A_{33} - 2A_{13} \right) \quad (1.51)$$

Where  $A_{11}$  and  $A_{33}$  are referred to as the Hamaker constant of the solid and the medium respectively, in vacuum. Here, the convention is that the first and the third character in the triplet subscript identify the two particles which are interacting through a liquid medium, identified by the second character. For two different particles (1 and 2) in medium 3, the Hamaker combining rule can be given by

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad (1.52)$$

Which can be written as,

$$A_{132} = (\sqrt{A_{11}} - \sqrt{A_{33}})(\sqrt{A_{22}} - \sqrt{A_{33}}) \quad (1.53)$$

Eq(1.49) suggests that the Hamaker constant  $A_{131}$ , is always positive (or zero). Therefore, two identical molecules or particles in medium 3 (ie liquid) always attract each other, although it can become zero, when  $A_{11}=A_{33}$ . For two different materials, the Hamaker constant,  $A_{132}$ . (Eq1.52), can be negative, when;

$$A_{11} > A_{33} > A_{22} \quad (1.54)$$

And when

$$A_{11} < A_{33} < A_{22} \quad (1.55)$$

Under which conditions the dispersion interaction energy becomes repulsive ie  $W(r)_{London} > 0$ . It should be emphasize that there is nothing contradictory in repulsive van der Waals forces. The London dispersion interaction between two molecules or particles (identical and different) in vacuum are always attractive. And the London interactions between two identical molecules or particles immersed in a liquid is always attractive, although it can be zero, when  $A_{11} = A_{33}$ . But when two different materials 1 and 2 interact, immersed in liquid 3, and when  $A_{11} \neq A_{22}$  and the conditions in Eq.(1.54) and (1.55) prevail, a net repulsion occurs.

Padday [11] demonstrated the applicability of Hamaker approach to n- alkanes by calculating the theoretical values of surface tensions ( $\gamma_{ii}$ ) of various n- alkanes using the following equations,

$$W_{ii} = 2\gamma_{ii} = \frac{A_{ii}}{12\pi_{ii}^2} \quad (1.56)$$

The calculated results have shown a good agreement with experimentally measured values of surface tension, which suggest that the intermolecular forces of n- alkanes are

mainly of dispersion type. It is clear that the calculated value of surface tension depends critically on the assumed value of  $r_{ii}$ . Horn [12] however pointed out that Hamaker [13] approach turns out to be not entirely accurate because of many body effect, i.e. the fact that the field which one atomic dipole creates at a neighbour is also influenced by other atoms in the vicinity, so that the total force is not obtained by simply adding up the effects of each pair of atoms.

Yildirim et al [14] added that Hamaker approach may not be applicable to some colloidal system, since, like the classical DLVO theory, it ignores the existence of hydrophobic interaction between particles of hydrophobic materials.

### **1.11 Purpose of the study**

Human immune deficiency virus (HIV) has been a serious problem to mankind. The failure of HAART, however triggered a research on finding alternative solution to HIV infection from the surface thermodynamic point of view. The purpose of this study includes.

- Relating the interaction that occurs between the HIV and lymphocytes during binding to surface thermodynamics.
- Determining the surface physio-chemical properties using the contact angle approach on HIV infected and uninfected blood.
- Understanding the degree of interaction existing between HIV and T-lymphocytes during infection by measuring the contact angles at the surface in contact with the probe liquids.
- Finding the interfacial free energy of adhesion and the combined Hamaker coefficient and hence predict possible isolation of the virus from the lymphocytes.

All these are aimed at establishing the conditions under which the HIV adheres to the surfaces of the lymphocytes (and possibly penetrate into the lymphocytes) or separate from the surface (and hence there will be no interaction).

### **1.12 Significance of the study:**

The contact angle approach is a vital tool to determining the absolute and combined Hamaker coefficients of HIV-blood interaction. The surface energies and surface free energies of adhesion obtained by this method at the interface where the HIV-blood and liquid meet is very important in study of their surface interactions. When the absolute Hamaker coefficient is positive, the attractive van der Waals forces between the HIV-lymphocyte prevail. But when combined Hamaker is negative, the van der Waals forces become repulsive and separation is predicted. Therefore, any agent or condition that will render the combined Hamaker negative will be recommended to the pharmaceutical industries as the alternative solution to the treatment of HIV infection from surface thermodynamics point of view.

### **1.13 Aim and Objectives:**

The study is aimed at understanding the degree of interaction between the HIV and blood upon binding. This interaction expressed as Hamaker coefficient ( $A_{132}$  or  $-A_{132}$ ) will be obtained using contact angle approach. To achieve this aim, the following objectives must be satisfied:

- The determination of the combined Hamaker coefficient of the interacting system which will give an idea of the nature of interactions.
- This will require the isolation of HIV infected blood and the determination of the contact angles on infected and uninfected blood samples.
- The determination of the CD4+ cell count for the sampled patients in order to know the degree of infection.
- The determination of the surface energy of the HIV infected and uninfected blood for the twenty sampled patients.
- The determination of the change in free energies of adhesion for both infected and uninfected blood.

#### **1.14 Scope/Research limitations:**

The scope of the work is limited to twenty samples each of HIV infected and uninfected blood obtained from Nnamdi Azikiwe Teaching Hospital Nnewi. The contact angles will be measured on these blood samples in whole and in separated form and the results will be used to determine the various interfacial energies. The results will therefore suggest whether there is attraction or rejection between the virus and blood cells. The contact angle data will also be used to estimate the Hamaker coefficient and hence predict the interaction between the HIV and blood. However, reasonable suggestions can be made to integrate the clinical approach to the contact angle (surface thermodynamic) in providing solution to HIV infections.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

Human immunodeficient virus (HIV) was first claimed to be the cause of AIDS in 1984 and the CD4+ count has been widely used to make treatment and diagnostic decisions, but the use of the CD4 count has been controversial, and recommendations regarding how to use them have changed many times over the years. Low CD4 T-cell counts are considered to be a marker of the progression of HIV infection and AIDS, and have been called the 'signature' of HIV [15]. Another finding that is common in people diagnosed HIV-positive is reduced lymphocyte activity and function. This calls for understanding of CD4+ dynamics during HIV- Blood interactions.

#### **2.1: HIV-Blood Interactions: CD4+ Lymphocytes Dynamics.**

Hraba and Dolezal [16] presented a mathematical model of CD4+ lymphocyte dynamics in HIV infection. The model incorporated a feedback mechanism regulating the production of T-lymphocytes and simulated the dynamics of CD8+ lymphocytes, whose production was assumed to be closely linked to that of CD4+ cells. Thus, because CD4+ lymphocyte counts are a good prognostic indicator of HIV infection, the model was used to simulate such therapeutic interactions as chemotherapy and active and passive immunization. The model also simulated the therapeutic administration of anti-CD8 antibodies; this intervention was assumed to activate T-cell production by activating a feedback mechanism blocked by the high number of CD8+ lymphocytes present in HIV-infected persons. This model concentrated on CD4+ lymphocytes because the depletion of the T-cell subpopulation and the parallel decrease in the helper activity of T-lymphocytes seemed to be major immune system defect caused by HIV infection.

The above model was purely on maintenance of T-cell population in HIV infections. Alderman [17] suggested that the depletion of CD4+lymphocytes might activate some homeostatic mechanism that would increase their production. This assumed that the homeostatic mechanism increased the production of both CD4+ and CD8+ lymphocytes and did not discriminate between the two T-cell subpopulations.

In their study, Bragardo et al [18] showed that HIV-1 glycoprotein120 induced CD4+ association with several molecules on the surface of CD4+ lymphocytes. One of the molecules was CD38, which is involved in lymphocyte/endothelium interactions. They therefore examined the possibility that glycoprotein120 binding altered the CD4+ T-cell interaction with vascular endothelium in vitro and in vivo. They were therefore able to confirm that glycoprotein120 induced CD4+ association with CD38 in peripheral blood CD4+ T-cells.

While most people know about the reports of lowered CD4 levels in people diagnosed HIV-positive, which continue to receive widespread press coverage, other reports concerning lowered CD4 counts in people who are HIV-negative have been widely ignored. These reports show that CD4+ counts commonly fall very low, especially if a person suffers from certain conditions. These conditions include a variety of viral illnesses, bacterial infections, parasitic infections, sepsis, septic shock, multiple organ system failure, tuberculosis, coccidioidomycosis, burns, trauma, transfusions, malnutrition, over-exercising, pregnancy, normal daily variation, psychological stress, and social isolation. In addition to lowered CD4 counts, these conditions result in other immunosuppressive changes that are also identical to those seen in people diagnosed HIV-positive, including reduced CD4/CD8 ratios, reduced lymphocyte function, anergy, atrophy of lymphoid organs, and general suppression of cell-mediated immunity.

## **2.2: Low CD4 counts in the intensive care unit**

Feeney et al [19] looked at CD4 counts in 102 consecutive intensive care unit (ICU) patients who were admitted for a variety of reasons, all of whom were HIV negative. The results demonstrated that acute illness alone, in the absence of HIV infection, can be associated with profoundly depressed lymphocyte concentrations. They also found that CD4 counts were linearly related to total lymphocyte concentrations [6] as reported for HIV-positive patients [19].

## **2.3: Low CD4 counts in Various Human Infections**

In 1983, about one year before HIV was first mentioned as a possible cause of AIDS, a study was published showing severely reduced CD4 counts in 146 consecutive people

with serious acute infections who were admitted to their hospital in New Mexico [20]. The infections included pneumonia, acute pyelonephritis, abscesses, infected wounds, cellulitis, deep tissue infections, and sepsis. This reveals that 31 of 45 (69%) had CD4 counts less than 500 cells/mm<sup>3</sup>, 19 of 45 (42%) had counts below 300, 13 of 45 (29%) had counts below 200, 6 of 45 (13%) had 100 or less, and 2 of 45 (4%) had values less than 50. The average CD4 count for all the people with pneumonia was 574. They also provide tables with clinical information and CD4 counts for 9 patients with soft tissue infections (STI) and 12 patients with sepsis/deep infections, all of whom had multiple T-cell abnormalities.

### **Low CD4 counts in malaria**

In 1999 a letter was published documenting severely lowered CD4 counts in African patients with malaria [21]. The author examined the CD4 count in 78 patients with malaria who were HIV-positive, and 19 who were HIV-negative. He was surprised to find that more HIV-negative malaria cases had severely lowered CD4 counts than did the HIV-positive cases, on average, with 8 of 19 (42%) HIV-negative cases being below 200, while only 31 of 78 (40%) HIV-positive cases had CD4 counts below 200. Seven HIV-negative malaria cases had CD4 counts below 100. In addition, 6 HIV-positive patients had normal CD4 counts, and the author states, "One may want to hypothesize that malaria reduces the CD4 count more than HIV infection".

### **Low CD4 counts in mononucleosis**

Mononucleosis is caused by cytomegalovirus (CMV) or Epstein-Barr virus (EBV), and usually results in prolonged cold and flu symptoms, swollen lymph nodes, and fatigue. In 1981 a group of researchers looked at CD4 and CD8 counts in ten consecutive patients with acute CMV mononucleosis, and compared their counts with those of ten healthy volunteers [22]. The CD4 counts were measured in nine of the ten patients, and the three with the lowest CD4 counts had 194, 202 and 255 cells/mm<sup>3</sup>. The authors also found that the T-lymphocytes of people with mononucleosis responded poorly to antigens, showing depressed function.

Five years later, a different set of researchers measured various lymphocyte subsets in acute EBV mononucleosis [23]. They took 17 consecutive patients who had recently been diagnosed, gave them an immunization designed to activate their B lymphocytes, and then took samples of blood. The immunization makes this study different from any of the other studies to be examined here. They did not find a statistically significant lowering of CD4 counts, but they did find significantly lowered CD4/CD8 ratios, with the ratios falling below 1 as is reported to occur in people diagnosed HIV-positive. The authors concluded that "these studies demonstrate that infection with EBV affects both B and T lymphocytes and causes a broad based transient immune deficiency".

### **Low CD4 counts in sepsis**

In 1986, a group of researchers from Osaka, Japan published a study where they examined various lymphocyte subsets in 9 consecutive patients admitted to the ICU with sepsis [24]. They examined their blood at weekly intervals for four weeks. The CD4 counts in these patients were markedly reduced, with averages beginning below 500 and staying there for the entire 4 week study period. They also found T-cell function to be diminished, especially in patients who did not survive, although there was no significant difference in CD4 counts between those that died and those that survived.

### **Low CD4 counts in pulmonary tuberculosis**

In 1985 a group of researchers in Indonesia examined the lymphocyte subsets in 26 patients newly diagnosed with pulmonary tuberculosis (TB) [25]. They undertook the study because of a previous report of lowered CD4 counts in HIV-positive patients with TB in which the authors assumed that the lowered CD4 counts were due to HIV. They found that in HIV-negative TB patients; CD4 counts were also significantly lowered, with an average of 748, compared to 1,043 in healthy controls. They also found significantly lowered CD4/CD8 ratios. Although the effects seen here were not as dramatic as in the studies reviewed previously, with only 5 of 26 patients having CD4 counts less than 500, the authors still felt their findings were highly significant to people

diagnosed HIV-positive. The authors also comment on some similar findings in leprosy, as well as in HIV-negative hemophiliacs.

#### **2.4: CD4 irregularities in hemophilia**

Hemophiliacs who are HIV-negative have been found to have lowered CD4 counts as well as lowered CD4/CD8 ratios, and it appears that this effect is caused by injections of factor VIII [26] stating in their conclusion that "our findings clearly indicate an impairment of immune function in hemophiliacs regardless of HIV infection. Depressed cell-mediated immunity was found to be independent of HIV status. [27].

#### **2.5: CD4 irregularities and injected drugs Interaction.**

Intravenous drug users (IVDUs) are another group with a high risk of being diagnosed HIV-positive. In an article published in 1987 in the journal, AIDS, lymphocytes were found to be reduced in HIV-positive injection drug users as a direct function of how many injections they received [28]. A similar finding in 1991, also published in the journal, AIDS, found that lymphocyte reactivity was much more significantly reduced in IVDUs who injected more frequently, regardless of whether or not they were HIV-positive [29]. Although the CD4 cell function was impaired, no difference was found in CD4 counts due to frequent injecting. They did find that HIV-positive IVDUs had lower CD4 counts than did HIV-negative IVDUs. The T-cell reactivity was 40-50% lower in IVDUs who were injecting 3 times a day for the preceding several months when compared to a similar group who had not injected in the preceding months, regardless of their HIV status. The authors write: "We conclude that lymphocyte reactivity is depressed by frequent injecting in both HIV-negative and HIV-positive drug users"[29].

In 1980, a report in the Journal of Immunology documented lowered T-lymphocytes in IVDUs from Georgia, Illinois, and Massachusetts [30]. The authors found that IVDUs in their study had about half to one third as many T-lymphocytes, expressed as a percentage, as control populations. Although they did not look specifically at CD4+ T-lymphocytes, it has been found that when total T-lymphocytes are reduced, CD4 counts are also normally reduced [31].

## **2.6: Irregularities caused by utero exposure to opiates**

In 1987, a study found that infants exposed to intravenous drugs in utero also have decreased CD4/CD8 ratios and reduced CD4 function, even when they are HIV-negative [32]. These results show that multi-factorial causes of low CD4 counts probably apply to all age groups, including newborns. This is especially true in the United States and in Europe where most newborns who are HIV-positive are born to women who use intravenous drugs. In Africa, malnutrition and other infectious diseases are more likely to contribute.

## **2.7: Low CD4 counts caused by injuries and burns.**

Several studies over the years have looked at the effects of severe injuries or burns on CD4 counts. An early report appeared in 1982, in which the authors looked at the percentage of CD4 counts in 30 patients admitted to their hospital's burn center [26]. They found that the severity of the burns was directly correlated with depressed CD4 percentages. Patients with greater than 25% of their body covered with third degree burns had the lowest percentages on admission, 37%, as compared to normals who had 63%. They found a similar pattern with the CD4/CD8 ratio, but do not report on absolute CD4 counts.

In 1985, a study was published that looked at two groups of patients with severe injuries, a group of 25 patients with burns, and a group of 21 patients with non-thermal injuries. Both groups had severely lowered CD4 percentages, which persisted until 50 days post-injury when the study was concluded. They also found that people with lower CD4 percentages were more likely to develop sepsis. Here are some of the author's comments:

The most important abnormality appears to be a reduction in CD4 positive cells in burn patients. A change in the ratio of CD4 to CD8 positive cells soon after injury is due to a reduction in CD4 positive cells, not an increase in CD8 positive cells. This paper is distinctive in that it attempts to explain a mechanism for the lowered CD4 counts, citing a study supporting the hypothesis that increased cortisol levels are responsible for the decline, and that increased cortisol is also a normal response to injury. The argument that

cortisol plays a key role in lowered CD4 counts will be encountered again in the section on psychological stress.

### **2.8: Low CD4 counts in normal human pregnancy**

A study in 1996, it was attempted to control for potentially confounding factors like the increased blood volume that normally occurs in pregnancy [33], they used CD4 percentages because of this variable, and determined that "Our CD4 cell findings for HIV-negative women are consistent with the majority of prior studies, which demonstrate a decline in CD4 levels during normal pregnancy". They also found that HIV-positive women had a more severe decline which did not correct post-partum as it did in HIV-negative women, although they fail to take into account other factors that can cause lowered CD4 counts.

In 1989 a study was published of normal pregnancy which found reduced CD4 percentages in the 1<sup>st</sup> and 2<sup>nd</sup> trimester, as well as reduced CD4/CD8 ratios in the 2<sup>nd</sup> trimester [34]. They comment on previous studies looking at a variety of lymphocyte changes during pregnancy, stating simply, "In these studies, variation in the number and proportion of CD4+ lymphocytes is the alteration most frequently reported [34]. They also claim that "we have accounted for all the presently known factors that can alter the concentrations of T-cell subsets in blood" [34], but in fact they did not consider any of the factors described in this paper, such as infections, trauma, overexercising, normal daily variation, or psychological stress. This demonstrates that even clinicians and researchers doing studies that focus specifically on CD4 levels are often unaware of how many different conditions cause low CD4 counts.

Reduced absolute CD4 counts, as well as reduced percentages of CD4+ T-cells in 76 women with normal pregnancies were found [35]. By the third trimester, the pregnant women had an average of only 543 + 169 CD4+ T-cells, compared to 1073 + 441 in non-pregnant women who served as controls.

## **2.9: Reduced CD4 Counts from over Exercising**

A report that, ten athletes were asked to over-train for three weeks [36]. Blood samples were taken immediately before starting, at the end of the three weeks, and again three weeks after returning to normal. The researchers found steady declines in the percentage of CD4+ T-cells, with the lowest amount occurring three weeks after returning to a normal exercise schedule. The authors also found reductions in the CD4/CD8 ratio, although these had normalized by the three week endpoint. Finally, the authors also checked levels before and five minutes after acute exercise, and again found reductions in CD4 percentages and in CD4/CD8 ratios, although these normalized by thirty minutes post-exercise. It is interesting that a stress as simple as over-exercising for three weeks could cause lowered CD4 counts, and that they did not correct for at least three more weeks after returning to a normal exercise schedule.

## **2.10: Low CD4 counts in Malnutrition**

Like the other conditions already discussed, malnutrition causes severe immunodeficiency with depletion of CD4+ T-cells and reduction of cell mediated immunity. One of the most recent studies is from India, where malnutrition is extremely common [37]. The authors found that reduced CD4 counts were a natural physiological effect of malnutrition, and commented that both HIV and malnutrition lead to a state of anergy with failure of cell-mediated immunity. They also pointed out that HIV usually occurs in conjunction with several other stressors of the immune system: "micronutrient abnormalities, concomitant infections, and genetic factors are some of the compounding co-factors which further contribute to the deterioration of immune functions in AIDS patients"[37].

Deficiencies of single essential nutrients with important roles in nucleic acid synthesis and metabolism appear to cause derangements in immunological functions that are quite similar to those seen in protein energy malnutrition. Both vitamin A and zinc deficiencies are characterized by lymphoid tissue atrophy and depressed cellular immunity [38].

## **2.11: Changes in CD4 Counts and Lymphocyte Function due to Psychological Stress and Social Isolation.**

A group of researchers [39] has done a great deal of work observing the effects of psychological and social stress on baboons and other primates, with most of their work focusing on the neurotoxicity that is caused by stress, with dementia and loss of neurons in the hippocampus [39]. In one study, however, they measured total lymphocyte counts and cortisol levels in a group of baboons that were invaded by a highly aggressive young male baboon, whom they named Hobbs [40]. Hobbs was particularly threatening to females in the group, and was apparently attempting to use fear, physical intimidation, and abuse to increase his chances of successful mating. Cortisol levels in the group nearly doubled after Hobbs joined the group, with a slightly greater increase among females. Interestingly, Hobbs, himself had the lowest number of lymphocytes in the entire group, and the highest cortisol level, suggesting that his behavior may have been taking an even greater toll on his system than it did on the victims of his aggression.

Whereas most studies of the effects of stress upon immunity examine functional indices of immune competence (e.g. mitogen stimulation tests, antibody generation, cytokine responsiveness), our field conditions limited us to this rather crude quantitative measure of numbers of cells [40]. Psychological variables, including loneliness, attachment and depression were related to the immune changes [41]. Another review, published in 1993, performed a meta-analysis of all studies that looked at psychological stress and the immune system [42]. In their discussion they mention their findings regarding CD4, helper T-cells. In terms of cell numbers, stress is reliably associated with a lower number of circulating B cells, helper cells, cytotoxic cells, and large granular lymphocytes. Stress is also reliably associated with a lower percent of lymphocytes that are T cells, helper T cells, and cytotoxic T-cells [42]. Almost any type of physical or mental stress can lead within minutes to greatly enhanced secretion of ACTH and consequently cortisol as well, often increasing cortisol secretion as much as 20-fold [43]. "Cortisol suppresses the immune system, causing lymphocyte production to decrease markedly. The lymphocytes are especially suppressed [43]. "Cortisol decreases the number of eosinophils and lymphocytes in the blood; this effect begins within a few minutes of injection of cortisol

and becomes marked within a few hours”. Indeed, a finding of lymphocytopenia or eosinopenia is an important diagnostic criterion for overproduction of cortisol by the adrenal gland.

In 1998, a group of researchers put these hypotheses to the test by checking CD4 counts and cortisol levels in people who were randomly assigned either to a bereavement support group intervention or to a wait-list control [44]. The intervention consisted of 10 weekly support group meetings, and blood samples continued to be taken periodically for a total of 6 months. Some of the group members were HIV-positive, and the authors stratified their data according to HIV status. They found that CD4 counts were increased in people receiving the support group intervention as compared to controls, and that these increases correlated with reduced levels of the stress hormone cortisol.

Results like these may help to explain why socially isolated people, when compared to people with high levels of social support, have been found in over eight studies to have between double and triple the death rates from all causes [45,36,46,37,47,38].

Further review of HIV interactions will be helpful in understanding the response of HIV the immune system and hence provide possible solution to the cure. HIV interactions with drugs used for HIV treatment will also be reviewed.

### **2.12: Drug interactions and Anti HIV Therapy**

Drug interactions associated with HIV medications can be broadly classified into Pharmacokinetics and pharmacodynamics [48]. Pharmacokinetics interactions refer to what happens to drugs in the body – their absorption, metabolism (processing) distribution to tissues and elimination.[49]. In an overview of antiretroviral and drug interactions said that pharmacokinetic interactions among drugs used in HIV therapy are often ‘multi-factorial,’ involving altered drug absorption, P-glycoprotein modulation, CYP450 induction or inhibition, changes in renal elimination and fluctuations in intracellular drug concentration. Table 2.1 shows some drugs used for Human antiretroviral therapy, their route of elimination and effect on CYP450.

Pharmacokinetics of antiretroviral drugs may be significantly altered in HIV positive people with hepatitis B or C, and that such impairment is more pronounced in those with

more advanced liver damage [50]. The conclusion was that liver dysfunction has a considerable impact on PI metabolism, but on the whole NNRTI and nucleoside reverse transcriptase inhibitor (NRTI) processing are minimally affected [51]. HIV positive people should be aware of potential for the interactions and inform all their health-care providers about all the drugs they are taking, including prescription and over-the-counter (OTC) medications, herbal remedies and supplements, and recreational or street drugs.

However, avoiding and managing interactions has become an increasingly important part of HIV medicine. While many drug interactions are of little clinical significance, others can lead to severe toxicities, loss of virological control of HIV, and emergence of drug resistance virus.

**Table 2.1: Clinical Significant Drugs Associated with HAART. Routes of Elimination of Antiretroviral Agents and the Effect on CYP450**

Drug	Elimination	Effect on CYP450 System
Zidovudine (AZT,ZDV)	Hepatic metabolism with renal excretion.	None
Didanosine (ddI)	Renal excretion 50%	None
Zalcitabine (ddC)	Renal excretion 70%	None
Stavudine(d4T)	Renal excretion 50%	None
Lamivudine (3TC)	Renal excretion 70%	None
Tenofovir(TDF)	Renal excretion 70-80%	None
Abacavir(ABC)	Hepatic	Insignificant
Emtricitabine(FTC)	Renal excretion 86%	None
Nevirapine (NVP)	Hepatic	CYP3A4 inducer
Delavirdine (DLV)	Hepatic	CYP3A4 inhibitor
Efavirenz(EFV)	Hepatic	CYP3A4 inducer/inhibitor
Saquinidone(SQV)	Hepatic	CYP3A4 inhibitor
Ritonavir(RTV)	Hepatic	CYP3A4 inhibitor CYP2D6 inhibitor (3A4 inhibitor > 2D6) 3A4 and CYP1A2 inducer.
Indinavir(IDV)	Hepatic	CYP3A4 inhibitor
Nelfinavir(NFV)	Hepatic	CYP3A4 inhibitor
Amprenavir(APV)	Hepatic	CYP3A4 inhibitor
Lopinavir/ ritonavir (LPV)/(RTV)	Hepatic	CYP3A4 inhibitor CYP2D6 inhibitor (3A4 inhibitor > 2D6) 3A4 and CYP1A2 inducer
Atazanavir(ATZ)	Hepatic	CYP3A4inhibitor,CYP1A2,CYP2C9 Inhibitor.
Fosamprenavir(FPV)	Hepatic	CYP3A4 inhibitor
Enfuvirtide(ENF)	Hepatic	None.

**Data from: [http://www.thebody.com/content/treat/art\\_13000.html](http://www.thebody.com/content/treat/art_13000.html)(8/24/2010)**

Pharmacodynamics interactions are those related to the combined clinical activity of two or more agents used together involving additive or synergistic anti- HIV therapy and a successful HAART relies on additive effect. An antagonistic effect happens when one agent reduces or cancels out the effect of another (ie  $1+1=0$ ). An additive effect refers to the combined effects of two or more agents added together (ie  $1+1=2$ ) while the synergist effect occurs when the overall effect of two or more agents used together is greater than the sum the of effects the compound would produce if used separately (ie  $1+1=3$ ).

The vast majority of drug interaction encountered in HIV medicine are pharmacokinetics in nature and occur as a result of a change in the absorption, distribution, metabolism or elimination of either the HIV medication itself or the concurrently administered medication [49] .Though numerous isoenzymes of CYP450 have been identified, the enzymes responsible for the elimination of the majority of drugs used in HAART are CYP3A4, CYP1A2, and CYP2D6 (table 2.1). Drug interaction is recommended as a good practice, if an antiretroviral regime does not seem to be working as well as it should ( eg, rising viral load, decreasing CD4 cell count).The mechanisms of interaction will enable the manufacturers to understand the wetting properties which is based on the surface tensions of drugs (Table 2.2). Low surface tension drugs wet easily and this encourages bioavailability of drugs to the target cells.

**Table 2.2: Surface tension of drugs and their manufacturers at 25°C**

Drugs	Manufactures	Surface tension (dynes/cm)
Cresofo	Prodonto Ltda	26.06
Camphorated paramonochlorophenol	Prodonto Ltda	25.12
Glutaraldehyde	Merck	59.38
Phenol	SS White	39.25
Neodex	Prodonto	59.67
Otosporin	Wellcom	40.90
Trieresol formalin	Prodonto Ltda	37.33
Eugenol	SS White	33.73
Xylol	Merck	29.85
Hydrogen peroxide 10%	Rio Quimica	70.42
Water with calcium hydroxide	Rio Quimica	66.82
Irrigocal	SS White	37.52
Chloroform	Merck	26.72
Citric acid 10%	Fermenta	68.34
Ethylene alcohol 96 GL	Fermenta	23.51
Eucaliptol	SS White	30.20
Dakin's Solution	SS White	71.34
Distilled water	SS White	72.73

**Prepared in the Endodontic Laboratory of the Dental School of Ribeirao preto.**

### **2.13: HIV - Monocyte Interaction**

Monocytes are vitally important in the immune system, as they are precursor cells to professional antigen- presenting cells (APCs), such as the dendritic cells (DCs) and Macrophages. These cells are responsible for a wide range of both innate and adaptive immune function[51].

Recent studies suggested the role of naturally occurring anti-HIV micro- RNA (miRNA) in suppressing HIV-1 replication in peripheral blood monocytes [52]. However, it has also been shown that HIV-1 is capable of suppressing some inhibitory mi RNA[53], which

may reflect an evolutionary interaction of HIV-1 and host factors. Zheng [54] showed that HIV- Tat encourages the survival of monocytes in situations where they would normally be cleared. Exogenous HIV -1 Tat has been shown to cause production of the cytokine interleukin (IL)-10 from monocyte in vitro [55]. Furthermore, up-regulation of IL-10 production of HIV/AIDS patients has been correlated with increased level of monocyte secreted myeloid differentiation- 2 and soluble CD14 [56]. High level of secreted CD14 have been associated with impaired responses to lipopolysaccharide (LPS) [57]. It has been proposed that the release of general immunosuppressant IL- 10 by monocytes facilitates the retroviral-mediated HIV-1 Nef expression in primary monocytes and promocytic cell line inhibits LPS- induced IL-12p40 transcription by inhibiting the JNK nitrogen – activated protein kinase[58].HIV-1 Nef is a multifunctional accessory protein that plays important role in viral pathogenesis[59].

The HIV-1 matrix protein (P17) regulates a number of cellular responses and interacts with the p17 receptor (P17R) expressed on the surface of the target cells [60]. Upon binding to the cell surfaces receptor P17R, exogenous HIV- 1 matrix protein causes secretion of the Chemokine protein-1(MCP-1, also known as CCL-2) from monocyte. MCP-1 potentially, increases monocyte recruitment to the sites of HIV-1 infection by increasing the available monocyte pool for infection of HIV -1. This recruitment may be of critical importance given the entirely low rate of infection of this cell type [61]. HIV and HIV derived factors have been shown also to reduce up- regulation of programmed death ligands -1 on monocyte in vitro [62]. This ligands, in complex with its receptor, programmed death-1 causes apoptosis of all T-cell types [63] and a loss of anti viral function in a manner similar to known immunosuppressive Cytokines [64].This suggest that HIV-1 can impair virus specific immunity by modulating immuno-regulatory molecules of monocytes and T-cells. However, the interactions between the virus and monocytes may contribute key functions in establishing Chronic HIV-1infection and facilitating the progression to AIDS. These outcomes are likely influenced by the altered immunological function of monocytes and their interactions with other types of HIV-1 target cells. Monocytes are implicated as a viral reservoir based on the detection of, or the recovery of infectious virus from monocytes isolated from HIV- positive individuals on antiretroviral therapy [65]. It is noted that CD16- positive monocytes (5% of monocytes

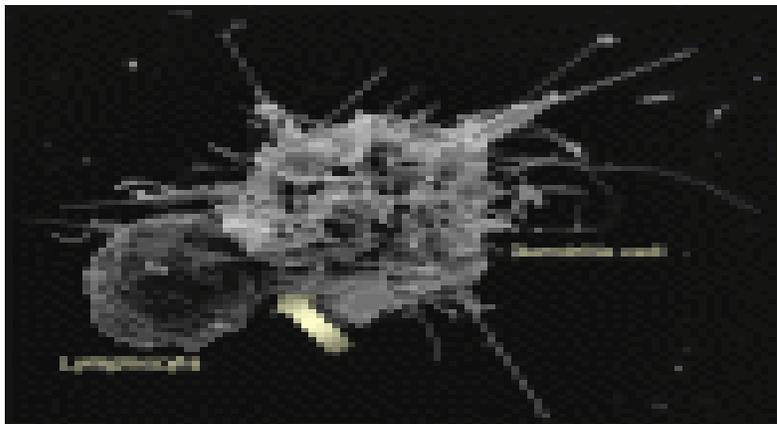
population) are both more susceptible to infection and preferentially harbor the virus long term [66, 57]. This explains why only small number of monocytes are infected by HIV in Vitro. When compared with activated CD4+ T-cell and macrophages, monocytes are known to have much lower level of cyclin T1( Cyc T1) expression [66] and they therefore lack functional positive transcription elongation factor b(P-TEFb).[(CycT1 and Cyclin-dependent kinase 9(CDK9) are collectively known as the positive transcription elongation factor b (P-TEFb)]. However, this is not the only factor responsible for the resistance of monocytes to HIV replication, as transient expression of CyT1 is not sufficient to restore HIV-1 Tat- mediated transactivation in monocytes, [67]. Phosphorylation of Cyclin- dependent kinase 9(CDK9) is known to be vital for the formation of a P-TEFb. CD14++ monocytes express high levels of low molecular weight form of APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide like 3G), whereas CD16+ monocytes express the high molecular weight form of APOBEC3G that has no anti- HIV activity [56].The study of HIV-1 latency in monocytes is challenging due to generally low viral integration and infection of monocytes[67] However, even when a HIV-1 proviral DNA construct is transinfected directly into monocytes, there is no infectious virus production. When monocytes differentiated into macrophages, they become increasingly susceptible to HIV-1 infection. Furthermore, the differentiation of monocytes into macrophages stimulates HIV -1 production in the infected monocytes. This suggest the role played by monocytes in both viral latency and reactivation.

In light of the evidence that suggests miRNAs play a role in the resistance on monocytes to HIV-1 infection, [54].It is of interest that a number of host miRNAs have been implicated in causing latency in resting primary CD4+ T-cells. Inhibitors of these miRNAs are now being touted as a new generation of treatment to be used in concert with current antiretrovirals [56] .Although much is known about the ways in which HIV-1 interacts with both monocytes and various types of DCs, some key questions remain to be answered to fully understand the pathogenesis and latency of HIV-1. Latency in HIV infection is a key area of study for understanding the pathogenesis and ultimate development of therapies or vaccinations against HIV/ AIDS. Unfortunately, there is no effective vaccine for AIDS currently available, and antiretroviral therapy is limited in its

ability to fully control viral replication in infected individuals. Recent progress suggest that understanding how HIV interact with host immune cells is vital for the treatment of infected cells [68]

#### **2.14: Dendritic Cell- HIV Interaction**

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) of heterogeneous population located throughout the blood, tissues, and lymphoid organs and are derived from different linages [69]. They are involved in the generation of both innate and acquired immune responses, including release of cytokines (such as IL-12, IL-10, and IFN $\alpha$ ), stimulation of naïve T lymphocyte clonal expansion [70]. The DC subtypes (myeloid DCs, plamacytoid DCs (pDC) and langerhans cells) are characterized based on their location surface maker and cytokine secretion profile(Fig 2.1).



**Fig.2.1: Interaction of a Dendritic Cell (right) having HIV Bound to its Surface (arrow) with a Lymphocyte (left)**

There is a report that productive HIV-1 replication occurs in human monocyte- derived DCs for up to 45days [71]. It was also found that only small percentage of circulating DCs is positive for HIV infected individuals [72]. Productive HIV-1 replication is dependent on fusion- mediated viral entry in monocyte- derived DCs [73] and observation showed that matured HIV- 1 particles are localized to a specialized tetraspinin- enriched sub-compartment within the DC cytoplasm [74]. In the presence of viral proteins, DCs acquire migratory phenotype, facilitating travel to the lymph nodes. It is possible therefore, that the DCs are trapped in the lymph nodes and unable to initiate a

protective immune response against the virus. Groot[75] reported that it has been demonstrated that pDCs are capable of inhibiting HIV-1 replication in T- cell when cultured together in vitro, showing the importance of pDCs for viral clearance. It has been confirmed that HIV-1 is capable of directly killing pDCs [76] illustrating that virus can remove a potential block to its replication and dissemination in pDCs. HIV- 1 envelope protein gp120 has also been shown to inhibit activation of T-cells by monocyte-derived DCs[77] suggesting that gp120 may also have a role in the suppression of T-cell function and progression to AIDS. HIV-1 has been shown to suppress the immune function of pDCs in general by suppressing activation of the anti-viral toll- like receptor 7( TLR7) and TLR8 and by blocking the release of the anti-viral interferon alpha[78]. Recent result [52] suggested that intercellular adhesion molecule-1 (ICAM-1),but not ICAM-2 or ICAM-3, is important for DC- mediated HIV transmission to CD4+ T-cells. The interaction between ICAM-1 on DCs and leukocyte function-associated molecule 1(LFA-1) on T-cell plays an important role in DC- mediated HIV-1 transmission. A report that the precise mechanism of virus from DCs to CD+ T cell has yet to be determined[79].In a study,a role for small lipid vesicle known as exosomes in immature and mature DC-mediated HIV transmission to CD4+ T cell was demonstrated [80]. According to the study, immature DCs are capable of constitutively releasing infectious virus associated with exosomes in the absence of CD+ T-cell. HIV and purified exosomes can be endocytosed by mature DCs into the same intracellular compartment and transferred to co- cultured CD+ T cell. HIV may also exploit an intrinsic exosome trafficking pathway in mature DCs to facilitate viral dissemination [81].Recent studies have also offered the intriguing possibility that HIV-1 can be transferred from cell- cell protrusions, with the virus either transmitting via cellular membrane nanotubes[82] or ‘surfing’ along the extracellular surface of the cytoplasmic membrane[83]. Indeed, the potential mechanisms of cell-cell mediated HIV transmission have yet to be investigated in the DC-T cell trans-infection model.

### **2.15: HIV- Protein Interactions**

Human Protein interaction data base (HHPID), currently comprises over 2,500 unique interactions [84]. Another report from the NCBI that HIV –human proteins are known to

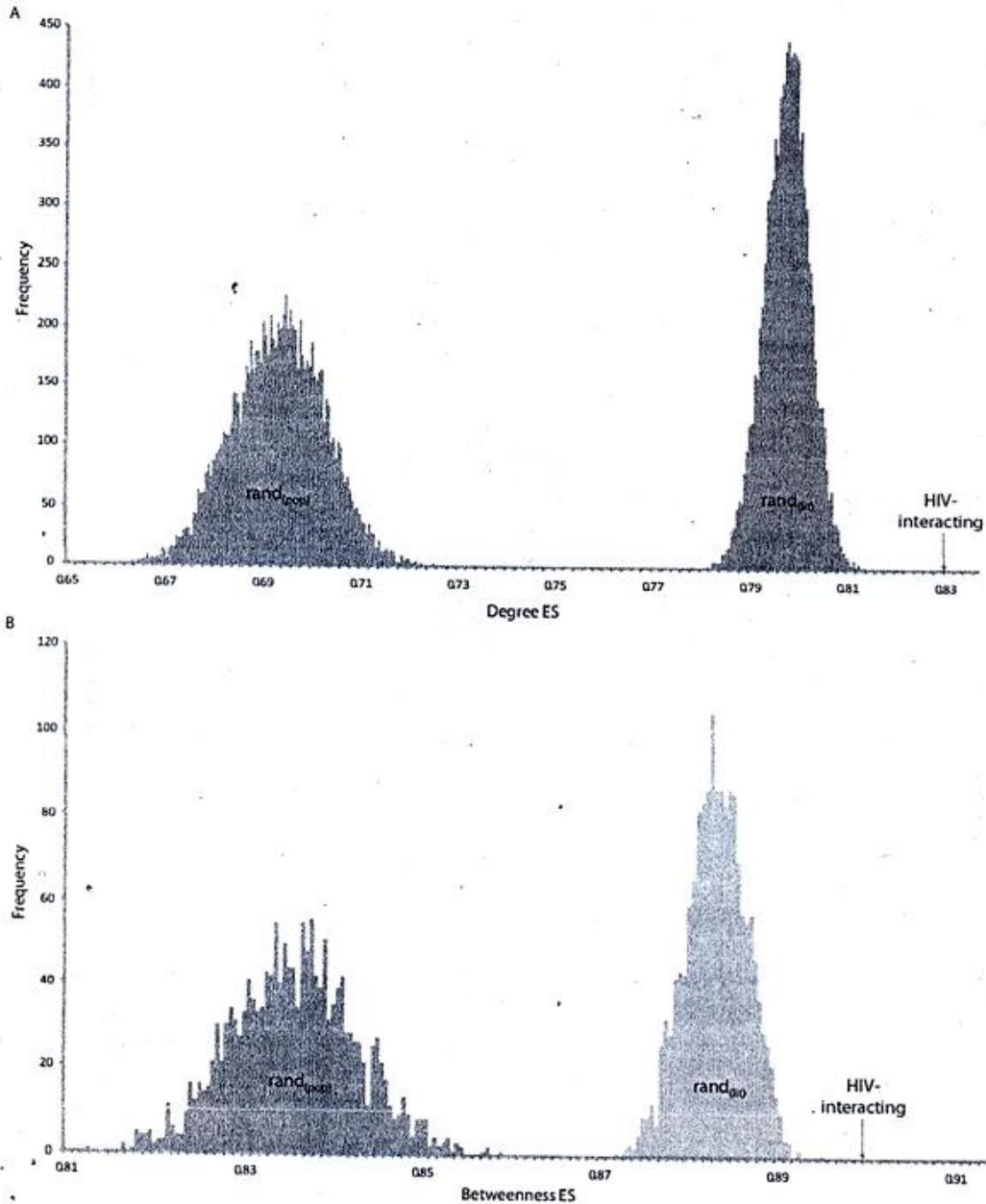
interact with approximately 6,000 other human proteins when integrated with the human protein interactions data showing high connected nature of the HIV-host interactions and their neighbor. However, the biological context of HIV- interacting human protein, rather than their individual properties has been the key determinant in the infection of host by the retroviruses.

Proteins with high degree (connectivity) are involved in a large number of interactions and have been previously shown to be associated with essentiality[85]. A Gen Set Enrichment Analysis(GSEA) algorithm had been used to determine the degree of distribution of HIV –interacting protein.[86]. The Enrichment scores (ES, from GSEA) for the degree of HIV interacting proteins, in addition to 10,000 random samples each of the same size) taken from the protein –coding gene population ( $rand_{pop}$ ) was calculated. The  $ES_{(degree)}$  for HIV interacting protein was 0.83, significantly higher than the average amongst the  $rand_{(pop)}$  sample, 0.69 (p-value of  $8.90 \times 10^{-48}$ ) grey distribution. Note, a higher ES denotes a stronger tendency towards higher degree. This result confirms a previously propensity for HIV to interact with highly connected proteins .The control set was also studied using the same 10,000 random samples taken to match the publication count distribution of the HIV sample ( $rand_{lit.}$ )[87]. The  $ES_{degree}$  of the HIV- interacting protein is 0.83 and the average amongst the  $rand_{pop}$  sample is 0.69 ( p-value of  $8.9 \times 10^{-48}$ ) while that of  $rand_{lit}$  is 0.80(p-value  $6.63 \times 10^{-15}$ ). Thus, we can confirm that HIV tends to interact with protein that high degree (Fig 2.2).

Betweenness centrality also has some significance for the properties of proteins [88]. Thus ES of the betweenness centrality was calculated with the same sample data sets. The  $ES_{betweenness}$  of HIV –protein is 0.90 and the average ES amongst the  $rand_{pop}$  sample is 0.84(p-value of  $1.98 \times 10^{-21}$ ), whilst that of  $rand_{lit}$  is 0.88(p-value of  $4.36 \times 10^{-8}$ ). Despite the significant difference between  $rand_{pop}$  and  $rand_{lit}$ , HIV-interacting protein can be shown to have a higher betweenness centrality than expected (fig 2.2).

Protein with higher degree was identified as a hub [89] while a protein is classified as a hub if it falls within the top 20% of proteins when sorted according to their degree [90]. Bottlenecks are generally a more accurate indicator of essentiality than degree or hub

propensity, despite the two being correlated. Eppig[91] reported that integrating homologous mouse genome knockout data with the HIV interactions reveals that HIV interact with 376(26.28%) essential protein. Furthermore, the mean number of essential proteins seen in  $\text{rand}_{\text{pop}}$  is only 143(9.99%). To investigate HIV's propensity to interact with key proteins, we determine the degree and betweenness centrality for proteins involved in the over-represented biological process GO terms, including immune and apoptotic process. Indeed, proteins involved in the over-represented biological process GO terms tend to be highly connected and central. Thus, HIV's propensity to interact with highly connected and central proteins is mainly a consequence of its interaction with particular functions, rather than being related to global network properties in any straightforward way.



Source:<http://www.biomedcentral/1752-0509/4/80>

**Fig. 2.2 Degree Enrichment amongst HIV and Randomised Data Sets**

### 2.16: HIV - Blood Interaction: Thermodynamic Approach

Achebe [4] recently measured absorbance using ultraviolet visible spectrophotometer for twenty samples of HIV infected and uninfected blood. The Hamaker constants  $A_{11}$ ,  $A_{22}$ ,  $A_{33}$  and the combined Hamaker coefficients  $A_{132}$  were obtained using the values of the

dielectric constant together with the Lifshitz equation. The harmonized Hamaker coefficients  $A_{132\text{har}}$  and the absolute Hamaker coefficient,  $A_{132\text{abs}}$  (an integral of all the values of the various Hamaker coefficients) for the infected blood samples were then calculated. The value of  $A_{132\text{abs}} = 0.2587 \times 10^{-21}$  Joule (i.e.  $0.2587 \times 10^{-14}$  erg) was obtained for HIV-infected blood [92,93,94,]. This value lies within the range of values derived by various researchers for other biological systems. Another significance of this result is the positive sense of the absolute Hamaker coefficient which implies net positive van der Waals forces indicating an attraction between the virus and the lymphocyte. This in effect suggests that infection has occurred thus confirming the role of this principle in HIV-blood interactions. A lower value of  $A_{131\text{abs}} = 0.1026 \times 10^{-21}$  Joule obtained for the uninfected blood samples is also an indicator that a zero or negative absolute combined Hamaker coefficient is attainable. As a first step to this, a mathematical derivation for  $A_{33} \geq 0.9763 \times 10^{-21}$  Joule which satisfies this condition for a negative  $A_{132\text{abs}}$  was obtained [4].

### **2.17: Measurement of Surface Tensions of Blood Cells and Proteins:**

Absolom et al [95] did extensive work on the measurement of surface tensions of blood cells and proteins employing different approaches. Among the various approaches used include; Contact angle approach, Adhesion experiments, Freezing-front technique, Stability of suspensions approach, and Phagocytic ingestion approach (Fig1.3). All these techniques yielded the same answer in comparison to equation of state approach. This goes to buttress the fact that the role of surface properties in various biological processes is now well established [96]. Interfacial tensions have been shown to play vital roles in phenomena as diverse as the critical closing and opening of vessels in the microcirculation[91] cell adhesion [97] protein adsorption, antigen-antibody interactions[98], and phagocytosis [99] obtained using the various techniques (Table 2.3).

**Table 2.3: Surface Tensions of Biological Entities in ergs/cm<sup>2</sup>/T=22°C [87]**

System	Contact Angle via Equation of State	Engulfment			Adhesion	Detachment	Suspending Stability
		Advancing Solidification	Phagocytic Ingestion				
			Granulocytes	Platelets			
Granulocytes (Human)	69.1	69.3	-	-	69.0	69.0	-
Lymphocytes (Human)	70.1	70.6	-	-	-	-	-
Erythrocytes (Human)	-	64.9	-	-	-	-	64.3
(Horse)	-	65.1	-	-	-	-	65.4
(Chicken)	-	64.8	-	-	-	-	65.2
(Turkey)	-	65.1	-	-	-	-	65.7
(Canine)	-	63.9	-	-	-	-	64.4
Platelets (Porcine)	67.2	-	-	67.9	-	-	-
Bacteria -E. Coli	69.7	-	69.6	69.3	69.6	-	-
- S. Aureus	69.1	-	68.7	68.8	69.3	-	-
- S. Epidermidis	67.1	-	66.9	67.3	66.0	-	-
- L. Monocytogenes	66.3	-	66.1	-	65.6	-	-
Proteins-B.Serum Albumin	70.2	-	-	-	-	-	-
- H. Serum Albumin	70.3	-	-	-	70.2	-	-
- H. Immunoglobulin G.	67.3	-	-	-	67.7	-	-
- H. Immunoglobulin M.	69.4	-	-	-	71.0	-	-
- H. a <sub>2</sub> Macroglobulin	71.0	-	-	-	71.0	-	-
- H. Transferrin	66.8	-	-	-	-	-	-

**Source:**American society of microbiology,Nov 1984.

\*(B=Bovine; H= Human)

The deductions from their extensive study can be summarized in the following sentences;

- Surface tensions of biological cells and proteins can be measured by a variety of techniques.
- The results obtained from the different techniques are in good agreement.
- Surface tensions of biological cells and proteins are relatively high; that is, these materials tend to be hydrophilic in their natural state.
- The interpretation of the experiments show that it could be concluded that surface tensions govern cell adhesion, protein adsorption, stability of suspensions and phagocytosis [96].

**2.18: Molecular interpretation of surface free energy/surface tension:**

The surface free energy( $\gamma_{sv}$ ) of a solid[100] is defined as the change in the total surface free energy(G) per unit change in surface area (A) at constant temperature (T), pressure (P) and mole (N), ie

$$\gamma_{sv} = \left(\frac{\partial G}{\partial A}\right)_{T,P,n} \quad (2.1)$$

For liquids, the surface area can be changed under the above conditions. For solids however, surface area cannot, in general, be changed without affecting its chemical potential. Therefore, in changing the area, work needs to be done against the elastic forces in the solid. In a given experiment involving stretching of solid surfaces, it is often difficult to delineate the effect of bulk and surface mechanics. The research carried out by Langmuir, Zisman and Adams stimulated other surface scientists [100 - 105] to investigate ways to determine directly the surface free energies of solid from contact angles. Gibbs [106] commented that the surface energies of solids cannot be derived from contact angles because there is virtually no way of estimating the interfacial free energy of solid and liquid. However, the simplification of Young's equation was actually possible as a result of Dupre's[107] equation; combining the work of adhesion at the liquid interface with the surface and interfacial tensions of the solid-vapour, solid-liquid and solid-vapour interfaces.

$$W_{SL} = \gamma_{SV} + \gamma_{LV} - \gamma_{SL} \quad (2.2)$$

The Dupre equation amounts to a conservation of total energy in a reversible process of adhesion and cohesion of two phases. The combination of Young and Dupre equation results in Eq.(2.3)

$$W_{SL} = \gamma_{LV}(1 + \cos\theta) \quad (2.3)$$

In this way the unknowns ( $\gamma_{SV}$  and  $\gamma_{SL}$ ) of the original Young's equation can be reduced to only term,  $W_{SL}$ . The left-hand side of Eq.(2.3) is actually a deformation term; it may be viewed as the strain energy of the liquid drop per unit area. Unlike solids, the strain energy is contributed by surface tension alone. The next major simplification of Young – Dupre equation was due to Good and Girifalco[101], who proposed analogous to the Berthelot[108] combining rule of intermolecular interaction, that the work of adhesion can be expressed as geometric mean of the surface tensions of the pure components;  $\gamma_{SV}$  and  $\gamma_{LV}$ .

$$W_{SL} = 2\Phi(\gamma_{SV}\gamma_{LV})^{0.5} \quad (2.4)$$

Where  $\Phi$  is a correction factor for intermolecular interaction.  $\Phi$  is equal to unity if the intermolecular forces acting across the surface are similar, such is the case with a hydrocarbon liquid interacting with a hydrocarbon solid.  $\Phi$  is less than unity when the intermolecular interactions that constitute cohesive and adhesive forces do not match, such as the case with a hydrogen – bonding liquid interacting with a pure hydrocarbon surface. Good and Girifalco [101] expressed  $\Phi$  in terms of the molecular level parameters of surfaces such as polarizability, ionization energy and dipole moments. The combination of Good and Girifalco equation and the Young – Dupre equation results in fundamental equation. Eq. (2.5)

$$\gamma_{LV}(1 + \cos\theta) = (\gamma_{SV}\gamma_{LV})^{0.5} \quad (2.5)$$

When the primary forces constituting the cohesive and adhesive interactions of dispersive type, Eq(2. 5) reduces to Eq. (2.6)

$$\gamma_{SV} = \gamma_{LV}(1 + \cos\theta)^2 / 4 \quad (2.6)$$

According to this Eq. (2.6)  $\cos\theta$  will be unity only when  $\Phi^2\gamma_{sv}$  is equal to  $\gamma_{LV}$ . Thus one obtains a relationship between  $\gamma_c$  and  $\gamma_{sv}$  as  $\gamma_c = \Phi^2\gamma_{sv}$ . The critical surface tension  $\gamma_c$  of wetting is equal to the surface free energy only when the interaction parameter  $\Phi$  is equal

to unity. The computation of  $\Phi$  depends upon the detailed knowledge of the chemical constitution of the solid and liquid as well as the model used to compute it.

Fowkes [102] provided a method of analyzing the energetic of the surfaces from the contact angles which does not require detailed knowledge of the surface compositions of solid. He considered that the total surface tension of a solid or a liquid can be decomposed into components corresponding to the specific types of intermolecular interactions.

$$\gamma = \gamma^d + \gamma^p + \gamma^i + \dots \quad (2.7)$$

The division of the surface tension into components allowed the work of adhesion to be expressed [103,104].

$$W_{12} = 2\sqrt{\gamma_1^d \gamma_2^d} + 2\sqrt{\gamma_1^p \gamma_2^p} + 2\sqrt{\gamma_1^i \gamma_2^i} \quad (2.8)$$

The surface tension components of the solid are determined by combining Eq.(2.3) and Eq.(2.8)

$$\gamma_{LV} (1 + \cos\theta) = 2\sqrt{\gamma_1^d \gamma_2^d} + 2\sqrt{\gamma_1^p \gamma_2^p} + 2\sqrt{\gamma_1^i \gamma_2^i} \quad (2.9)$$

Wu [105] used harmonic – mean combining rule of the surface tension components to approximate Young's equation.

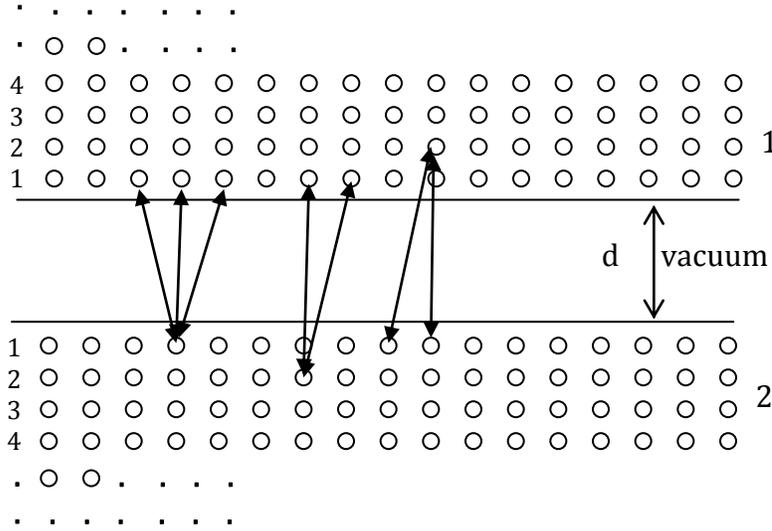
$$\gamma_{LV} (1 + \cos\theta) = 4 \left\{ \gamma_{SV}^d \gamma_{LV}^d / (\gamma_{SV}^d + \gamma_{LV}^d) + \gamma_{SV}^p \gamma_{LV}^p / (\gamma_{SV}^p + \gamma_{LV}^p) \right\} \quad (2.10)$$

### 2.19: Microscopic approach to Surface interfacial interactions.

Good and Girifalco [102] early used pairwise additivity to compute interaction energies across condensed phases. The energy of interaction between two semi – infinite flat slabs (Fig 2.3) is given by Eq.(2.11).

$$G_{12} = \int_{v_1} dv_1 \int_{v_2} n_1 n_2 g_{12} dv_2 \quad (2.11)$$

Where  $dv_1$  and  $dv_2$  are the volume elements of bodies 1 and 2 with respect to  $v_1$  and  $v_2$ ;  $n_1$  and  $n_2$  are the number densities of the oscillators in bodies 1 and 2, and  $g_{12}$  is the interaction energy between two oscillators of bodies 1 and 2.



**Source:** M.K Chaudhury- interfacial interaction between low energy surface,page 102.

**Fig.2.3: Interaction of Two Semi-infinite Solid Bodies, 1 at a Separation,  $d$  in Vacuum**

The interaction energy between two flat slabs was calculated rigorously by Good and Girifalco by considering the Debye, Keesom and London forces. The heuristic derivation of the Good – Girifalco equation using McLachlan's [109] equation according to which  $g_{12}$  can be expressed as:

$$g_{12} = -\left(6kT/R^6\right) \sum_{n=0}^{\infty} \alpha_1(i\omega_n) \alpha_2(i\omega_n) \quad (2.12)$$

Where  $(i\omega_n)$  is the polarizability of the oscillator expressed along the complex frequency axis  $i\omega_n$ . For  $g_{12}$ , the overall interaction energy can be written as:

$$G_{12} = -A_{12}/(12\pi d^2) \quad (2.13)$$

Where  $A_{12}$  is the Hamaker constant and  $l$  is the separation distance,  $A_{12}$  is obtained using Eq.(2.14).

$$A_{12} = 6\pi^2 n_1 n_2 kT \sum_{n=0}^{\infty} \alpha_1(i\omega_n) \alpha_2(i\omega_n) \quad (2.14)$$

The polarizability appearing in the summation can be decomposed into two terms: one arising from zero frequency (d.c photon) interaction and the other from the higher frequency interaction:

$$\alpha_1(i\omega_n) = \left( \mu^2 / 3kT \right) / \left( 1 + \omega_n / \omega_{rot} \right) + \alpha_e(0) / \left[ 1 + \left( \omega_n / \omega_e \right)^2 \right] \quad (2.15)$$

Where  $\mu$  is the dipole moment,  $\omega_{rot}$  is the rotational frequency of the dipole,  $\alpha_e(0)$  is the electronic polarizability and  $\omega_e$  is the electronic excitation frequency.

The zero frequency of Eq(2.14) can be written as:

$$A_{12} I_{n=0} = \pi^2 n_1 n_2 \left[ \left( \mu^2 \mu_2^2 / 3kT \right) + \left\{ \mu_1^2 \alpha_{e2}(0) + \mu_2^2 \alpha_{e1}(0) \right\} + 3kT \alpha_{e1}(0) \alpha_{e2}(0) \right] \quad (2.16)$$

The first term of the above equation is due to the classical dipole – dipole interaction, the second term is due to a dipole – induced dipole, and the third term is due to the Casimir–Polder interaction[110]. The Casimir–Polder interaction is due to electrically neutral atoms or molecules in unexcited state. This formula agrees with the calculations in quantum electrodynamics for the interaction of two atoms at large distances. The higher frequency component of the Hamaker constant can be written as:

$$A_{12} I_{n>0} = (3/2) \pi^2 n_1 n_2 h \omega_{e1} \omega_{e2}(0) \alpha_{e2}(0) / (\omega_{e1} + \omega_{e2}) \quad (2.17)$$

This corresponds to the classical London dispersion interaction.

## 2.20: Macroscopic theory of Lifshitz:

Lifshitz [111] used a macroscopic model, where the interaction energy between two surfaces is calculated from the fourier transform of the normal component of the electromagnetic stress tensor. In a simplified form, the Hamaker constant of interaction at short distance can be expressed according to Lifshitz's theory as follows:

$$A_{12} = 1.5kT \sum_{n=0}^{\infty} \sum_{j=1}^{\infty} \left( \left\{ \left[ \frac{(\epsilon_1(i\omega n) - 1)}{(\epsilon_1(i\omega n) + 1)} \right] \left[ \frac{(\epsilon_2(i\omega n) - 1)}{(\epsilon_2(i\omega n) + 1)} \right] \right\} \right)^j / j^3 \quad (2.18)$$

Here,  $\epsilon_1(i\omega n)$  is the dielectric susceptibility of the material  $m(m\epsilon)$  expressed along the complex frequency axis  $i\omega n$ .

Lifshitz's theory of interaction provides protocol to calculate the interaction energy between condensed phases in terms of the dielectric susceptibility of the materials which are continuum properties that is valid for large separation distance. The continuum approach of Lifshitz does not rigorously apply to wetting and adhesion where the separation distance is comparable with molecular sizes. The required corrections are of second order for dispersion forces. Fowkes[102,112], Isrealachvili[113]Hough and White[114]as well as van Oss et al[115,116,]calculated the Hamaker constant of a number of non-polar liquids and solids, and found that the dispersion component of the surface tension and Hamaker constants form a ratio which is approximately constant for a number of different materials. The Lifshitz's theory has been used to estimate just how much of the interfacial interactions of the polar liquids is contributed by the dipolar interactions.

### 2.21: Surface Tension and Dipole Moments:

The zero frequency term of the lifshitz's equation, which is contributed by the dipolar interactions, predicts that this is almost negligible in comparison with the higher frequency – dispersion interactions. The ratio of the zero frequency term to the sum of higher frequency terms gives the ratio of  $\gamma^p$  to  $\gamma^d$ . For a single component, Eq(19) can be approximated as:

$$A_{11} = 1.5kT \sum_{n=0}^{\infty} \left[ \frac{(\epsilon_1(i\omega_n) - 1)}{(\epsilon_1(i\omega_n) + 1)} \right]^2 \quad (2.19)$$

The ratio  $\gamma^p / \gamma^d$  for a single component is then given by;

$$\gamma^p / \gamma^d = \left[ \frac{(\epsilon_1(0) - 1)}{(\epsilon_1(0) + 1)} \right]^2 / 2 \sum_{n=0}^{\infty} \left[ \frac{(\epsilon_1(i\omega_n) - 1)}{(\epsilon_1(i\omega_n) + 1)} \right]^2 \quad (2.20)$$

The lifshitz's calculation agrees with contention of Fowkes [112], according to which,  $\gamma^p$  of water is negligible because the conflicting dipolar fields cancels each other. It has been noted from Fowkes' idea to ignore the dipolar forces for surface interaction; but it should be borne in mind that the dipoles have certain degree of orientation at the air–solid or solid-solid interfaces, and thus have uncompensated electrostatic fields, the effect of

which may not be negligible. For liquids however, no simple correlation between surface (or interfacial) and molecular dipole moments can be found.(Table 2.4).

**Table2.4: interfacial tension and dipole moments**

Compound	Contacting phase	$\mu$ (Debye)	$\gamma$ (mNm <sup>-1</sup> )
Water	Air	1.85	72.8
Chloroform	Air	1.01	27.13
Ethanol	Air	1.7	22.4
Acetonitrile	Air	3.4	29.3
Benzene	Air	0	28.9
Toluene	Air	0.4	28.5
Benzene	Water	0	35
Heptane	Water	0	50.2
Nitrobenzene	Water	3.9	25.7
Ethanol (dissolves)	Water	1.7	0

**Source:** M.K Chaudhury – interfacial interaction between low energy surfaces, page 107.

**2.22: Role of Surface Free Energy in the Adhesion under Liquid: Stability of particles suspension:**

Neumann et al [117] devised several methods to estimate the surface free energy of solid, which are based on adhesion under liquid media. The central theme of these method is based on the following equation.

$$\Delta F_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \quad (2.21)$$

Where  $\Delta F_{132}$  denotes the free energy of adhesion of two surfaces 1 and 2 in liquid 3. In principle, the free energy of adhesion can take any value from negative to positive, depending upon the relative magnitude of the interfacial tension of the three interfaces.

When 1 and 2 denote the same particles, Eq (2.21) reduces to;

$$\Delta F_{131} = -2\gamma_{13} \quad (2.22)$$

In Neumann's studies, it has been assumed that the interfacial tension between a solid and liquid can only be positive or zero. If the interfacial tension is positive, the particles will coagulate spontaneously to an unstable suspension. This kind of situation arises for hydrophobic particles suspended in a hydrophilic liquid, such as water. If now, the surface tension of the liquid is slowly decrease by adding a low surface tension liquid to the first, a situation may arise when  $\gamma_{13}$  becomes zero. In that case, there is no driving force for the particles to adhere, and a stable suspension results. The surface tension of the liquid mixture at which this occurs was taken by Neumann et al as the surface free energy of the particle [56, 118]. They used this technique to determine the surface tensions of several polymer particles, including biological and bacteria.

### 2.23: Repulsive Van der Waals interaction between particles:

Neumann et al [117] studied the repulsive van der Waals interactions between two dissimilar materials in liquid as predicted by Lifshitz theory. They studied this phenomenon with a number of polymeric particles in naphthalene and found that the particles are either rejected or engulfment by the solidification front. Engulfment of the particles depends on the speed of solidification. At lower speed, particles are rejected while at higher speed, the particles are engulfed due to hydrodynamic drag forces. Using dimensional analysis, Neumann et al[117] developed Eq.(2.23).

$$\Delta F^{adh} = 2.64 \times 10^5 \frac{\rho_L^{0.847} T^{0.280} k_B^{0.720}}{\mu^{0.127} (\rho_P C_P)^{0.441}} D^{0.407} V_c^{0.847} \quad (2.23)$$

Eq.(2.23) provides a novel to estimate the free energy of adhesion  $\Delta F$  from the advancing solidification front. The free energy of adhesion can further decomposed according to Eq.(2.24).

$$\Delta F^{adh} = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \quad (2.24)$$

Neumann used Eq.(2.24) to determine the surface free energy of several polymer particles (Table 2.5).

**Table: 2.5: Surface tension values of several polymers obtained from advancing solidification.**

Particle material (mJ/m <sup>-2</sup> )	Surface free energy
Acetal	44.3
Nylon - 12	40.6
Nylon- 6,12	34.0
PVC	32.7
PMMA	35.3

**Source:** M.K Chaudhury- interfacial interaction between low energy surfaces,page 112.

This technique was found to be applicable not only to various polymers but with biological cells at the interface. The interfacial tension between two surfaces can be written.

$$\gamma_{12} = \gamma_{12}^{LW} + \gamma_{12}^{AB} \quad (2.25)$$

Eq.(2.25) can be reduced as

$$\gamma_{12} = \left( \sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}} \right)^2 + \left( \gamma_1^{AB} + \gamma_2^{AB} - W_{12}^{AB} \right) \quad (2.26)$$

The LW component of the interfacial tension is always positive, whereas the AB component of the interaction can have a negative value, since neither acid nor a base interacting with each other are self-associative. In this case the AB component of the interfacial tension is negative.

Chuadhury [116] stated that a negative interfacial tension implies that the interface will disintegrate accompanied by chaotic and dissipation transport unless there are other mechanisms to stabilize the surface.

#### **2.24: Donor – acceptor interaction at surfaces:**

Fowkes [112], as well as Bolger and Micheals[118] stated that the interaction between surfaces can be thought of consisting of two major components; dispersion forces and acid – base interactions. Drago’s et al [119] gave a quantitative estimate of the Lewis acid – base interaction energy. The enthalpy ( $\Delta H$ ) of acid – base interactions can be estimated

from certain parameters that expresses the basics (B) and acidic (A) properties of the two surfaces. Eq.(2.27)

$$-\Delta H = C_A C_B + E_A E_B \quad (2.27)$$

where C and E represent the covalent and electrostatic interactions (Table 2.6).

Fowkes[112] suggested that if the number of acid – base pairs at an interface is known, the free energy of the interfacial interaction can be obtained using Eq.(2.28)

$$\Delta G = \eta_{ab} \Delta H \quad (2.28)$$

**Table 2.6: Drago's C and E parameters (KJmol<sup>-1/2</sup>)**

Acid	C <sub>A</sub>	E <sub>A</sub>	Base	C <sub>B</sub>	E <sub>B</sub>
Chloroform	0.31	6.77	Ethylacetate	3.56	1.98
Phenol	0.90	8.85	Benzene	1.8	0.75
Water	0.67	5.01	Acetone	4.76	2.02
Butanol	0.61	4.17	Tetrahydrofuran	8.73	2.00
Boron trifluoride	6.30	16.3	Pyridine	13.09	2.39
Iodine	2.04	2.04	Triethylamine	22.67	2.02
Silica	8.98	2.33	Diethylsulfide	15.13	0.70
Rutile	11.6	2.09	PMMA	1.39	1.96

Source: M.K Chaudhury- interfacial interaction between low energy surface, page 113.

### 2.25: Acid-Base interaction in Adsorption and Adhesion.

Drago et al[119] estimated the C and E parameters from the heat of solution. For powders and flat surfaces, heat of adsorption ( $\Delta H$ ) can be estimated from the temperature – dependent adsorption constants according to Eq (2.29)

$$-\Delta H = -\Delta H_{ads} = RT_1 T_2 / (T_2 - T_1) \ln \left( \frac{k(T_2)}{k(T_1)} \right) \quad (2.29)$$

Where  $k(T_2)$  and  $k(T_1)$  are the adsorption constants at temperatures  $T_2$  and  $T_1$  respectively. These constants are generally determined from Langmuir's adsorption plots or using Eq(2.27).

$$\frac{C}{\Gamma} = \frac{1}{\Gamma_m k(T)} + \frac{C}{\Gamma_m} \quad (2.30)$$

Where  $\Gamma$  is the surface concentration,  $\Gamma_m$  is the maximum value for  $\Gamma$  and  $C$  is the bulk concentration of the adsorbent. The value of  $\Gamma_m$  provides an estimate of the number of acid or base sites present on a solid substrate. Once these values are known, the acid – base component of the work of adhesion for two dissimilar materials can be estimated using Eq.(2.27) and (2.28).

Fowkes also devised a method to determine the acid – base properties of flat solid surfaces using the contact angle. He measured the contact angles of a dispersive liquid mixed with a small amount of acid or base on a solid surface. Using the Gibbs adsorption equation, the change of contact angle due to adsorption can be written as

$$d(\gamma_{LV} \cos\theta) = d(\gamma_{SV} - \gamma_{SL}) = \Gamma_i d\mu_i \quad (2.31)$$

The surface excess quantity can be obtained as

$$\Gamma_i = d(\gamma_{LV} \cos\theta) / d\mu_i \quad (2.32)$$

From the surface excess quantities, the adsorption constants and the heat of adsorption can be determined using Eq (2.29) and (2.30).

Fowkes proposed that adsorption of polymers from organic solvents onto inorganic surfaces is a process that involves dispersion forces and acid – base interactions. Because of the polarizabilities of the liquids and polymers, the dispersion force contribution to adsorption is normally very small, and hence the acid – base interaction becomes the dominant contributor to adhesion.

### **2.26: Intermolecular Interactions/Van der Waals Interaction**

J.D van der Waals, when studying (1873) the deviation of a real gas behavior from the ideal gas law, proposed the idea that there exist non- covalent and non- electrostatic interactions (apolar interactions) between neutral atoms and molecules. These electrostatic intermolecular forces collectively called van der Waals forces originated from three distinct interactions, Keesom, Debye and London. While these three kinds of interactions have distinct origins, they have in common the fact that their interaction energies decay rapidly with the sixth power of their inter-atomic or molecular distance

Namely:

$$W(r)_{keesom} = -\frac{U_1^2 U_2^2}{3(4\pi \epsilon_0)^2 kT r^6} \quad (2.33)$$

$$W(r)_{Debye} = -\frac{\alpha U^2}{(4\pi \epsilon_0)^2 r^6} \quad (2.34)$$

$$W(r)_{London} = -\frac{3h\nu\alpha^2}{(4\pi\epsilon_0)^2 r^6} \quad (2.35)$$

Equations (2.33- 2.35) are referred to as interaction in vacuum. Of the three interactions, Keesom and debye interactions require that molecules have permanent dipole moments and are therefore not present in all materials. The already small Keesom interaction is virtually completely screened out, especially in aqueous media which contain electrolytes. London [120] treated the interacting two atomic systems as dynamic and attributed van der Waals to the dispersion effect. The dispersion effect is the interaction between the instantaneous dipoles formed in the atoms by their orbiting electrons. London dispersion forces are the dominant and the most forces in many system. The dispersion forces play a role in a host of important phenomena such as adhesion, surface tension, wetting, physical adsorption, the flocculation of particle, the properties of gases, liquids and thin films, the strength of solids and others. The energy of interaction between atoms *i* and *j* separated by a distance *H* is given as;

$$E = -\frac{\lambda_{ij}}{H^6} \quad (2.36)$$

Where  $\lambda_{ij}$  is the London constant, whose value depends on the atomic numbers of the two interacting atoms. Equation (3.45) is valid only at distances less than the wavelength of the major electronic adsorption band for the gas due to the transition from the ground state to an excited electronic state. At separations greater than this, retardation effects become importance and the attractive energy is inversely proportional to *H*. Retardation effects are caused by the fact that the electromagnetic field has to travel farther at greater separations. By the time the field influences the neighboring atom, the original atomic

dipole has changed its orientation. This effect causes the interaction to be slight out of phase. The attraction energy is still attractive but has been reduced.

Van der Waals forces exist not only between individual atoms and molecules but also between particles. Hamaker [121] used the additivity concept proposed by London [120] to determine the equations for the van der Waals forces between particles. The additivity concept allows the force to be calculated based on the interaction between individual atoms making up the particles. The non-retarded energy between two particles, 1 and 2, of volumes  $v_1$  and  $v_2$  containing  $q_1$  and  $q_2$  atoms per  $\text{cm}^3$  is shown in Fig 2.3. and Eq.(2.37)

$$E = - \frac{\int v_1^d v_1 \int v_2^d v_2 q_1 q_2 \lambda_{1,2}}{H^6} \quad (2.37)$$

The van der Waal force will be given by:

$$F_{vdw} = \frac{\partial E}{\partial H} \quad (2.38)$$

Eq.(2.37) combined with Eq.(2.38) has been solved for the van der Waals force equations between bodies of regular geometric form.[121]

**Case 1:** For two spheres of radii  $R_1$  and  $R_2$

$$F_{vdw} = \frac{AR}{12H^2} \quad (2.39)$$

where  $R$  equals the reduced radius or

$$R = \frac{2R_1 R_2}{R_1 + R_2} \quad (2.40)$$

and  $A$  is called Hamaker's coefficient (or constant) and equals (for Hamaker's development).

$$A = \pi^2 q_1 q_2 \lambda_{1,2}. \quad (2.41)$$

**Case 2:** For a sphere of radius  $R$  and a plane surface;

$$F_{vdw} = \frac{AR}{6H^2} \quad (2.42)$$

**Case 3:** For two plane surfaces, the solution of equation (2.36 and (2.37) is expressed as a pressure,  $p$ , or van der Waals force per unit area of contact:

$$F_{VDW} = \frac{\partial F_{VDW}}{\partial A} = \frac{A}{6\pi H^3} \quad (2.43)$$

Eq. 2.41, 2.42 and 2.43 describe non- retarded van der Waals forces for perfectly smooth surfaces. The approach of Hamaker assumes complete additivity of forces between individual atoms and is called the microscopic approach to van der Waals forces.

Lifshitz [122] developed the macroscopic theory (also called the modern or continuum theory) of van der Waals forces between and within continuous materials. He argued that the concept of additivity was unsatisfactory when applied to closely packed atoms in a condensed body. He attributed the non- additivity to the thermodynamic fluctuations always present in the interior of a material medium.

### 2.27: Lifshitz - van der Waals Interaction

The surface tension  $\gamma_i$  ie the surface free energy per unit area of a liquid in vaccum is equal to one half he free energy of cohesion ( $\Delta G_{ii}$ ) and opposite in sign;

$$\gamma_i = -\frac{1}{2}\Delta\gamma_{ii} \quad (2.44)$$

For solids, equation (2.44) is equally true but solids differ from liquids in that  $\Delta\gamma_{ii}$  is not their free energy of cohesion, but just the free energy available for interacting with liquids. Fowkes proposed that surface free energy of materials could be considered to be the sum of the components resulting from each class of intermolecular interaction ie

$$\gamma_i = \sum_j \gamma_{ij} \quad (2.45)$$

where the  $\gamma_j$  term represent the specific contributors eg hydrogen bonding, dipolar, dispersion etc.

Using the Lifshitz approach for van der Waals interaction in condensed media, Chaudhury,[116] experimentally demonstrated that dispersion (London), induction (Debye) and dipole (Keesom) contributions to the Lifshitz-van der Waals or (apolar) components of the surface tension  $\gamma^{LW}$  are additive .

$$\gamma^{LW} = \gamma^L + \gamma^D + \gamma^K \quad (2.46)$$

Thus, it follows that on a macroscopic level, the three types of van der Waals interactions; (Keesom, Debye and London) can be treated together as the total of apolar, or Lifshitz- van der Waals (LW) interaction. The interfacial tension  $\gamma_{12}$  between two different materials 1 and 2 is one of the most important concept in colloidal and surface science as it leads directly to a quantitative expression for the free energy of inter-particle or intermolecular interaction in condensed phase system. Interfacial tensions between two reasonably immiscible liquids can be measured, but interfacial tensions between solid and liquid and between solid and solids cannot be determined directly. It thus becomes important to arrive at these interfacial tensions  $\gamma_{12}$  via the surface tension  $\gamma_1$  and  $\gamma_2$  of the interacting materials 1 and 2. Good and Grifalco and Fowkes demonstrated experimentally that if only dispersion interaction forces are available between two condensed phase materials e.g a solid and a liquid, the interfacial tension between them is given by the following equations;

$$\gamma_{12}^{LW} = \left( \gamma_1^{LW} - \gamma_2^{LW} \right)^2 \quad (2.47)$$

or,

$$\gamma_{12}^{LW} = \gamma_1^{LW} + \gamma_2^{LW} - 2\sqrt{\gamma_1^{LW} \gamma_2^{LW}} \quad (2.48)$$

equation 3.60 is referred to as Good- Grifalco- Fowkes combining rule.

If we recall Eq(2.44), the apolar component of the free energy of cohesion of material 1 is;

$$\gamma_{ii}^{LW} = -2\gamma_1^{LW} \quad (2.49)$$

The free energy of interaction between materials 1 and 2 in vacuum is related to the surface tension by the Dupre equation,

$$\Delta\gamma_{12}^{LW} = \gamma_{12}^{LW} - \gamma_1^{LW} - \gamma_2^{LW} \quad (2.50)$$

The substituting equation 2.48 into equation 2.49, we obtain;

$$\Delta\gamma_{12}^{lw} = -2\sqrt{\gamma_1^{LW} \gamma_2^{LW}} \quad (3.51)$$

This equation states that the atoms at an interface are pulled by those in the neighboring phase. Since the Lifshitz-van der Waals forces are universal and always available at the surface. Eq(2.51) also suggest that the energy of interaction is negative, ie the interaction energy between two purely polar condensed phase is always attractive. Similarly, the interaction energy between molecules of particles of material 1 immersed in a liquid 2 is;

$$\Delta\gamma_{121}^{LW} = -2\gamma_{12}^{LW} \quad (2.52)$$

The two different objects (1 and 2) immersed in a liquid 3 are related to the interfacial tensions by;

$$\Delta\gamma_{132}^{LW} = \gamma_{12}^{LW} - \gamma_{13}^{LW} - \gamma_{23}^{LW} \quad (2.53)$$

Using Eq(2.48) and (2.50) to expand the interfacial surface tensions in Eq(2.53) gives;

$$\Delta\gamma_{132}^{LW} = -2\gamma_3^{LW} - 2\sqrt{\gamma_1^{LW} \gamma_2^{LW}} + 2\sqrt{\gamma_1^{LW} \gamma_3^{LW}} + 2\sqrt{\gamma_2^{LW} \gamma_3^{LW}} \quad (2.54)$$

Since from equation (2.51), it follows from Eq 2.54, that

$$\Delta\gamma_{132}^{LW} = \Delta\gamma_{33}^{LW} + \Delta\gamma_{12}^{LW} - \Delta\gamma_{13}^{LW} - \Delta\gamma_{23}^{LW} \quad (2.55)$$

This is the confirmation of the Hamaker combining rule obtained via a purely surface thermodynamic treatment.

## 2.28: Polar or Lewis acid –base interactions

For some time, it was thought that the Keesom dipole – dipole interactions should be rated separately from the debye and London interactions. Because of the dipolar nature of Keesom phenomena, the term polar was applied to these interactions, in contrast to the apolar, Debye and London interactions. The distinction between all the three apolar electrostatic forces impeded progress in the search for the true polar surface interaction. After Chaudhury [116] showed that the three, apolar, electrostatic forces are simply additive, and should be treated as a single entity, it became possible to

examine the nature of the polar (Lewis) properties of surfaces as an entirely separate phenomena from their electrostatics (Lewis) apolar properties.

In the past, significant advances have been made in the thermodynamic treatment and interpretation of interfacial tension between two interacting surfaces ie solid and liquid. It is now clear that in aqueous media, and especially for solid surfaces which are rich in oxygen such as silicates minerals, the principal polar interaction is hydrogen bonding, involving donors and acceptors. As this type of interaction can be treated as occurring between a Bronsted acid (the hydrogen donor) and a Bronsted base(the hydrogen acceptor), the polar interaction must account for the dual nature of such interaction. Moreover, polar surface interactions are not restricted to hydrogen bonding, so that the polar concept has been extended to include all the electrons donating and electron accepting phenomena, as encompassed in the more general acid- base paradigm of Lewis. To emphasize the (Lewis) acid- base character of the polar interactions, the designation AB has been used.

As the polar and apolar components of the surface tension are additive

$$\Delta\gamma = \Delta\gamma^{LW} + \Delta\gamma^{AB} \quad (2.56)$$

Where,  $\Delta\gamma^{LW}$  is the free energy due to Lifshitz – van der Waals interaction and  $\Delta\gamma^{AB}$  is the same due to acid- base interactions.

Rewriting equation (2.44) as

$$\Delta\gamma_{ii} = -2\gamma_i \quad (2.57)$$

It followed that;

$$\gamma_i^{Total} = \gamma_i^{LW} + \gamma_i^{AB} \quad (2.58)$$

where,  $\gamma_i^{LW}$  and  $\gamma_i^{AB}$  refer to the apolar ( Lifshitz-van der Waals) and polar (acid-base) component of surface tension material  $i$ , respectively.

Van Oss et al[115] suggested, based on Fowkes' acid-base interaction approach, that electron acceptor(Lewis acid) and electron donor(Lewis base) interaction are essentially asymmetrical in the sense that of a given polar substance  $i$ , the electron acceptor and electron donor parameters are usually quite different and play different roles in the

interfacial interactions. Therefore, they must be described by two distinct parameters. This is very different from the LW interaction where, for example, recalling Eq. (3.63)

$$\Delta\gamma_{12}^{LW} = -2\sqrt{\gamma_1^{LW}\gamma_2^{LW}} \quad (2.59)$$

Such a simple combining rule is not applicable to AB interactions. For the AB interactions, the free energy of interaction between two materials, i and j is defined as

$$\Delta\gamma_{ij}^{AB} = -2\sqrt{\gamma_i^\theta\gamma_j^\varphi} - 2\sqrt{\gamma_j^\theta\gamma_i^\varphi} \quad (2.60)$$

Where the electron donor parameter is designated  $\gamma^\theta$  as (basic component) and the electron acceptor parameter is designated as  $\gamma^\varphi$  (acid component). There are two terms in Eq. (3.72) because there two kinds of interactions each of which must be accounted for ie an electron donor  $i$  interacting with an electron acceptor of  $j$  (the first term) and an electron donor of  $j$  interacting with an electron acceptor of  $i$  ( the second term).The negative sign is made imperative by the thermodynamic convention that a negative sign for  $\Delta\gamma$  signified an attraction, given that  $\Delta\gamma_{ij}^{AB}$  is always attractive, or zero, while the two right hand terms under the square root signs are positive, or zero. Either terms in Eq.(2.58) may be zero because, for example surface i, may exhibit electron donating properties ( $\gamma_i^\theta > 0$ ) but no electron accepting properties ( $\gamma_i^\varphi = 0$ )

Such material is termed monopolar. For this situation.

$$\gamma_i^{AB} = 0 \quad (2.61)$$

$$\gamma_i^{AB} = \gamma_i^{LW} \quad (2.62)$$

which is precisely what one would expect for a purely apolar surface. Thus, the condition in Eq(3.74) should not be taken as an indication of the apolar nature of the material, indeed monopolar substances can strongly interact with bipolar materials, and with monopolar materials of the opposite polarity, notwithstanding the seemingly apolar nature of their surface tension. The polar AB free energy of cohesion of material I is the defined as;

$$\Delta\gamma_{ii}^{AB} = -4\sqrt{\gamma_i^\theta\gamma_i^\varphi} \quad (2.63)$$

Since  $\Delta\gamma_{ii} = -2\gamma_i$  is the polar components of the surface tension of material I is defined as,

$$\gamma_i^{AB} = -2\sqrt{\gamma_i^\theta} \gamma_i^\varphi \quad (2.64)$$

The factor of 2 in Eq (2.58) and (2.74) is needed to maintain the values of a comparable order of magnitude for  $\gamma_i^\theta \gamma_i^\varphi$  and  $\gamma_i^{AB}$

From Dupre equation which is applicable for any type of interaction, one may define,

$$\Delta\gamma_{12}^{AB} = \gamma_{12}^{AB} - \gamma_1^{AB} - \gamma_2^{AB} \quad (2.65)$$

We can express the interfacial tension  $\gamma_{12}^{AB}$  between substances 1 and 2 as follows.

$$\gamma_{12}^{LW} = \Delta\gamma_{12}^{AB} + \gamma_1^{AB} + \gamma_2^{AB} \quad (2.66)$$

Substituting the values for  $\Delta\gamma_{ij}^{AB}$  from equation 3.65 and the values for  $\gamma_1^{AB}$

and  $\gamma_2^{AB}$  from equation 3.75 gives

$$\gamma_{12}^{AB} = 2\sqrt{\gamma_1^\theta} \gamma_1^\varphi + \sqrt{\gamma_2^\theta} \gamma_2^\varphi - \sqrt{\gamma_1^\theta} \gamma_2^\varphi - \sqrt{\gamma_2^\theta} \gamma_1^\varphi \quad (2.67)$$

which can be written as in Eq(2.75)

$$\gamma_{12}^{AB} = 2\sqrt{\gamma_1^\varphi} - \sqrt{\gamma_2^\varphi} \cdot \left( \sqrt{\gamma_1^\theta} - \sqrt{\gamma_2^\theta} \right) \quad (2.68)$$

which is equivalent to equation (3.59) for Liftshitz-van der Waals (LW) interactions.

Examination of the expression for the AB components of the interfacial tension Eq( 2.68)

shows that  $\gamma_{12}^{AB}$  is not restricted to the positive values or zero, as is the case for  $\gamma_{12}^{LW}$ ,

rather  $\gamma_{12}^{AB}$  will be negative when either,  $\gamma_1^\varphi > \gamma_2^\varphi$  .and  $\gamma_1^\theta < \gamma_2^\theta$

$$\gamma_1^\varphi < \gamma_2^\varphi \text{ .and } \gamma_1^\theta > \gamma_2^\theta \quad (2.69)$$

Fowkes' surface tension components approach can be applied to interfacial tension as follows

$$\gamma_{12} = \gamma_{12}^{LW} + \gamma_{12}^{AB} \quad (2.70)$$

Therefore, since AB and LW components of the interfacial tension are additive, the total expression for the interfacial tension between two condensed phases is

$$\gamma_{12} = \left( \sqrt{\gamma_1^{LW}} - \sqrt{\gamma_2^{LW}} \right)^2 + 2 \left( \sqrt{\gamma_1^\theta} \gamma_1^\theta + \sqrt{\gamma_2^\theta} \gamma_2^\theta - \sqrt{\gamma_1^\theta} \gamma_1^\theta - \sqrt{\gamma_1^\theta} \gamma_2^\theta \right) \quad (2.71)$$

which can be re-written as;

$$\gamma_{12} = \left( \sqrt{\gamma_1^{Total}} + \gamma \sqrt{\gamma_2^{Total}} \right)^2 - 2 \sqrt{\gamma_1^{LW}} \gamma_2^{LW} - 2 \sqrt{\gamma_1^\theta} \gamma_2^\theta - \sqrt{\gamma_1^\theta} \gamma_2^\theta \quad (2.72)$$

Dupre' equation for three condensed media (of which at least one, must be a liquid), gives;

$$\Delta \gamma_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \quad (2.73)$$

Expanding this in terms of the *AB* and *LW* components yields;

$$\begin{aligned} \Delta \gamma_{132} = & 2 \left( \sqrt{\gamma_1^{LW}} \sqrt{\gamma_3^{LW}} + \sqrt{\gamma_2^{LW}} \sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}} \sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}} + \sqrt{\gamma_3^\theta} + \sqrt{\gamma_1^\theta} + \sqrt{\gamma_2^\theta} - \sqrt{\gamma_3^\theta} \right) \\ & + \sqrt{\gamma_3^\theta} \left( \sqrt{\gamma_1^\theta} + \sqrt{\gamma_2^\theta} - \sqrt{\gamma_3^\theta} \right) - \sqrt{\gamma_1^\theta} \gamma_2^\theta - \sqrt{\gamma_1^\theta} \gamma_2^\theta \end{aligned} \quad (2.74)$$

Similarly, the interaction energy between two identical materials, 1, immersed in liquid,3, gives;

$$\Delta \gamma_{131} = 2 \sqrt{\gamma_{13}} = 2 \left( \sqrt{\gamma_1^{LW}} - \gamma_3^{LW} \right)^2 - 4 \left( \sqrt{\gamma_1^\theta} \gamma_3^\theta - \sqrt{\gamma_2^\theta} \gamma_3^\theta - \sqrt{\gamma_1^\theta} \gamma_3^\theta + \sqrt{\gamma_3^\theta} + \sqrt{\gamma_1^\theta} \right) \quad (2.75)$$

Lastly, the interaction between two different substance 1 and 2 in vacuum is

$$\Delta \gamma_{12} = -2 \left( \sqrt{\gamma_1^{LW}} \gamma_2^{LW} + \sqrt{\gamma_1^\theta} \gamma_2^\theta + \sqrt{\gamma_2^\theta} \gamma_1^\theta \right) \quad (2.76)$$

It is clear from equation (2.76) that the sign of the interaction energy between any two materials in vacuum is always negative ie there is an attraction between them and cannot be zero because  $\gamma^{LW}$  for all materials is finite and positive. When in aqueous system, low energy substances interact with each other,  $\gamma_{13}$  is positive and  $\Delta \gamma_{131}$ , negative giving rise to an attraction. The opposite is true for high surface energy substances. Similar analysis can be done for  $\Delta \gamma_{132}$  interactions. The sign would determine whether the interaction eg adsorption, between substances 1 and 2 is thermodynamically possible or not. For example, a negative  $\Delta \gamma_{132}$  would indicate a feasible adsorption reaction whereas a

positive  $\Delta\gamma_{132}$  would indicate that no adsorption would be possible. On the other hand, the magnitude of a negative  $\Delta\gamma_{132}$  is indicative of the strength of the adsorption interaction- the larger the magnitude, the stronger the interaction.

### **2.29: Repulsive Van Der Waals Interactions: Their Role in Various Separation Methods.**

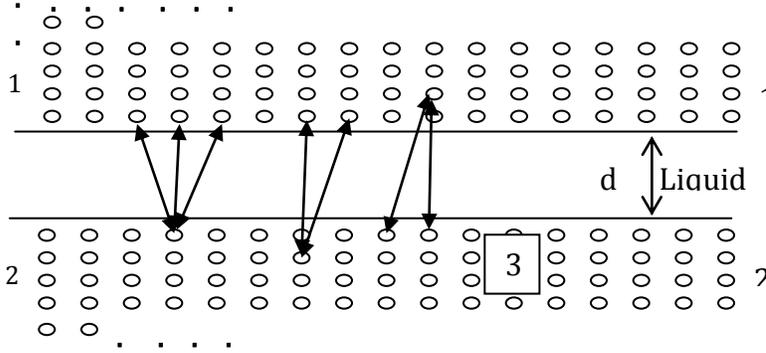
Hamaker H.C [121] in his classical papers stated that “If two particles are embedded in a fluid and the London-van der Waals force between particles and fluid is greater than between the particles themselves, it might be thought that the resultant action will be repulsion rather than an attraction”. Owing to a peculiar property of the London–van der Waals forces, the resultant force is generally attractive even when the particles are surrounded by fluid. This is a matter of considerable interest which warrants a detailed discussion.

Viser[123,114] in a more recent review on Hamaker constants stated explicitly: “When two materials are immersed in a liquid medium and the interactions of each of these materials with that of the liquid medium is larger than the interaction between these materials themselves spontaneous separation can occur due to dispersion forces only”. Fowkes [112] demonstrated the existence of such a repulsive interaction with poly-(tetrafluoroethylene)-glycol-iron oxide.

In more recent work Omenyi et al[124,115], have shown theoretically and experimentally that the sign of the net van der Waals interaction between two different solid bodies or between two different dissolved macromolecules in liquids, often is negative (i.e. they repel one another) even if they are electrically neutral and even when they are immersed in polar liquids. They also went further to test the methodology arising from these considerations on the dissociations of antigen-antibody bonds of the van der Waals-type and on the elution of proteins from hydrophobic chromatography columns[125] The results of both studies confirmed the validity of the theory and the entire practicality of the ensuing experimental procedures [126].

Clearly, the new capability to change the attraction between different (even neutral) solids submerged in liquids, and/or dissolved macromolecules into repulsion, has considerable implications for a variety of novel as well as traditional separation methods.

The theory involved is basically same as has been introduced earlier. The assumption here for simplicity is the interaction between two different solids (or dissolved) bodies 1 and 2 in a liquid 3 and may be represented as an interaction between semi-infinite slabs.



**Source:** M.K Chaudhury – interfacial interaction between low energy surfaces.

**Fig.2.4:** Interaction of Two Solid Bodies, 1 and 2 at a Separation,  $d$  in liquid 3.

Considering the Hamaker expression for the free energy for that case:

$$\Delta F(d) = \left[ -\frac{A_{132}}{12\pi d^2} \right] \quad (2.77)$$

Assuming a minimum separation distance  $d_0$ , and that Eq (2.77) is still valid for such a small separation distance, the Hamaker coefficient can be expressed thus;

$$A_{132} = -12\pi d_0^2 \Delta F(d_0) \quad (2.78)$$

The Hamaker coefficient  $A_{132}$  for the interaction between two different bodies in a liquid can be calculated from Eq.(2.78) once the free energy of adhesion between the two bodies is known for which:

$$\Delta F_{132}^{adh} = \gamma_{12} - \gamma_{13} - \gamma_{23} \quad (2.79)$$

The values of  $\gamma_{12}$ ,  $\gamma_{13}$ , and  $\gamma_{23}$  can be obtained using the equation of state approach.

Alternatively,  $A_{132}$  can be determined by the use of Eq. (2.80);

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23} \quad (2.80)$$

For this approach  $A_{12}$ ,  $A_{13}$  and  $A_{23}$  are obtained from;

$$A_{ij} = 12\pi d_0^2 \Delta F_{ij}(d_0) \quad (2.81)$$

And  $A_{33}$  can be derived from the free energy of cohesion:

$$\Delta F_{ij}^{coh} = -2\gamma_{iv} \quad (2.82)$$

The  $\gamma_{3v}$  for the liquid 3 was measured using the pendant drop method or with the Wilhelmy method.

A positive value of  $A_{132}$  implies that the net van der Waals interaction between particles 1 and 2 immersed in liquid 3 (Fig 2.4) is attractive, while a negative value means that the net van der Waals forces is repulsive

Actually, it can be easily shown [123] that  $A_{132}$  (considering Eq. (2.80) always is negative when:

$$A_{11} > A_{33} > A_{22} \quad (2.83)$$

Or when:

$$A_{11} < A_{33} < A_{22} \quad (2.84)$$

Which (compares eqn 2.84) is the same as stating that  $A_{132}$  will always be negative when:

$$\Delta F_{11} > \Delta F_{33} > \Delta F_{22} \quad (2.85)$$

or when:

$$\Delta F_{11} < \Delta F_{33} < \Delta F_{22} \quad (2.86)$$

or (see eqn 2.7), when:

$$\gamma_{1v} > \gamma_{3v} > \gamma_{2v} \quad (2.87)$$

or when:

$$\gamma_{1v} < \gamma_{3v} < \gamma_{2v} \quad (2.88)$$

However if the absolute value of  $A_{132}$  becomes closer to zero than  $\approx \pm 3.5 \times 10^{-15}$  ergs ( $3.5 \times 10^{-22}$  J), an exact prediction of attraction or repulsion based on whether  $A_{132}$  is positive or negative may no longer be reliable[126]. This then calls for different separation methods.

### **Separation of Particles by Advancing Solidification Fronts**

The engulfing, or rejection, of solid particles suspended in a melt, by advancing solidification front can be of great importance in science and engineering as a separation method. Various surface thermodynamics as well as fluid dynamics, treatment of particle engulfment or rejection phenomena at solid- liquid interfaces have been published [124,126]. In biology, the engulfment of bacteria by phagocytes (fig 1.2) is major defensive mechanism against infective invaders, whilst the ability of bacteria to be

rejected by phagocytes is the principal mechanism by which many pathogenic bacteria achieve virulence[124,127].The advancing solidification front method has also been proposed as a procedure for separating particles according to size [128]. Particles with diameters varying between 0.2 and 0.01mm, consisting of acetal, nylon-6,nylon-6,6,nylon-12, nylon-6,10, nylon-6,12,polystyrene particles, Teflon, and siliconized glass were used. However, the surface tensions of solid particles and Hamaker coefficient  $A_{132}$  determined by contact angle method (using equation of states) for particle 1,suspended in liquid naphthalene 3, and with solidified naphthalene 2, was found with the help of equation 2.3 and 2.4. Table 2.7 below compares the combined Hamaker coefficient  $A_{132}$  found for various particles, with an advancing solidification front, with an advancing naphthalene solidification front with their engulfment, or rejection by the solidification melt.

**Table 2.7: Hamaker Coefficient  $A_{132}$  at 80°C, Compared with Rejection or Engulfment of Various Particles by an Advancing Solidification Front of Naphthalene[118]**

Nature of particle	$A_{132}$ in 10 <sup>-14</sup> ergs	Particle behavior
Acetal	-3.27	Rejection
Nylon-6	-2.81	Rejection
Nylon-6,6	-2.67	Rejection
Nylon-12	-1.97	Rejection
Nylon-6,10	-0.92	Rejection
Nylon-6,12	+ 0.35	Engulfment
Polystyrene	+ 2.01	Engulfment
Teflon	+ 6.43	Engulfment
Siliconed glass	+ 8.28	Engulfment

**Source:** The concept of Negative Hamaker coefficient;

**Separation of polymer in solution by phase separation:**

Many studies have been published on the phase separation of polymer solutions [129] The same treatment used in the interpretation of engulfment versus rejection of particles

by a solidification front was applied to the prediction of compatibility or separation of polymer pairs in solution [127]. A negative Hamaker coefficient  $A_{132}$  for the interaction of two different polymers 1 and 2 in the solvent 3 implies repulsion between the two types of polymer molecules. As  $A_{131}$  and  $A_{232}$  are always, like molecules will always attract each other. Thus, a negative  $A_{132}$  favors phase separation.

Evidently, in all cases where the combined Hamaker coefficient  $A_{132}$  is unmistakably positive, the polymer pairs exert a van der Waals attraction on each other, in their respective solvents, and are compatible. Clearly, polymer compatibility or separation in ternary systems also conforms well to the theory developed by van der Waals. Attraction allows compatibility or separation of polymer pairs dissolved in a given common solvent, whilst a negative (repulsive) net van der Waals interaction results in polymer separation.

#### **Separation of proteins by Hydrophobic Chromatography:**

Polymeric biological substances, such as protein and polysaccharides have their surface tensions lower than water which is their natural solvent. They will be less attracted to the hydrophobic “low energy” surfaces, in water due to a net positive van der Waals interaction. The biopolymers can be made to adhere to the hydrophobic surfaces as a result of van der Waals interactions. They can also be made repulsive by lowering the surface tension of water to a value below that of a polymer and thus causing it to elute. This mechanism of separation method is called hydrophobic chromatography [125]. This research had earlier revealed that elution of protein from hydrophobic surfaces could be enhanced by the addition of organic solvent such as ethylene glycol [130] and detergents [131].

Phenyl-sepharose as well as octyl-sepharose had also be used in hydrophobic chromatography of proteins [132]. A study was made of the elution of proteins from whole human serum, after adsorption onto phenyl-sepharose, and from the concentrations (and thus the surface tensions) of ethylene glycol corresponding to the maximum concentration of each of the eluted protein fractions [130]. The free energy of detachment  $\Delta F_{132}$  and the values of combined Hamaker coefficients for each eluted protein, were calculated (Table 2.8).

**Table 2.8: Hydrophobic Chromatography on Phenyl-Sepharose of Whole Human Serum [120]**

Eluted Protein	$\gamma_{1V}$ Protein (dynes/cm)	$\gamma_{3V}$ of eluant (dynes/cm)	$\Delta F_{132}$ Protein (ergs/cm <sup>2</sup> )	$A_{132}$ Protein ( $\times 10^{-14}$ ergs)
$\alpha_2$ macroglobin	70.6	67.3	+ 3.5	- 4.3
Serum albumin	70.2	64.0	+ 5.4	-6.6
$\alpha_2$ HS glycoprotein	68.1	59.0	+ 5.6	-6.8
$\beta_{1C}$ - $\beta_{1A}$ (C3)	67.8	56.0	+ 5.8	-7.1
Immunoglobulin G	67.2	54.0	+ 5.6	-6.8
Transferrin	66.8	53.0	+ 5.3	-6.5

\*\* Assuming the separation distance  $d_0 \approx 1.8\text{\AA}$

The  $\gamma_{2V}$  of the adsorbent (phenyl-sepharose) = 40.9 dynes/cm ( $4.09 \times 10^{-2}$  N/m).

The six proteins came off the column in the order of their decreasing surface tension  $\gamma_{1V}$  as the surface tension  $\gamma_{3V}$  of the eluant solution decreased. The free energies of detachment  $\Delta F_{132}$  values were all positive which favoured detachment. The Hamaker Coefficients  $A_{132}$  were all negative which imply a net van der Waals repulsion and higher values are in comparison with those obtained in earlier treated phenomena.

This reveals that hydrophobic chromatography in which solutes (and/or particles) are attached to the adsorbent surface occurs principally by van der Waals attraction. Here the surface tension of the liquid medium is higher than those of the solutes (and/or particles) and the adsorbent. Elution thus occurs when the van der Waals attraction is changed into a repulsion by lowering the surface tension of the liquid to a value intermediate between that of the solutes (and/or particles) and the adsorbent.

Also, solutes may be attached to the adsorbent when the surface tension of the liquid is lower than that of both solutes and the adsorbent. In this case, elution can occur by increasing the surface tension of the liquid to a value in between the surface tensions of the solutes and the adsorbent.

In polar liquids, like water, electrostatic interactions are inevitable. A means of partially eliminating this effect is by the addition of salt. However, too high a salt concentration in

the elution step should be avoided since most salts can create an increase in the surface tension.

### **Separation of Antigens and Antibodies:**

Antigen-antibody bonds are principally Coulombic (electrostatic) and/or van der Waals-London bonds [133]. Some antigen-antibody systems interact solely by van der Waals interactions [134,135] while others do so through a combination of Coulombic and van der Waals bonds. The combination method is always more operative due to the small separation distances between antigenic determinant and antibody active site [135]. Hapten3-azopyridine ( $P_3$ ) when coupled to rabbit serum albumin ( $P_3A$ ) precipitates quite well with rabbit anti- $P_3$  (which is elicited with  $P_3$  coupled to bovine gamma globulin) [134].

Thus the precipitating  $P_3A$ -anti- $P_3$  system was extensively studied, as an exclusively van der Waals-London interaction system and compared with a typical combined van der Waals and Coulombic precipitating system namely bovine serum albumin-goat anti-bovine serum albumin (BSA-anti-BSA) [136].

The following discoveries were made:  $P_3A$ -anti- $P_3$  precipitates can be dissociated at neutral pH at  $\gamma \approx 50$  dynes/cm ( $5 \times 10^{-2}$  N/m). The prevention of  $P_3A$ -anti- $P_3$  precipitate formation is attained at  $\approx 62$  dynes/cm ( $6.2 \times 10^{-2}$  N/m). Lower surface tension is needed for the complete dissociation of  $P_3A$ -anti- $P_3$  precipitates than for the prevention of their formation because once an antigen-antibody precipitate is formed; part of the interstitial liquid between antigenic determinant and antibody-active site is expelled. This condition tends to strengthen the antigen-antibody bond [135], so that it requires more energy to dissociate such a bond, once formed, than to prevent its formation [136].

Precipitates of the combined Coulombic-van der Waals-London system BSA-anti-BSA could not be dissociated at neutral pH at surface tensions of the liquid medium below even 48 dynes/cm ( $4.8 \times 10^{-2}$  N/m). Likewise, the precipitation of BSA-anti-BSA at neutral pH could not be prevented at surface tensions of the medium below 52 dynes/cm ( $5.2 \times 10^{-2}$  N/m). Also, at the surface tension of water and pH values as low as 3 or as high as 9.5, BSA-anti-BSA precipitates could not be dissociated. Only lowering the surface tension of the liquid to  $\approx 50$  dynes/cm ( $5 \times 10^{-2}$  N/m) and lowering the pH at 4.0 or raising it to 9.5, would result in dissociation of BSA-anti-BSA [136,127]. By electrophoresis it

could be demonstrated that in mixed systems as these, the dissociation of antigen-antibody complexes by this method is quite complete [137].

The surface tension of the liquid medium can conveniently be lowered by the addition to the buffer of ethylene glycol, dimethyl sulfoxide or propanol. Concentrations of propanol  $\approx 0.25$  to  $0.50\%$  which lowers the surface tension of water to respectively 59 and 52 dynes/cm ( $5.9 \times 10^{-2}$  N/m and  $5.2 \times 10^{-2}$  N/m) generally suffice. Propionic acid is popular and efficacious in the dissociation of antigen-antibody bonds as its low concentrations readily lower the pH of water to below 4.0 and its surface tension below 44 dynes/cm ( $4.4 \times 10^{-2}$  N/m). Citric acid, acetic acid and acid glycine act in the same manner.

$P_3A$ -anti- $P_3$  complexes dissociated with ethylene glycol solutions will re-precipitate upon removal of the ethylene glycol by dialysis [136]. With the  $P_3A$ -anti- $P_3$  system the  $\gamma_{1V}$  of the antibody-active site probably is close to  $\approx 65$  dynes/cm ( $6.5 \times 10^{-2}$  N/m) and  $\gamma_{2V}$  of the antigenic determinant as low as  $\approx 40$  dynes/cm ( $4 \times 10^{-2}$  N/m).

The deductions here is that in immunochemical systems also, van der Waals interactions can be given a net negative value (i.e. they can be changed from attractive to repulsive interactions) by lowering the surface tension of the liquid medium to a value intermediate between those of the two different interacting sites. This approach to the dissociation of antigen-antibody bonds has a considerable impact on a variety of analytical and preparative immunochemical procedures; it opens new possibilities in the determination of antigen-antibody ratios in circulating antigen-antibody complexes from animals or patients with immune complex disease [137], the quantitative elution of blood group antibodies from erythrocytes [137], the study of the influence of various salts on the dissociation of coulombic antigen-antibody bonds under conditions of zero (or slightly negative) van der Waals attraction [137], and finally, in the improvement of the methods used in immuno-adsorption as earlier discussed in affinity chromatography.

### **2.30: Thermodynamic considerations in particles separations**

Thermodynamically, Omenyi [124] stated the condition for particle engulfment is that the net change in free energy,  $\Delta F_{NET}$ , for the process of particle engulfing is less than zero, ie, if

$$\Delta F_{NET} < 0$$

there will be particle engulfment, and if it is larger than zero, ie, if

$$\Delta F_{NET} > 0$$

There will be particle rejection.

From the thermodynamic model given below,  $\Delta F_{NET}$  for the process the engulfment of a sphere of unit surface area is given by,

$$\Delta F_{NET} = \Delta F_b + \Delta F_c \quad (2.89)$$

The free energy of engulfing of a particle by the solid phase now reduces to

$$\Delta F_{NET} = \gamma_{PS} - \gamma_{PL} \quad (2.90)$$

And the free energy of adhesion of a particle, originally suspended in the liquid, to the solid/liquid interface is,

$$\Delta F^{adh} = \gamma_{PS} - \gamma_{PL} - \gamma_{SL} \quad (2.91)$$

where  $\gamma_{ij}$  is the interfacial free energy.

Adhesion is expected when the free energy change from above equations is negative. If it is positive, repulsion is predicted (Table 2.9). An experimental verification of this thermodynamic prediction was carried out using the experimental rig on Teflon, Polystyrene, Nylon, Siliconed glass and Acetal particles in biphenyl and naphthalene matrices. An agreement was obtained between actual observation and theory (Table 2.9) .

**Table2.9: Change in free energy and thermodynamics predictions.**

Matrice materials	$\Delta F_{NET}$ (mJ/m <sup>2</sup> )	Prediction
Biphenyl/silicone glass	-3.4	Engulfing
Biphenyl/Teflon	-2.6	Engulfing
Biphenyl/polystyrene	-0.1	Engulfing
Biphenyl/nylon	+2.5	Rejecting
Biphenyl/acetal	+2.7	Rejecting
Naphthalene/silicone glass	-3.5	Engulfing
Naphthalene/Teflon	-2.7	Engulfing
Naphthalene/polystyrene	-0.4	Engulfing
Naphthalene/nylon	+2.1	Rejecting
Naphthalene/acetal	+2.3	Rejecting

Source: The concept of Negative Hamaker Coefficient.

For the situation at which there is particles rejection is operative, the particle remain at the advancing solid – liquid interface. But with increasing rates of solidification, however, a viscous drag force is generated which opposes the thermodynamic repulsive force. When the two effects become equal, engulfment occurs.

### **2.31: Methods of contact angle measurement:**

The contact angle technique is one of the most convenient ways of determining the energetic of the surface. However, there are various methods that can be used to measure contact angle at the three phase boundary. These include Wilhemy plate method, capillary rise methods, Douy ring method and Sessile drop methods,[138]. All these methods relates the measured contact angle to the surface tension of the liquids and other surface characteristics and discussed below.

**Wilhelmy plate method:** The Wilhelmy plate consist of a thin glass, platinum plate or pre-wetted paper, usually on the order of a few centimeters square, attached to an electrobalance via thin metal wire and used to measure equilibrium interfacial tension at an air-liquid or liquid-liquid interface. The metal plate must be cleaned from organic contaminants or test solutions, therefore the plate is flamed before the experiment to avoid contamination and to help maintain good wetting of the plate by the test liquid. The plate is then immersed and retracted into and out of the test solution contained in a beaker on a mechanical stage. During these cycles the force acting on the plate versus depth of immersion are recorded. The meniscus formed at the solid-liquid interface is characterized by the contact angle. Two contact angles are measured, an advancing contact angle and a receding contact angle and the surface tension is calculated from the resulting force. The static wetting force on the plate is used to calculate the static surface tension,  $\gamma$  using the Wilhelmy equation.

$$\gamma = \frac{F}{l \cdot \cos \theta} \quad (2.92)$$

Where F is the difference in wetting force upon immersion and withdrawal in mN/m,  $l$  is the wetted perimeter of the Wilhelmy plate and  $\theta$  is the advancing or receding contact

angle between the liquid phase and the plate. This method has been extensively developed and used by scientist in the pharmaceutical industry.

With this technique, it is possible to measure and control interfacial properties in granulation and tableting,[139], polymeric surfactant, emulsions and foams, protein-phospholipid interaction[1140], interfacial tension of tropical skin formulations [141], bioadhesive forces between mucosal tissue and microsphere drug delivery system [142].

**DuNoüy ring method:** This method is a traditional method used to measure static or interfacial tension. The measurement simply requires the ring to be wetted by the liquid and then pulled through the interface while measuring the force exerted on the ring. Wetting properties of the surface or interface have little influence on this measuring technique. As in the case of Wilhelmy plate, the ring, with a diameter of 2-3 cm, is usually made up of platinum or iridium is submerged into liquid and then pulled through the liquid-air interface. Maximum pull exerted on the ring by the surface is measured which is directly proportional to the surface tension value at equilibrium [143]. With this method, it is possible to measure the interfacial tension at both liquid –air and liquid – liquid interfaces. Surface tension can be calculated using the equation below

$$\gamma = \frac{F}{p \cos \theta} f \quad (2.93)$$

Where p is the perimeter of the three-phase contact line, is the correction factor between each measurement. One major difference between the Douy ring method and Wilhelmy's plate is the way in which the surface tension measurement is carried out. The ring moves through the interface whereas the plate is static at the interface. Both ring and plate geometries can be used with the force balance type of tensiometer. A single instrument is normally capable of performing either Wilhelmy plate or Douy ring measurement [144]

**Capillary rise method:** This method is based on measuring the penetration time needed for a liquid to rise to a certain height when the end of a capillary is immersed into the solution. When the meniscus is ideally 'hemi-cylindrical' concave in shape, the height at which the solution reaches inside the capillary is related to the surface tension. The wicking of a solvent vertically through a powder is described by Washburn equation.

$$x^2 = \frac{r\gamma \cos\theta}{2\eta}t \quad (2.94)$$

Where  $t$  is the time required for solvent to rise  $x$  millimeters above the solvent through the capillary tube  $\gamma$  and  $\eta$  are the surface tension and viscosity of the solvent,  $\cos\theta$  is the cosine of the contact angle and  $r$  is the internal radius of the capillary [145]. Capillary rise method is a routine measurement for contact angle study of powder and porous materials [146]. However, it has been found that this method tends to overestimate the contact angle value. The best alternative is to measure the contact angle on a compacted powder surface with the use of sessile drop technique.

**Sessile drop method:** The sessile drop method is based on the analysis of the profile of the drop placed on a solid substrate (fig 3.1). This method for contact angle determination is, in principle simple, but great care must be taken to make accurate measurement. The liquid is contained in a syringe from which a droplet is deposited onto the substrate, and a high resolution camera captures the image. The drop can then be analyzed either by eye (using a protractor) or using image analysis software to calculate contact angle, surface and interfacial tension, wettability and absorption [147]. The sessile drop technique can be used to measure contact angle between solid, liquid and vapor phases and characterize the solid surface properties by solving Young's equation:

$$\cos\theta = \frac{\gamma_{SL} - \gamma_{SV}}{\gamma_{LV}} \quad (2.95)$$

### 2.32: Measurement of Contact Angle on Topical and Transdermal Delivery Systems

Contact angle measurement and calculation of surface free energy show that different parts of the skin on the body have different characteristics in term of polarity which is due to the distribution of sebum glands on the skin, [148]. High contact angle value results on poor wettability of the skin surface.

In general, surface tension of clean and dry human skin is 27-28 dyne.cm<sup>-1</sup>. For any substrate to adhere to the surface of the skin, its surface energy must be equal to or less than that of human skin. To design skin adhesives used in bandage, wound healing and transdermal systems, the considerations are; the product has to adhere to the skin for 24

hours to seven days, it should allow removal without excessive trauma to skin, leave no residue on skin upon removal.

In order to meet these challenges, contact angle measurements were performed, [149]. The main obstacle for transdermal drug delivery is the stratum corneum that forms a permeation barrier of drugs. Several techniques have been used to increase drug penetration across the skin including the use of penetration enhancers that disrupts the stratum corneum. Chemical penetration enhancers, such as surfactants, interact with keratin, swell stratum corneum and extract the intercellular lipid matrix of the stratum corneum. Reducing drug/skin interfacial tension improves the contact between the drug and skin and leads to enhanced permeation of the drug through stratum corneum. Some drugs, such as ibuprofen, have shown ionic surfactant activity; therefore acting as self-penetration enhancers [150].

Therefore, the composition of a drugs vehicle should be considered in its nomenclature. Some products have low surface tension and spread rapidly and easily on the surface of skin, while others are difficult to apply to the surface. Study of the physical properties such as surface tension of topical products can be used to provide a more scientific basis for the classification of topical dosage forms, and as a guide for physicians when prescribing topical drugs (Table 2.2) [151].

### **2.33: Measurement of the Surface Free Energy of Bacterial Cell Surfaces and Its Relevant for Adhesion:**

Hendrik et al [152] experimentally determined the contact angles using sessile drop techniques on bacterial layers deposited on cellulose triacetate filters. The filters are completely and homogeneously covered with bacteria. Measurements with water, water-n-propanol mixtures, and  $\alpha$ -bromonaphthalene were employed to calculate surface free energies of various bacteria. Differences of 30-40 ergscm<sup>-2</sup> were obtained for four different bacterial species isolated from the human oral cavity. Methods of calculation yielding  $\gamma_s^d, \gamma_s^p$  together with spreading pressure  $\pi e$  and  $\gamma_{SV}$  separately were employed. The polar and dispersion components of the liquids are known. The results of the contact angle measurement is shown in table 2.10 while the surface energy is shown in table 2.11

**Table 2.10: Contact angle of water, water-n propanol mixtures, and  $\alpha$ -bromonaphthalene on deposits of oral bacteria.**

Liquids	V.alcalescens VI	S.sanguis CH3	S.salivarius HB	S.mitiior T6
Water	20	42	26	55
n-propanol	15	41	26	52
$\alpha$ -Bromonaphthalene	57	41	44	31

Contact angles were corrected for a slight time dependent by linear extrapolation.

**Table 2.11: Surface free energies of oral bacteria**

Bacteria	$\gamma_{SV}$	$\gamma_s^d$	$\gamma_s^p$	$\gamma_s$	$\pi_e$
V.alcalescens VI	60 $\pm$ 1	27 $\pm$ 4	74 $\pm$ 1	101 $\pm$ 4	42 $\pm$ 3
S.sanguis CH3	45 $\pm$ 1	34 $\pm$ 2	52 $\pm$ 2	86 $\pm$ 1	44 $\pm$ 1
S.salivarius HB	58 $\pm$ 2	33 $\pm$ 2	72 $\pm$ 3	105 $\pm$ 5	49 $\pm$ 2
S.mitiior T6	33 $\pm$ 2	38 $\pm$ 1	30 $\pm$ 6	69 $\pm$ 6	33 $\pm$ 5

Values are in ergs per centimeter squared,  $\pm$  the standard deviation.

# CHAPTER THREE

## MATERIALS AND METHODS

### 3.1: Experimental Determination of CD4+ Cell Count

This experiment was carried out to determine the immune system depletion during HIV infection.

#### Materials Used:

- Partec Flow Cytometry instrument (eg CyFlow® SL-3, Code No. CY-S-1023):  
This instrument displays automatically the number of CD4+ T cells per  $\mu\text{l}$  whole blood and as percentage of CD4+ with 2-colour analysis of CD4/CD45, the absolute number of leucocyte, lymphocyte. Like a computer, the partec Cytoflow machine must be backed up with UPS
- Partec test tube (Code No. 04-2000):  
The test tube serves as a reactor where the blood is mixed with other reagents during the test.
- Micropipettes and pipette tips (e.g. Eppendorf, Code No. 3112000.029 and 3111000.0165):  
The micropipettes are used to measure the required volume of the reagents (buffer 1 and 2) into the test tube.
- Power-free latex gloves (e.g. Safeskin, Code No. 545-950-06):  
The glove is used as a safety gadget that ensures that our skin is well protected during the experiment.
- Venous Blood Collection System with EDTA as anticoagulant (e.g. Greiner Bio-One: Vacuette® EDTA Tubes, K3E/EDTA K3, 3ml, Code No. 454217, Vacuette® Blood Collection Needles 38x0.8mm, Code No. 450076, vacuette® Tube Holder, Code No. 450201): The collecting system that serves as a syringe and used to draw blood from the vessel during the test experiment.
- 100ml Buffer 1: This is a solubilization test reagent.
- 100ml Buffer 2: This is a solubilization test reagent.

- 1000 $\mu$ l CD4 mAb PE(MEM-241, PE-Dy647-conjugated monoclonal antibody to human CD4): This recognizes the human CD4 antigen, a transmembrane glycoprotein (55 kDa) of the immunoglobulin supergene family, present on a subset of T-lymphocytes and also expressed at a lower level on monocytes.
- 1000 $\mu$ l CD45 mAb PE-Dy647 (MEM-28, PE-Dy647-conjugated monoclonal antibody to human CD45): The mouse monoclonal antibody HI-28 reacts with all alternative forms of the human CD45 antigen (Leucocyte Common Antigen), a 180 – 220 kDa single chain type 1 transmembrane protein expressed at high level on all of hematopoietic origin.

**Experimental procedure:** A 20 $\mu$ l of whole blood (EDTA as anticoagulant) was added to a Partec test tube. 10 $\mu$ l of CD4 mAb PE and 10 $\mu$ l CD45 mAb PE-Dy647 was added and mixed gently. The mixture was incubated for 15 minutes at room temperature protected from light. 400 $\mu$ l of Buffer 1 was added and gently shaken. Prior to the measurement, 400 $\mu$ l of Buffer 2 was added and analyzed immediately (within 10 minutes) on a Partec CyFlow device.

In order to find the actual CD4+% in the blood. Immediately after the incubation, 400 $\mu$ l of buffer 1 are gently mixed to stabilize the reaction. The CD4+ percentage script is opened but before the program is run, buffer 2 is added.

### **3.7: Experimental Determination of Contact Angles on HIV –Blood Surfaces.**

This experiment was carried to measure the contact angles on samples of HIV infected and uninfected blood.

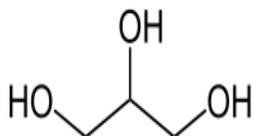
#### **Materials used for the experiment.**

(a). **Test Liquids.** Three liquids (called probe liquids) are used for the experiments. These liquids are, water, glycerine and diiodomethane (Table 3.1)

**Water.** An important feature of water is its polar nature. The water molecule forms an angle, with hydrogen atoms at the tips and oxygen at the vertex. Since oxygen has a higher electronegativity than hydrogen, the side of the molecule with the oxygen atom has a partial negative charge. An object with such a charge difference is called a dipole meaning two poles. The oxygen end is partially negative and the hydrogen end is

partially positive, because of this, the direction of the dipole moment points towards the oxygen. The charge differences cause water molecules to be attracted to each other (the relatively positive areas being attracted to the relatively negative areas) and to other polar molecules. This attraction contributes to hydrogen bonding, and explains many of the properties of water, such as solvent action. Water molecules stay close to each other (cohesion), due to the collective action of hydrogen bonds between water molecules. These hydrogen bonds are constantly breaking, with new bonds being formed with different water molecules; but at any given time in a sample of liquid water, a large portion of the molecules are held together by such bonds. Water also has high adhesion properties because of its polar nature. On extremely clean/smooth glass, the water may form a thin film because the molecular forces between glass and water molecules (adhesive forces) are stronger than the cohesive forces. Water has a high surface tension of 72.8 mN/m at room temperature, caused by the strong cohesion between water molecules, the highest of the non-metallic liquids. Some of these properties make water unique as a contact angle liquid.

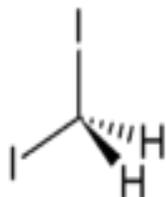
**Glycerine:** Glycerol (or glycerine) is a simple polyol compound. It is a colourless, odourless, viscous liquid that is widely used in pharmaceutical. Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol backbone is central to all lipids known as triglycerides. Glycerol is sweet-tasting and of low toxicity.



Glycerol is shown to reduce the coefficient of friction of polymer coated surfaces by several orders of magnitude. This effect is attributed to the enhanced viscosity of glycerol-water solutions as compared to pure water. Like ethylene glycol and propylene glycol, glycerol is a non-ionic kosmotrope that forms strong hydrogen bonds with water molecules, competing with water-water hydrogen bonds. This disrupts the crystal lattice formation of ice unless the temperature is significantly lowered. It is used as contact

angle liquid because of high cohesive forces within the molecule. The minimum freezing point temperature is at about  $-37.8\text{ }^{\circ}\text{C}$  corresponding to 60–70% glycerol in water.

**Diiodomethane:** Diiodomethane or methylene iodide, commonly abbreviated "MI", is a liquid organoiodine compound. It is insoluble in water, but soluble in ether and alcohol. It has a relatively high refractive index of 1.741, and a surface tension of 50.8 mN/m.



Diiodomethane is a colourless liquid; however, it decomposes upon exposure to light liberating iodine, giving samples brownish colour. Because of its high density, diiodomethane is used in the determination of the density of mineral and other solid samples. It is also used as a contact liquid for refractometers; for example when testing the authenticity of diamonds, with which the compound shares a similar density. Diiodomethane is a reagent in the Simmons – Smith reaction serving as a source of methylene.

**Table 3.1: Physical properties of the contact angle liquids.**

Liquids	Molecular formular	Density (g/cm <sup>3</sup> )	Boiling point( <sup>0</sup> C)	Dipole moment(D)	Surface tension(mJ/m <sup>2</sup> )
Water	H <sub>2</sub> O	1	100 1 atm	1.8546	$\gamma^{LW} = 21.8$ $\gamma^{-} = 25.5$ $\gamma^{+} = 25.5$
Diiodomethane	CH <sub>2</sub> I <sub>2</sub>	3.32	181 1 atm	[1.08]	$\gamma^{LW} = 50.8$ $\gamma^{-} = 0$ $\gamma^{+} = 0$
Glycerol	C <sub>2</sub> H <sub>8</sub> O <sub>3</sub>	1.261	290 1atm		$\gamma^{LW} = 34$ $\gamma^{-} = 3.92$ $\gamma^{+} = 57.4$

**(b) Samples of HIV infected blood and Uninfected blood**

**(i) Sample collection.** Twenty 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 is used and operates 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. Three slides were prepared for each of the twenty samples on different blood component since three liquids are needed for a test. The samples were allowed to dry naturally in 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 5.0 $\mu$ l capacity:** Each of these liquids was dropped on the surface of the prepared slides using a microliter syringe of 5.0 $\mu$ l capacity.

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

**Digital Camera (CANON ZOOM LENS 3.4X):** The spreading process is 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.

**Sessile Drop Technique.**

The sessile drop technique is considered in this work for contact angle measurements. This is simply because;

- When HIV infected blood or uninfected blood is smeared on a glass slide and allowed to dry for contact angle measure; the best approach will 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, and also because of other precipitates in the blood such as the bicarbonate ions.
- In absence of a powerful photomicroscope with optical graticule, the best approach to contact angle determination will be of the high resolution camera to capture the drop profile, making it amenable to use with 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[153]
- The liquid used must not spread on the cast surface of blood components

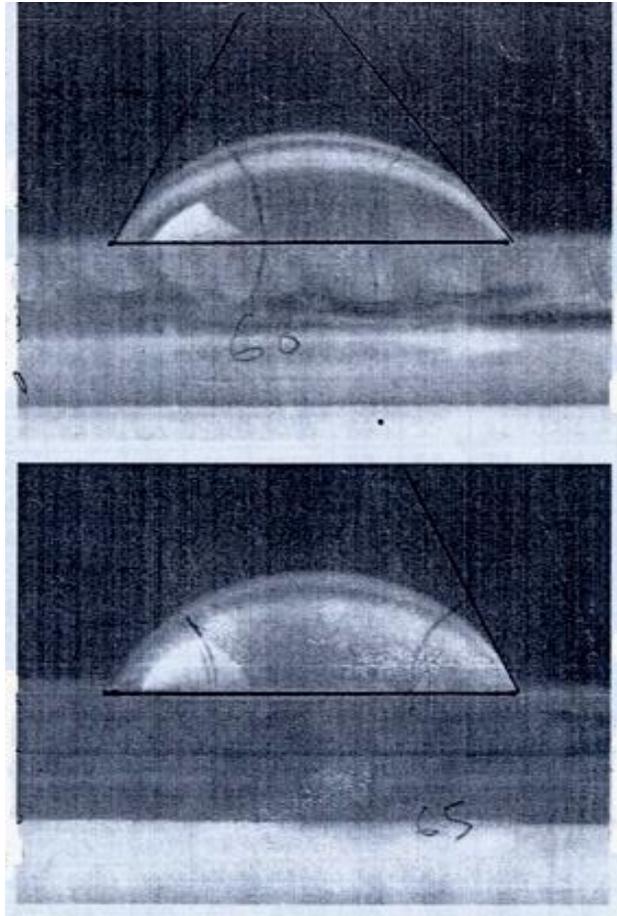
### **Contact Angle Measurement on Whole Blood**

The whole blood contains the mixture of components; white blood, plasma and red blood cells. Each of three test liquids is dropped on the surface (whole blood) of the prepared slides using a microliter syringe of 5.0 $\mu$ l capacity. The tip of the syringe was positioned a few micrometers 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 is 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 3.3).

### **Contact Angle Measurement on Blood Components**

Each of three test liquids is dropped on the surface of the prepared slides of the separated blood components (HIV infected and uninfected Plasma, Leucocytes and erythrocytes) using a microliter syringe of 5.0 $\mu$ l 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 is 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 3.1).



**Fig 3.1: Contact Angle Measurement on Blood Components**

### **3.8: Test of Reliability of Results in Table 4.1**

Reliability is concerned with the ability of an instrument to measure consistently [154]. It should be noted that the reliability of an instrument is closely associated with its validity. An instrument cannot be valid unless it is reliable. The most widely used objective measure of reliability is the Cronbach's alpha. Alpha was developed by Lee Cronbach

[155] to provide a measure of the internal consistency of a test scale; it is expressed as number between 0 and 1. The formula used to calculate the reliability Coefficient;  $\alpha$  is as follows;

$$\alpha = \left( \frac{N}{N-1} \right) \times \left( \frac{\text{Total variance} - \text{Sum of individual variance}}{\text{total variance}} \right) \quad (3.57)$$

$$N = 20$$

Using table 3.4; the test for reliability was done with the results of contact angle measurement on HIV infected and uninfected WBC for each of the twenty samples. This is shown in Appendix F.

$$\begin{aligned} \text{Total variance} &= (\text{score} - \text{Average score})^2 \text{ for the total group} \\ &= 1674.22 \end{aligned}$$

$$\text{Sum of individual variance} = 837.19$$

Substituting in Eq.(3.57), the test for reliability,  $\alpha = 0.526$ .

It is expected that a test of other pairs of sample will not give a result far different from the above value. However, if the items in a test are correlated to each other, the value of alpha is increased though a high coefficient alpha does not mean a high degree of internal consistency because alpha is also affected by the length of the test.

Thus, the calculated value of 0.526 is within accepted range and this shows that the data obtained in this work are valid and reliable. The maximum alpha value of 0.90 has been recommended while the minimum alpha value of 0.70 is recommended for social sciences and 0.50 for natural sciences [156].

### 3.9: Further Validation of Results

Validity is concerned with the extent to which an instrument measured what it is intended to measure. It is related to reliability of the instrument and hence the validity of the experimental results shown in table 3.4 as stated as follows:

- The result of contact angles measured on blood cells as seen from literature shown on table 2.3 agreed with experimental values of contact angle on infected blood using water as the test liquid.
- Also the contact angle measured on some bacteria like B.coli (table 2.3) on substrate has been measured by Hendrik et al and the results show a good

agreement with the experimental values obtained from HIV infected blood using water.

- The equation of states by Neumann show a good agreement with results in table 4.1.
- The reliability coefficient, 0.526 is a close measure of validity.

# CHAPTER FOUR

## RESULTS AND DISCUSSION

**Table 4.1: Result of Contact Angle Measurement Using Probe Liquids**

INFECTED BLOOD CONTACT ANGLE ( $\theta$ ) Results.													UNINFECTED BLOOD CONTACT ANGLE ( $\theta$ ) Results.												
	Whole			WBC			SERUM			RBC				Whole			WBC			SERUM			RBC		
CD4	Wat	Gly	Dio	Wat	Gly	dio	wat	gl	Di	Wat	gly	Dio	CD4	Wa	Gly	Diio	Wat	gly	Diio	Wat	gly	dio	wat	gly	Diio
438	75	62	35	80	58	65	69	59	54	78	57	56	4500	52	47	30	59	45	42	54	45	50	64	42	40
278	66	60	40	78	59	65	69	58	60	65	55	50	6000	55	38	34	78	51	65	60	53	50	65	55	50
282	73	58	50	76	66	50	75	55	62	69	59	56	8000	60	47	33	50	47	32	50	40	45	70	49	48
682	79	59	31	64	67	63	72	65	60	69	59	56	4900	65	49	31	57	48	43	58	55	48	69	43	56
606	74	60	32	70	68	63	70	62	54	63	60	52	5000	59	55	32	63	48	53	58	50	38	63	58	62
20	80	54	38	85	59	60	72	60	48	76	60	66	4500	57	54	38	64	50	50	61	49	47	76	40	65
613	67	63	42	71	68	60	60	70	47	67	67	55	4000	58	46	35	60	48	43	50	50	45	60	47	43
468	76	66	33	76	73	50	60	56	49	60	53	50	6200	59	55	38	63	51	48	58	53	44	60	56	52
853	65	63	45	74	69	54	59	54	50	71	58	50	4900	50	40	39	58	55	54	63	50	52	63	49	40
356	80	63	25	75	65	60	70	58	65	68	62	60	4800	50	45	40	60	50	47	58	52	50	50	44	37
268	68	55	30	64	61	52	65	50	50	68	55	55	5000	55	48	36	64	50	48	58	51	42	73	46	38
625	70	53	37	75	60	41	69	63	57	60	60	56	4000	53	42	32	60	61	40	59	58	57	58	50	35
230	72	54	41	69	63	42	70	63	65	68	66	65	4000	51	40	36	63	50	35	57	53	50	69	55	42
246	70	55	37	70	60	57	57	50	56	64	61	60	4000	58	50	36	64	53	49	62	52	50	60	58	45
339	67	52	34	71	65	63	66	58	50	62	57	55	6000	60	50	40	55	48	48	58	48	51	50	47	39
316	78	64	32	66	63	50	60	59	58	60	57	50	4400	55	50	45	65	51	45	62	51	47	52	50	45
220	73	60	29	73	60	46	65	54	41	63	60	55	4700	53	40	38	58	52	47	54	54	40	55	53	34
374	68	62	50	69	66	50	50	49	48	65	62	60	4800	56	47	43	59	46	38	50	49	43	62	52	47
593	82	59	46	65	60	52	60	58	42	64	60	50	4300	55	50	46	58	50	45	50	48	42	54	42	40
372	75	57	43	78	65	55	70	63	44	72	68	58	6000	52	51	45	60	49	40	50	53	44	57	50	38

The average contact angle for infected and uninfected blood for each test liquid are listed in table 4.1(a-d).

**Table 4.1a: Average contact angle data on whole blood (Appendix A)**

Test liquid	Water	Glycerine	Diiodomethane
Infected	72.9±5.19	58.85±3.91	37.5±7.02
Uninfected	55.65±3.9	47.2±5.08	37.35±4.78

**Table 4.1b: Average contact angle data on white blood cell.(Appendix B)**

	Water	Glycerine	Diiodomethane
Infected	72.45±5.61	63.75±4.09	54.9.5±7.38
Uninfected	60.5±5.41	50.15±3.47	45.60±7.25

**Table 4.1c: Average contact angle data on Plasma.(Appendix C)**

	Water	Glycerine	Diiodomethane
Infected	65.4±6.36	58.2±5.35	53±7.23
Uninfected	56.5±4.47	50.85±3.63	46.75±6.63

**Table 4.1d: Average contact angle data on Red blood cell.(Appendix D)**

	Water	Glycerine	Diiodomethane
Infected	66.6±5.03	68.5±4.88	55.25±4.25
Uninfected	61.9±7.7	49.3±5.46	45.3±8.47

From the tables above, the contact angles increased as the blood components are being infected by the virus; hence a decrease in CD4+ counts. However, infected blood has higher contact angle than the uninfected blood. In general, Water gave the highest contact angles for both the infected and uninfected blood cell prepared slides. Appendix A-D presents the CD4+ cell count and corresponding contact angles with estimated physiochemical properties using different model and measuring liquids. Appendix E is a validation of the physiochemical properties using SCILAB software.

#### 4.1: HIV infection and State of the Cells.

The results shown in Table (4.1) and summarized in (Table 4.2), clearly indicate that infected bloods have high measured contact angle than the uninfected blood in all cases. That is to say that infected cells are poorly wetted. Thus, HIV infection has the tendency to increase the hydrophobicity of the blood as against increase in hydrophilicity for uninfected surface. Since infection is by HIV, it is therefore valid to suggest that HIV surface is hydrophobic.

**Table 4.2: Relationship between the average contact angle and the state of the cells**

Liquids	Water ( $\theta$ )		Glycerine ( $\theta$ )		Diiodomethane ( $\theta$ )	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Whole blood	72.9±5.19	55.65±3.9	58.95±4.07	47.2±5.08	37.5±7.02	37.35±4.78
Leucocytes	72.45±5.61	60.5±5.41	63.75±4.09	50.15±3.47	54.95±7.38	45.60±7.25
Plasma(serum)	65.4±6.36	56.5±4.47	58.2±5.35	50.85±3.63	53±7.23	46.75±6.63
Erythrocytes	66.6±5.03	61.9±7.7	68.5±4.88	49.3±5.46	55.25±4.25	45.3±8.47

Table 4.2 above summarizes the effects of HIV on different components of the blood (Appendix A-D) using the contact angles and standard deviation. At this juncture, it is important to understand how contact angle varies with the CD4+ cell count. CD4+ count gives indication of severity of HIV infection. HIV reduces the CD4+ cell count and the lower the count, the more severe is the HIV attack. This study on HIV-blood interaction is informed by the fact that HIV does not attack other components except T4 lymphocytes. Fig 4.2 shows a plot of contact angles against CD4+ cell counts for infected and uninfected patients. The relationship between the contact angle and wetting with regard to surface constitution is shown in fig 4.1. In all discussions using SPSS ANOVA table, letter a represents infected blood while letter b represents uninfected blood.

\***Contact Angle:** Surface Energy, Change in free energy of adhesion and Hamaker coefficient.

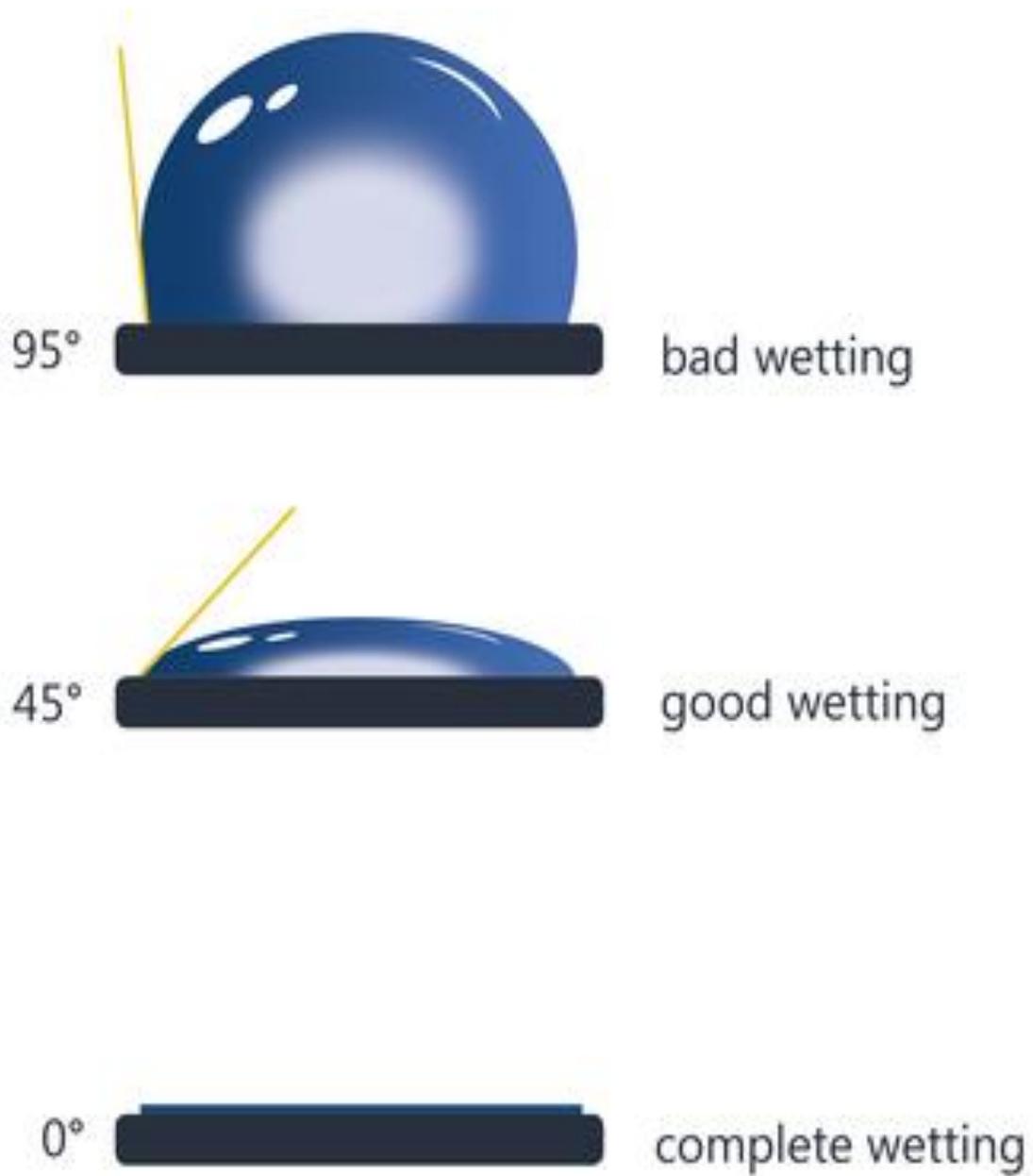
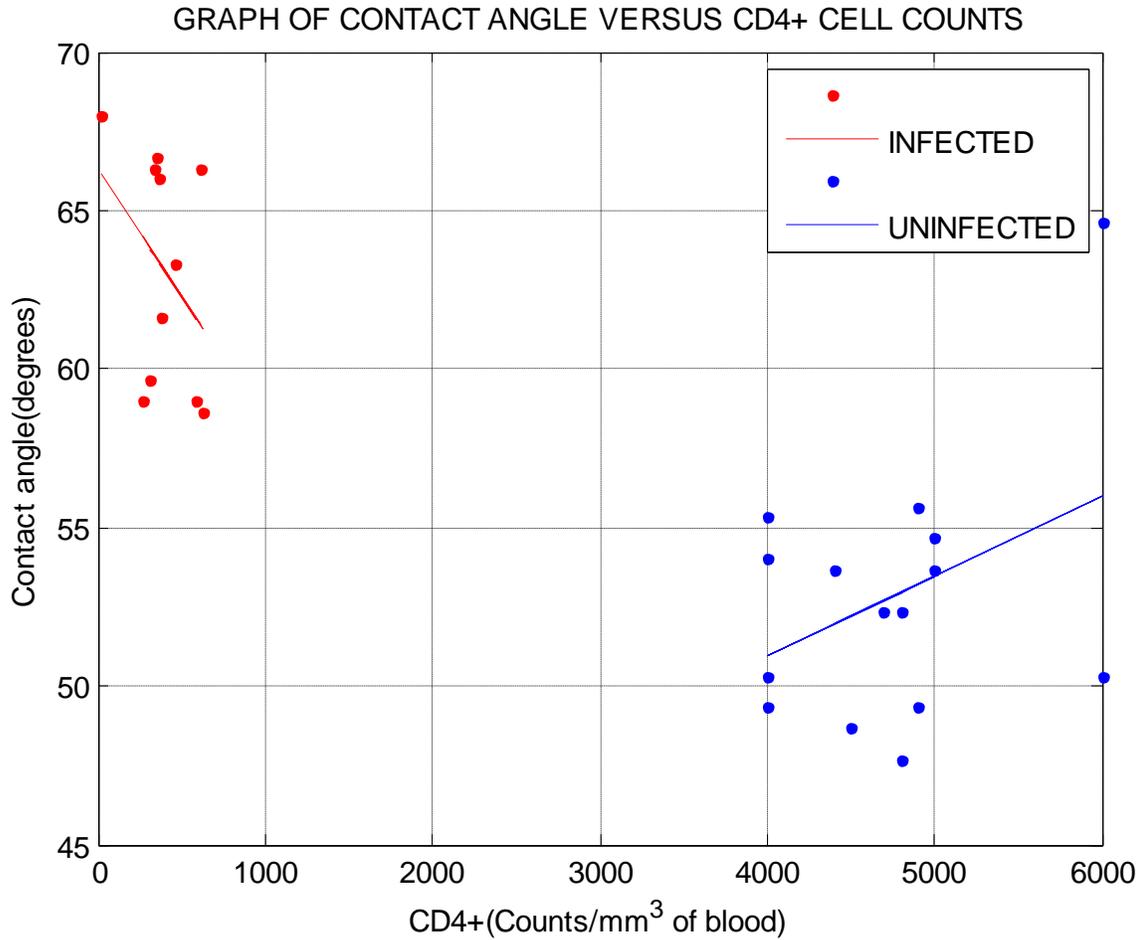


Fig 4.1: Sketch of three degree of wetting and corresponding contact angle.



**Fig4.2: Linear plots of Contact Angle Vs CD4+**

**Table 4.2a(i):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.657	.432	.400	3.725

*The independent variable is CD4+(Counts/mm<sup>3</sup>).*

**Table4.2a(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	189.971	1	189.971	13.689	.002
<i>Residual</i>	249.790	18	13.877		
<i>Total</i>	439.761	19			

*The independent variable is CD4+(Counts/mm<sup>3</sup>).*

**Table 4.2a(iii):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>CD4+(Counts/mm<sup>3</sup>)</i>	-.003	.001	-.657	-3.700	.002
<i>(Constant)</i>	64.876	1.005		64.564	.000

**Table4.2b(i):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.721	.520	.476	1.601

*The independent variable is CD4+(counts/mm3).*

**Table 4.2b(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	30.496	1	30.496	11.894	.005
<i>Residual</i>	28.203	11	2.564		
<i>Total</i>	58.699	12			

*The independent variable is CD4+(counts/mm3).*

**Table 4.2b(iii):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>CD4+(counts/mm3)</i>	.002	.000	.721	3.449	.005
<i>(Constant)</i>	33.067	2.300		14.374	.000

The summary in table 4.2 shows the variation in contact angles and standard deviations obtained from the contact angle measurement on infected and uninfected blood samples using the three test liquids. The contact angle results on HIV infected blood components are in agreement with the literature results (Table 2.3) for contact angle measurements on blood cells, proteins and bacteria. Table 4.2 however, indicated a highest increase in contact angle when water is used as a test liquid. As expected, water has the highest surface tension (72.8N/m) compared with other

two liquids (Table 3.1). The behavior of water is not far fetched. It is a polar solvent whose properties are attributed to the hydrogen bonding. The greater proportion of the polar groups (O-H groups) in water molecules, the stronger the attractive forces between them. Stronger attractive forces give rise to a high surface tension and hence the cohesive force in water molecules is always greater than the adhesive force to the solid (HIV) surface and hence increases in contact angle (Fig 4.1). In the same vein, diiodomethane is completely dispersive whose cohesive force is weaker than the adhesive force to the solid (HIV) surface. The glycerin is an intermediate whose contact angles are in between the water (polar) and diiodomethane (dispersive). The properties of these liquids had also been discussed earlier in chapter three. It is also interesting to observe from Table 4.1, that the contact angle obtained on infected white blood cells are greater than other components of blood irrespective of the liquid used. The linear plots of infected WBC in Fig 4.2 shows that contact angle increases with decrease in the CD4+ cell count when the test liquids are considered on average contact angle. This simply means that the energy at the surface is independent of the liquid used but on the surface characteristics. Meanwhile, the linear plots for uninfected WBC shown in fig 4.2 imply that CD4+ cell counts increase as contact angles increase indicating little or no infection for the twenty sampled patients. The  $R^2$  values for all the trends; polynomial, linear, exponential plots are generally very low. These cannot therefore be used to explain the regression model. This is because the CD4+ count varies according to patient's degree of infection and HIV infection is not the only cause of low CD4+ count as mentioned in the literature. The ANOVA tables (4.2aii and 4.2bii) show that the contact angle and CD4+ count have a limited acceptable level of significance since the values fall below 0.05 levels.

Thus, from all indication, HIV infected surfaces tend to be hydrophobic in nature and hence poor wetting shown by increase in contact angle (Fig 4.1). Such surfaces are regarded as “apolar” or “low energy” surfaces. But as the contact angle decreases for uninfected cells, the surfaces tends to hydrophilic state and such surfaces are regarded as “polar” or “high energy” surfaces with good wetting properties[93]. Appendix A-D show the twenty samples of HIV infected and uninfected blood components with their measured contact angles and corresponding CD4+ counts, neutrophil, PcV.

### Surface free energies and State of the Cells.

The surface energy is a measure of workdone on the surface. When the surface energy is high, the contact angle is usually low for wetting or ‘polar’ surfaces. But when the surface energy is low, contact angle is usually very high for non-wetting ‘apolar’ surfaces (Fig 4.1).The surface free energies determined from contact angle data, for both infected and uninfected blood components are summarized( Table 4.3). Appendix G1 and G2 show the relationship between the surface free energies of infected and uninfected blood components respectively with corresponding CD4+ cell count using the probe liquids. Appendix G3 show average values of surface free energies of the individual blood components with corresponding CD4+ cell counts for twenty patients. This averaging is informed by the fact that surface energy is actually independent of the liquids used. .

**Table 4.3: Summary of Average Surface energies (mJ/m<sup>2</sup>) for twenty blood samples.**

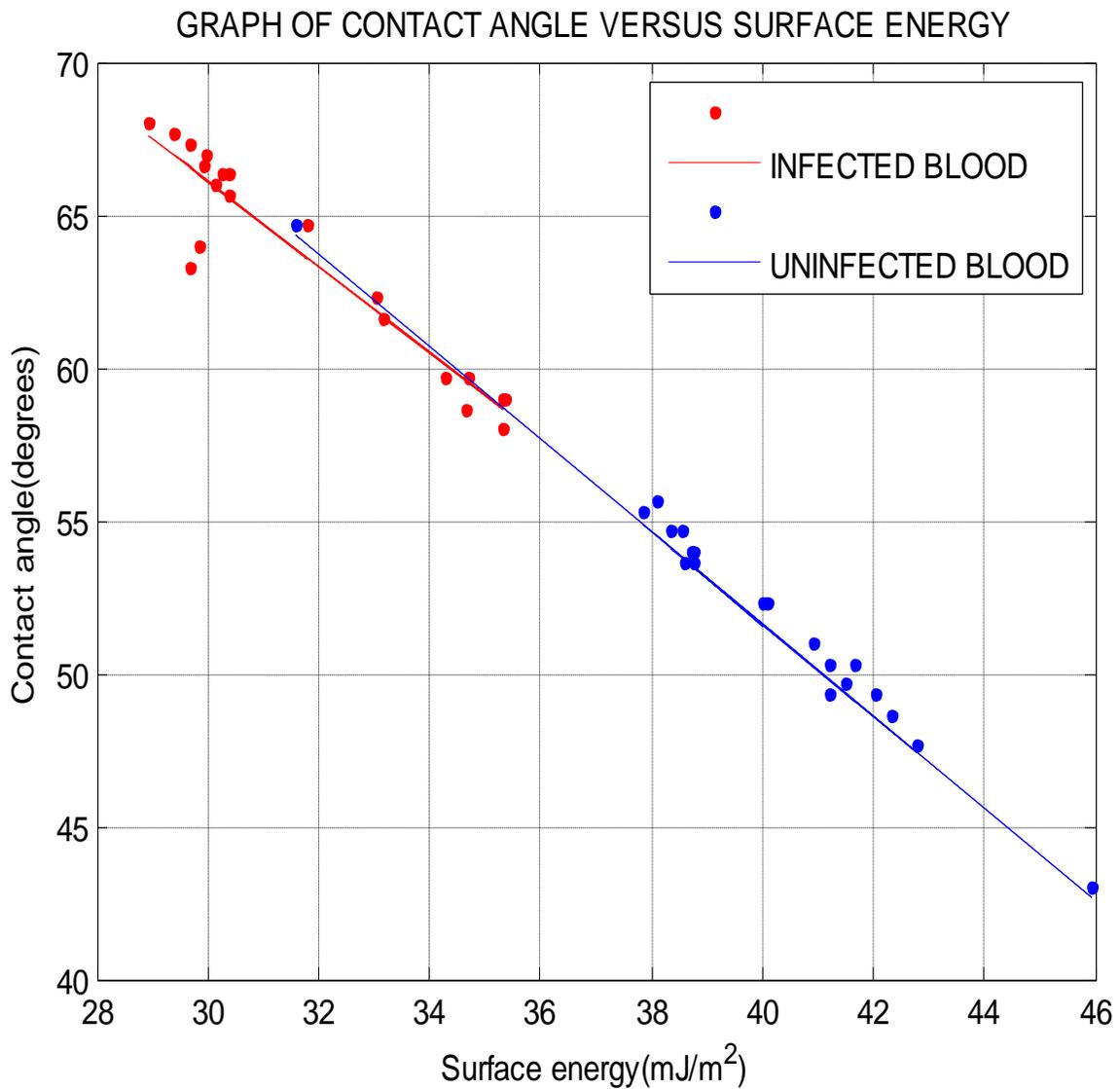
State of the cells	Whole Blood	WBC	SERUM	RBC
Infected	36±1.89	31.81±2.36	35.47±3.29	34.10±2.56
Uninfected	43.53±2.17	39.94±2.82	40.82±2.26	40.09±3.36

Table 4.3 shows that the surface free energies of infected blood components are lower than the uninfected. Tha is to say, that HIV infection has the surface energy reducing capacity, and therefore reduces the work done on the surface. The reduction in surface free energy on WBC is the greatest, by about 20%. While the reduction in Serum and RBC are 13% and 15% respectively. It then shows that WBCs are more greatly attacked by the virus.

Now that it is well known that HIV attacks the WBC; the reduction (difference on infected and uninfected) in surface free energies of Serum and RBC could be as a result of film of HIV on RBC and its presence in the serum. The film of HIV prevents it from penetrating the cell wall of RBC and hence no infection.

Since HIV attacks WBC, penetrates them, destroys their RNA and replicates in the lymphocytes (WBC), further considerations will be on interaction between infected WBC (here assumed to represent the HIV) and the uninfected WBC (lymphocytes) with Serum as the suspending medium.

It could be then deduce from Table 4.3, that infected blood components have low surface free energies than the uninfected blood, whereas for uninfected blood components; WBC, Serum and RBC have fairly the same surface free energy of  $40\text{mJ/m}^2$ . However, it is observed that the surface free energy reduction in infected components is greatest in WBC ( $31.81\text{mJ/m}^2$ ). This shows that WBC is mostly attacked by HIV as confirmed physically. Thus, further interactions between HIV and WBC should be considered. Also, since Table 4.3 show that HIV interacts more with WBC; the interest goes to HIV-WBC relationship as illustrated in Fig 4.3.



**Fig 4.3: Linear plot of contact angle Vs surface energy**

**Table 4.3a(i):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.977	.954	.952	.778

*The independent variable is Surface Energy(mJ/m<sup>2</sup>)*

**Table 4.3a(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	227.761	1	227.761	376.596	.000
<i>Residual</i>	10.886	18	.605		
<i>Total</i>	238.647	19			

*The independent variable is Surface Energy (mJ/m<sup>2</sup>).*

**Table 4.3a (iii): Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>		
<i>Surface Energy(mJ/m<sup>2</sup>)</i>	-1.463	.075	-.977	-19.406	.000
<i>(Constant)</i>	110.247	2.405		45.840	.000

**Table 4.3b(i):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.993	.986	.985	.528

*The independent variable is Surface energy(mJ/m<sup>2</sup>).*

**Table 4.3b(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	344.023	1	344.023	1234.497	.000
<i>Residual</i>	5.016	18	.279		
<i>Total</i>	349.040	19			

*The independent variable is Surface energy(mJ/m<sup>2</sup>).*

**Table 4.3b(iii):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Surface energy(mJ/m<sup>2</sup>)</i>	-1.489	.042	-.993	-35.135	.000
<i>(Constant)</i>	111.759	1.699		65.784	.000

Fig 4.3 shows that contact angle increases when the surface free energy decreases for infected WBC. It should be recalled that at low CD4 count (HIV infected blood), the contact angle tends to increase as a result of decrease in surface energy (Fig4.1) caused by the

presence of the virus. For uninfected cell in Fig 4.3, the contact angle decreases when the surface free energy increases.

$R^2$  – square values of both infected and uninfected WBC show a good curve fit and therefore, can represent a good model for the contact angle and surface energy due to HIV infection. The ANOVA tables (4.3aii and 4.3bii) and coefficients indicate that regression equations are within the significance level.

**Change in Free Energy of Adhesion and State of the cell:**

The surface energy described is the determinant to cell adhesion. When the change in free energy of adhesion is negative; adhesion is thermodynamically favorable. Adhesion is therefore governed by attractive van der Waal forces and hence increases in contact angles. When HIV attaches itself to the surface of the lymphocytes in a liquid medium, there is the tendency of the CD4+ count to be depleted. However, more adhesion leads to more depletion of CD4+ cell and thus, increases in contact angle. Results obtained using different models for the twenty sampled patients are shown in Appendix H1 and summarized in Table 4.4.

**Table 4.4: Average  $F^{adh}$  and State of the Cell.**

Change in free energy of adhesion, $\Delta F^{adh}$ (mJ/m <sup>2</sup> )				
	WU	FOWKES	NEUMAN	G&G
Infected WBC	-63.419±4.94	-58.49±4.33	-23.518±2.29	-36.02±3.22
Uninfected WBC	-57.54±3.84	-51.246±3.57	-15.57±2.91	-25.13±3.82

Table 4.4 shows that the change in free energies of adhesion are all negatives indicating that the net van der Waals forces are attractive. The change in free energy of adhesion is observed to be higher for infected WBCs than uninfected WBC. In other words, presence of HIV increases the change in free energy of adhesion. The change in free energies of adhesion  $\Delta F^{adh}$  increases with increase in contact angle and decrease with CD4+ cell count and also tend to increase with decrease in surface energy. Analysis of the results from table 4.4 shows that change in free energy of adhesion interaction term  $\Delta F_{132}^{adh}$ , using Neumann model (equation of state) is within the range of -41.21mJ/m<sup>2</sup> ( $\approx$ -40mJ/m<sup>2</sup>).



**Table 4.4a(i): Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.975	.950	.947	.813

*The independent variable is Change in free energy of Adh.*

**Table4.4a(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	226.744	1	226.744	342.899	.000
<i>Residual</i>	11.903	18	.661		
<i>Total</i>	238.647	19			

*The independent variable is Change in free energy of Adh.*

**Table4.4a(iii)Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Change in free energy of Adh</i>	-1.508	.081	-.975	-18.518	.000
<i>(Constant)</i>	28.230	1.924		14.673	.000

**Table4.b(i)Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.887	.787	.776	2.031

*The independent variable is Change in free energy of Adh(mJ/m<sup>2</sup>).*

**Table 4.4b(ii): ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	274.817	1	274.817	66.647	.000
<i>Residual</i>	74.222	18	4.123		
<i>Total</i>	349.040	19			

*The independent variable is Change in free energy of Adh(mJ/m<sup>2</sup>).*

**Table 4.4b (iii): Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Change in free energy of Adh(mJ/m<sup>2</sup>)</i>	-1.304	.160	-.887	-8.164	.000
<i>(Constant)</i>	31.906	2.529		12.618	.000

The results obtained using different models are presented in appendix H1. The table shows an increase in attraction ( $-\Delta F^{\text{adh}}$ ) for HIV infected WBC when compared to uninfected WBC irrespective of the model used to obtain the change in free energies of adhesion. The negative values suggest that the interaction is driven by attractive forces. Although attraction is more on infected WBC than uninfected WBC but the negative signs on uninfected cell is an indication that HIV infection is not the only cause of lower CD4 count as observed in appendix H1.

Table 4.4 shows an average values of change in interfacial free energy of adhesion for the twenty samples of infected and uninfected WBC. The table however, shows that attraction is more on infected WBC and less on uninfected WBC. That is to say that HIV infection increases the van der Waals forces of attraction and this minimizes the surface area at the phase boundary. Here, the particles at the surface will try to reduce the free energy by interacting with the particles at the adjacent phase and hence decrease in surface energy.

The linear plot in Fig 4.4 shows that as the contact angle increases as change in free energy of adhesion increases and hence decrease in CD4+ cell count for infected cells and decreases as the change in free energy of adhesion decreases for uninfected cells. Meanwhile, among the models shown in Appendix H1, Neumann indicated lowest attraction (low Change in free energy of adhesion) indicated by the degree of negative signs. And since the low surface energy has been noted for hydrophobic (apolar) surfaces; it suggests that, Neumann model (equation of states) would give a better approximation between an empirical formulations (obtained by macroscopic observations and well documented assumptions) and contact angle data.. Results indicated that equation of state was capable of good prediction, whereas the surface tension approach is limited to higher surface tension of solid surfaces.

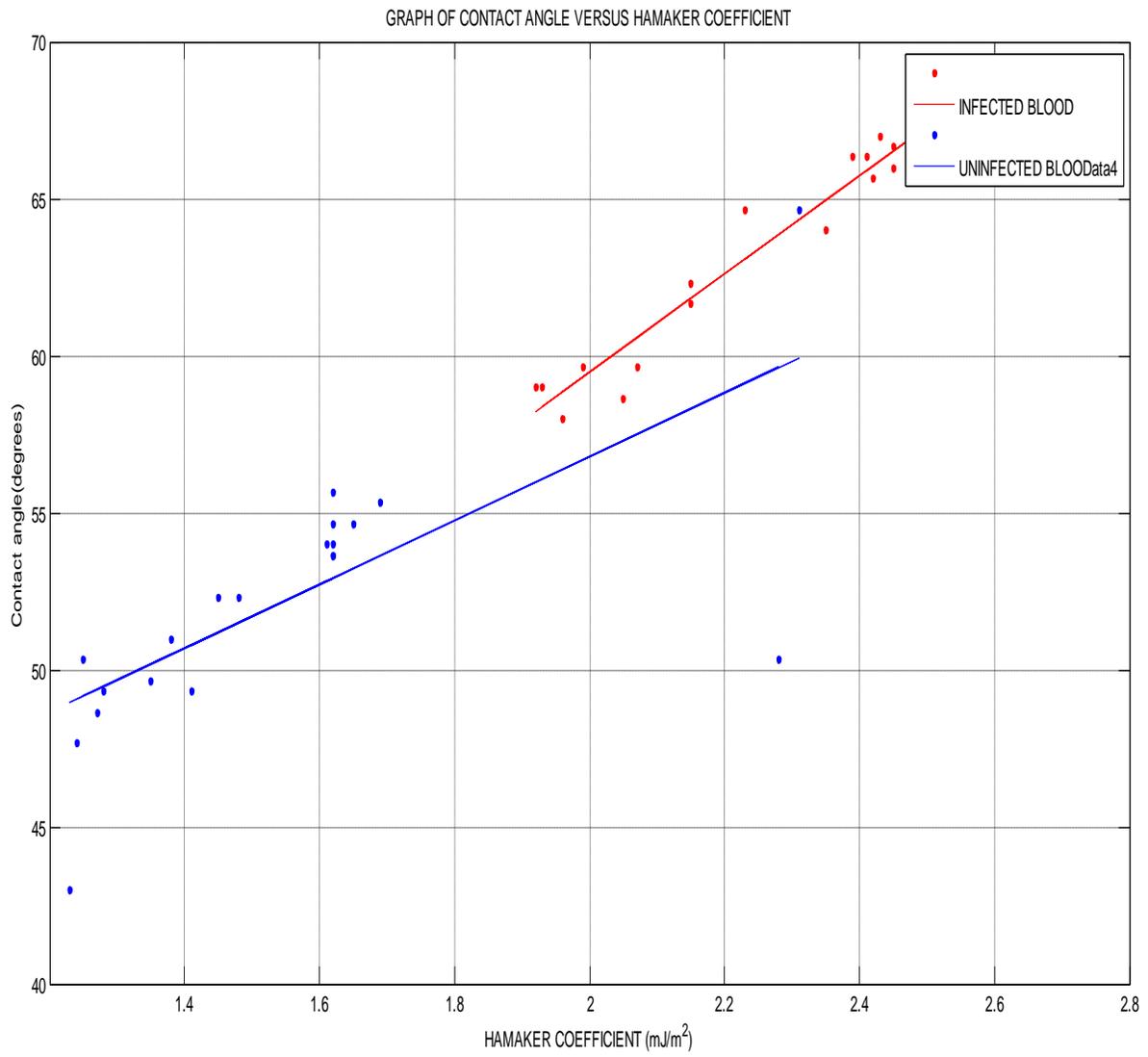
### **Hamaker Coefficient and State of the Cell.**

The energies of interaction can be expressed as a Hamaker constant. It is obtained from the value of surface energy and change in free energy of adhesion from the contact angle data. The positive value of the absolute Hamaker coefficient indicates that the interaction is governed by attractive forces. Appendix I show the Hamaker coefficients for the twenty sampled patient and the average shown in Table 4.5.

Table 4.5: Average  $A_{132}$  using different models on the state of the cell.

Hamaker coefficient $A_{132}(\text{mJ}/\text{m}^2)$				
	WU	FOWKES	NEUMAN	G&G
Infected WBC	6.24E-17±2.43E-18	5.73E-17±2.37E-18	2.27E-17±2.22E-18	3.49E-17±3.19E-18
Uninfected WBC	5.63E-17±2.28E-18	5.08E-17±3.63E-18	1.76E-17±7.61E-18	2.21E-17±4.65E-18

From table 4.5, the Hamaker coefficients are all positive suggesting that the van der Waals forces are attractive. Infected WBCs have higher Hamaker coefficient than uninfected WBCs. Amongst all the models, Wu model indicated highest Hamaker coefficient ( $6.24 \times 10^{-17} \text{mJ}/\text{m}^2$ ) while Neumann model indicated lowest attraction ( $2.27 \times 10^{-17} \text{mJ}/\text{m}^2$ ).



***Fig 4.5: Contact angle Vs Hamaker Coefficient***

**Table4.5a(i):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.975	.950	.947	.816

*The independent variable is Hamaker coefficient(mJ/m<sup>2</sup>).*

**Table4.5a(ii):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	226.654	1	226.654	340.170	.000
<i>Residual</i>	11.993	18	.666		
<i>Total</i>	238.647	19			

*The independent variable is Hamaker coefficient(mJ/m<sup>2</sup>).*

**Table 4.5a(iii):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Hamaker coefficient(mJ/m<sup>2</sup>)</i>	15.617	.847	.975	18.444	.000
<i>(Constant)</i>	28.238	1.931		14.622	.000

**Table 4.5b(i): Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.950	.902	.897	1.378

The independent variable is Hamaker Coefficient  $mJ/m^2) \times 10^{-17}$ .

**Table 4.5b(ii): ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	314.884	1	314.884	165.945	.000
<i>Residual</i>	34.155	18	1.898		
<i>Total</i>	349.040	19			

The independent variable is Hamaker Coefficient  $mJ/m^2)$

**Table 4.5b(iii) Coefficients**

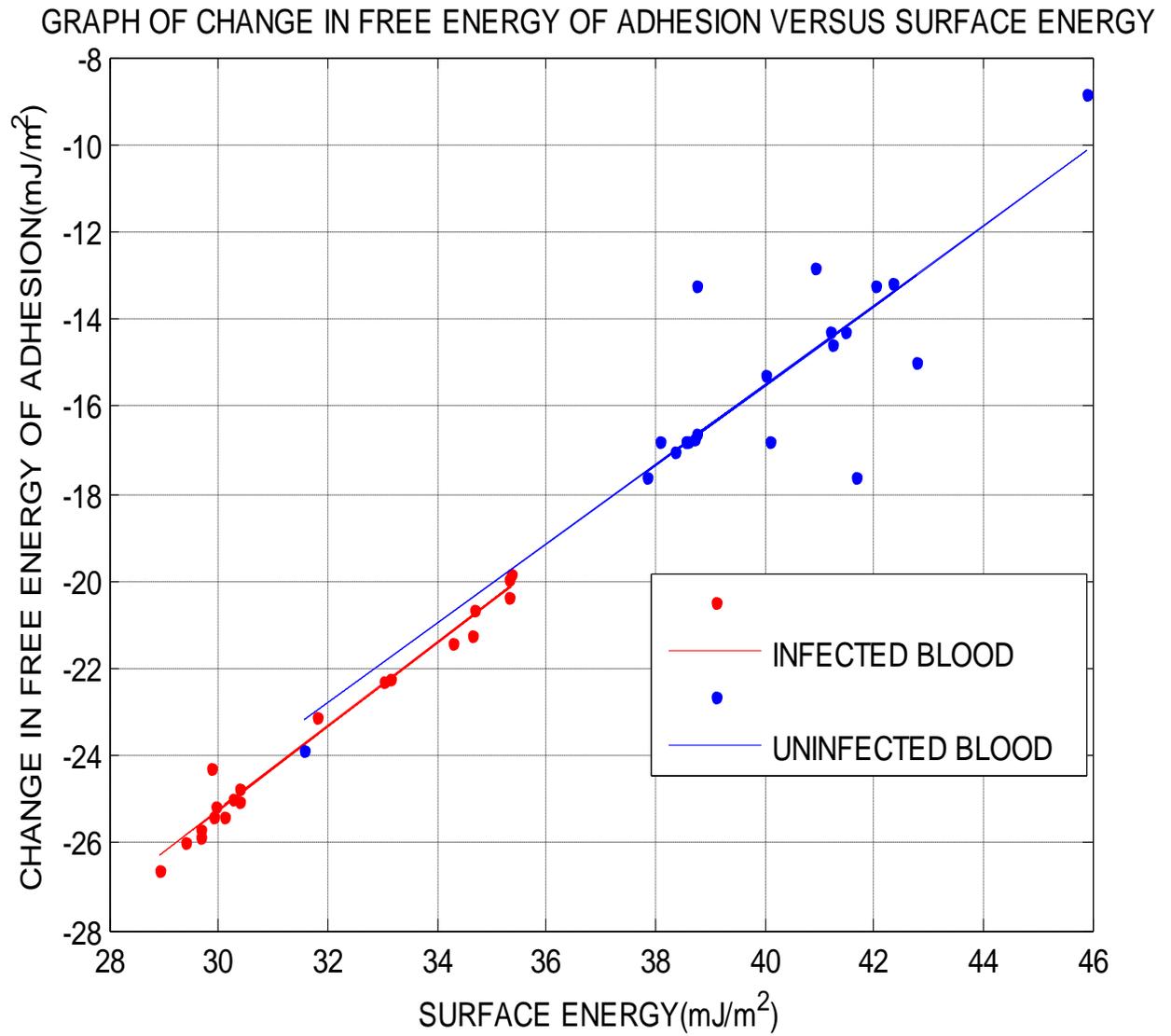
	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Hamaker Coefficient <math>mJ/m^2) \times 10^{-17}</math>,</i>	16.089	1.249	.950	12.882	.000
<i>(Constant)</i>	28.119	1.896		14.834	.000

Fig 4.5 shows that for, infected WBC, contact angle increases as the Hamaker coefficient increases. While for uninfected WBC, contact angle decreases with corresponding decrease in Hamaker coefficient.  $R^2$  – square values for infected and uninfected WBC show a better linear relationship and the ANOVA tables show a predictable significance between the variables.

Appendix I shows the relationship between the Hamaker coefficient and CD4+ counts. Hamaker coefficient is seen to increase as the cell is being infected by the virus. Based on the state of the cells; HIV infected WBC have higher Hamaker coefficients than uninfected WBC. The increase in interaction for infected WBCs may be attributed to the presence of HIV. However, uninfected WBCs have lower Hamaker coefficients at higher values of CD4+ counts which indicated little or total absence of viral infection. The positive values for uninfected WBC indicates that attraction is possible due the presence of other infections, since HIV infection is not the only cause of low CD4+ cell count. The linear plot in fig 4.4 show that Hamaker coefficient  $A_{132}$  increases as the WBC is being infected. The results shown in appendix I indicate that the values of the Hamaker coefficients are within the range of  $\times 10^{-16} \text{mJ/m}^2 \approx \times 10^{-19} \text{J/m}^2$ . The result obtained by Neumann (Table 4.4) is a good approximation of the resent result [4]. The difference may be due to the differences in the degree of HIV infections. The values of Hamaker coefficients,  $A_{132}$  for particle interactions are also reported in the literature ranging from  $\times 10^{-14} \text{J}$ – $\times 10^{-24} \text{J}$ .

#### **CONTACT ANGLE: Surface Energy, Change of energy of adhesion and Hamaker Coefficient**

Surface free energy, Change in free energy of adhesion and Hamaker coefficient are interrelated and analysis show that they agreed to the the measured contact angle data. But in actual sense, the nature of the surface is the determining factor. From the on-going analysis, the HIV surface seems to be hydrophobic “low energy” and this gives rise to increases in contact angles irrespective of the liquid used. Fig 4.1 simply explained this relationship. The change in free energy of adhesion increases with increases in contact angle and decrease in surface free energy (low CD4+). The Hamaker coefficient increases with increase in contact angle and decrease in surface energy. The energies of interaction could be shown graphically in two dimensional (2D) (Fig 4.6a – 4.6b) and three dimensional (3D) planes (Fig 4.7a - 4.7c)



**Fig 4.6a: Change in free energy of adhesion Versus Surface energy**

**Table 4.6ai(1):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.989	.977	.976	.355

*The independent variable is Surface Energy(mJ/m<sup>2</sup>).*

**Table 4.6ai(2): ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	97.432	1	97.432	772.995	.000
<i>Residual</i>	2.269	18	.126		
<i>Total</i>	99.701	19			

The independent variable is Surface Energy(mJ/m<sup>2</sup>).

**4.6ai(3): Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Error</i>
<i>Surface Energy(mJ/m<sup>2</sup>)</i>	.957	.034	.989	27.803	.000
<i>(Constant)</i>	-53.964	1.098		-49.150	.000

**Table 4.6aii(1): Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.879	.772	.759	1.431

The independent variable is Surface energy(mJ/m<sup>2</sup>)

**Table 4.6aii(2): ANOVA**

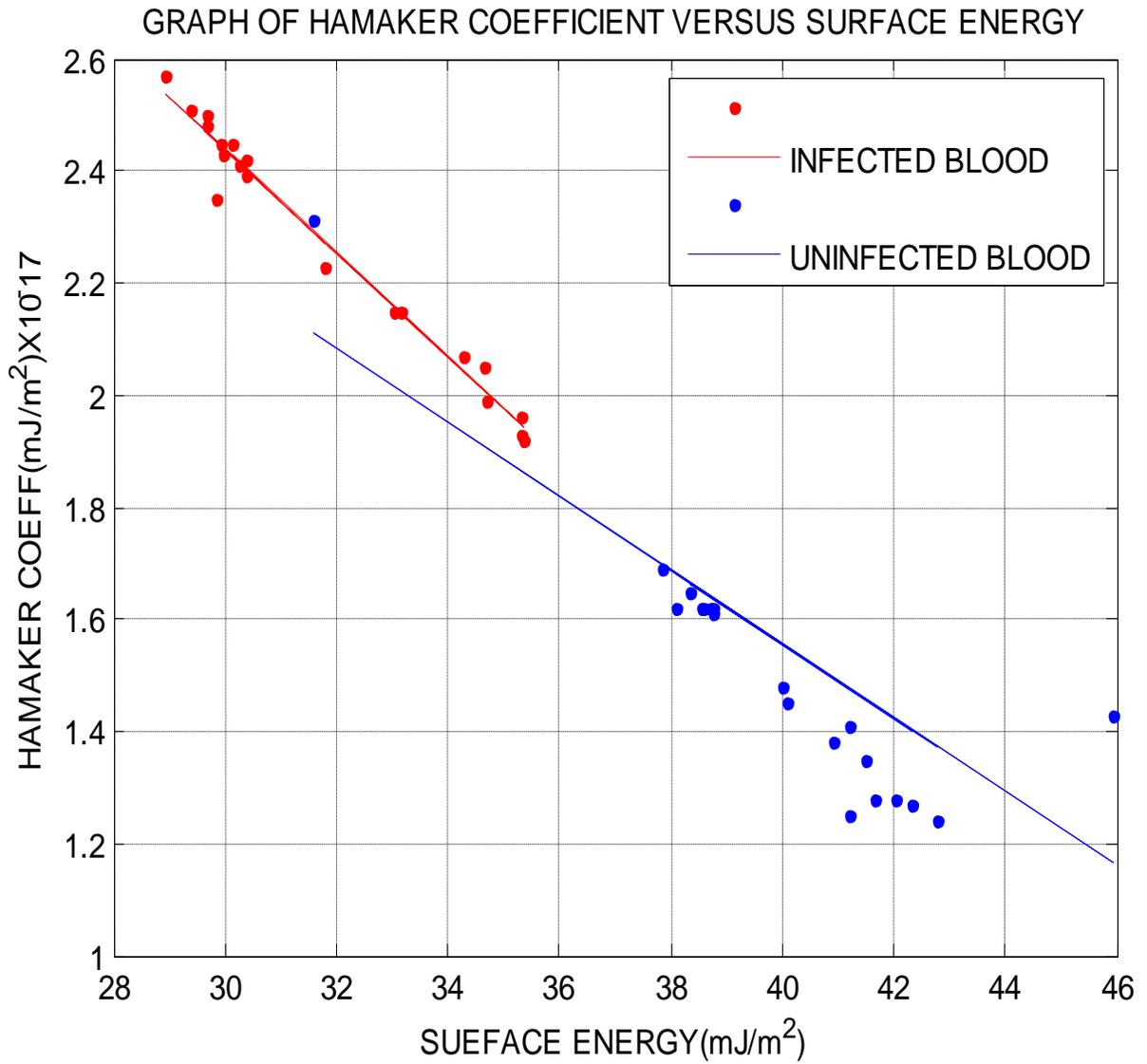
	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	124.836	1	124.836	60.990	.000
<i>Residual</i>	36.843	18	2.047		
<i>Total</i>	161.678	19			

*The independent variable is Surface energy(mJ/m<sup>2</sup>).*

**Table 4.6aii(3): Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Surface energy(mJ/m<sup>2</sup>)</i>	.897	.115	.879	7.810	.000
<i>(Constant)</i>	-51.446	4.604		-11.174	.000

For infected WBC (fig 4.6ai), the change in free energy of adhesion increases as the surface energy decreases. For uninfected WBC (fig 4.6aii), the change in free energy of adhesion decrease also as the surface energy increases. R<sup>2</sup> – square values for infected WBC show a better linear relationship between change in interfacial free energy of adhesion and surface energy. However, infected WBC gives a better fit and can be used to model the system. The ANOVA tables show a good predicted significance.



**Fig 4.6b: Graph of Hamaker coeff Vs Surface energy**

**Table 4.6bi (1): Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.990	.979	.978	.033

*The independent variable is Surface Energy(mJ/m<sup>2</sup>).*

**Table 4.6bi(2):ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	.910	1	.910	830.790	.000
<i>Residual</i>	.020	18	.001		
<i>Total</i>	.929	19			

The independent variable is Surface energy(mJ/m<sup>2</sup>).

**Table 4.6bi(3):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>t</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Surface Energy(mJ/m<sup>2</sup>)</i>	-.092	.003	-.990	-29.058	.000
<i>(Constant)</i>	5.213	.102		51.347	.000

**Table 4.6bii(1):Model Summary**

<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
.974	.949	.946	.059

The independent variable is Surface energy(mJ/m<sup>2</sup>).

**Table 4.6bii(2);ANOVA**

	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Regression</i>	1.166	1	1.166	335.403	.000
<i>Residual</i>	.063	18	.003		
<i>Total</i>	1.229	19			

The independent variable is Surface energy(mJ/m<sup>2</sup>).

**Table 4.6bii(3):Coefficients**

	<i>Unstandardized Coefficients</i>		<i>Standardized Coefficients</i>	<i>T</i>	<i>Sig.</i>
	<i>B</i>	<i>Std. Error</i>	<i>Beta</i>	<i>B</i>	<i>Std. Error</i>
<i>Surface energy(mJ/m<sup>2</sup>)</i>	-.087	.005	-.974	-18.314	.000
<i>(Constant)</i>	4.963	.190		26.156	.000

Fig 4.6bi shows that the Hamaker coefficient increases as surface energy decreases for infected cells. Here, there is drastic increase in interaction because of the viral load. The CD4+count, however decreases due to the infection. Fig 4.6bii shows that the Hamaker coefficient decreases with increase in surface energy. The energies of interactions tend to decrease at a high CD4+ count. Because there is little or no infection. The R<sup>2</sup> square value for both infected and uninfected show good curve fit. The ANOVA tables indicate a predictable level of significance between the interactive energies and surface energy.

**Contact Angle:** Surface energy, Change in free energy of adhesion and Hamaker Coefficients shown in 3- dimensional plane.

The 3 - dimensional surface plots show trends in values across two dimensions in a continuous curve and the third value which represent the z-axis( 4.6ai – 4.6aiii) for infected blood and (4.6bi -4.6biii) for uninfected blood.

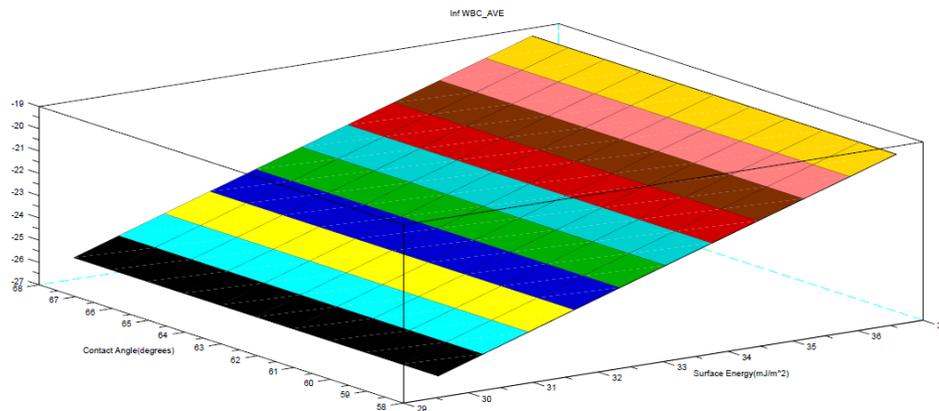


Fig 4.7a(i): 3-dimensional plot of Surface Energy, Contact Angle, Change in Free of Adh.(inf)

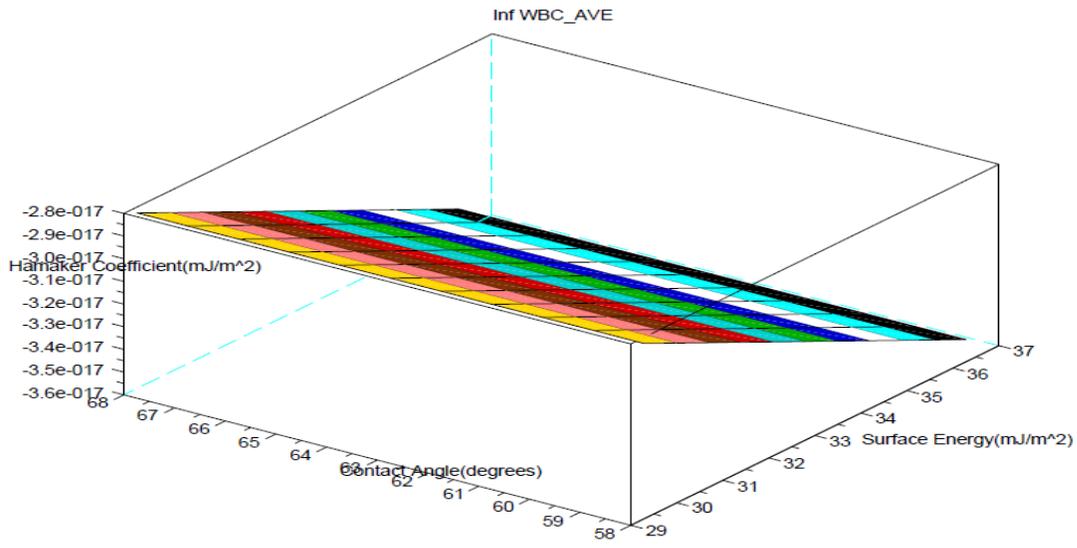


Fig 4.7a (ii):3- dimensional plot of Surface Energy, Contact Angle, Hamaker Coefficient.(inf)

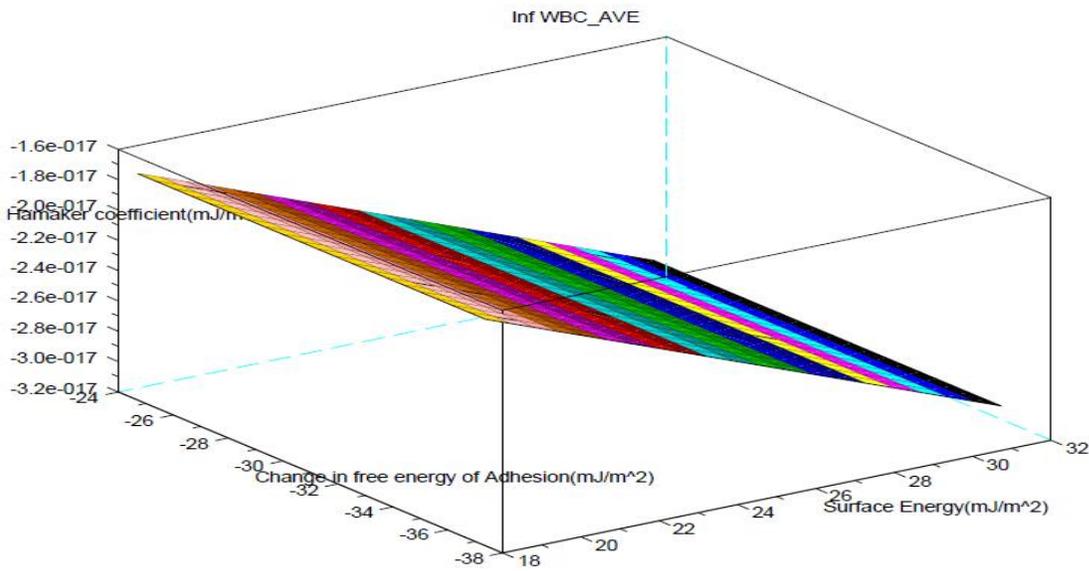


Fig 4.7a (iii):3- dimensional plot of Surface Energy, Change in Free Energy of Adh, Hamaker Coefficient.(inf).

The 3-D plot in Fig 4.7a shows the interrelationships between the surface properties of HIV infected cells. The contact angle approach is used to characterize the surface. Fig 4.7a indicates that as the contact angle increases due to decrease in surface energy, the change in free energy of

adhesion and Hamaker coefficient increases. Then considering the axis of rotation in x,y,z plane, one point can be fixed while others are allowed to move along axis . The plot means the surface properties have equal matrices and can explain the relationship.

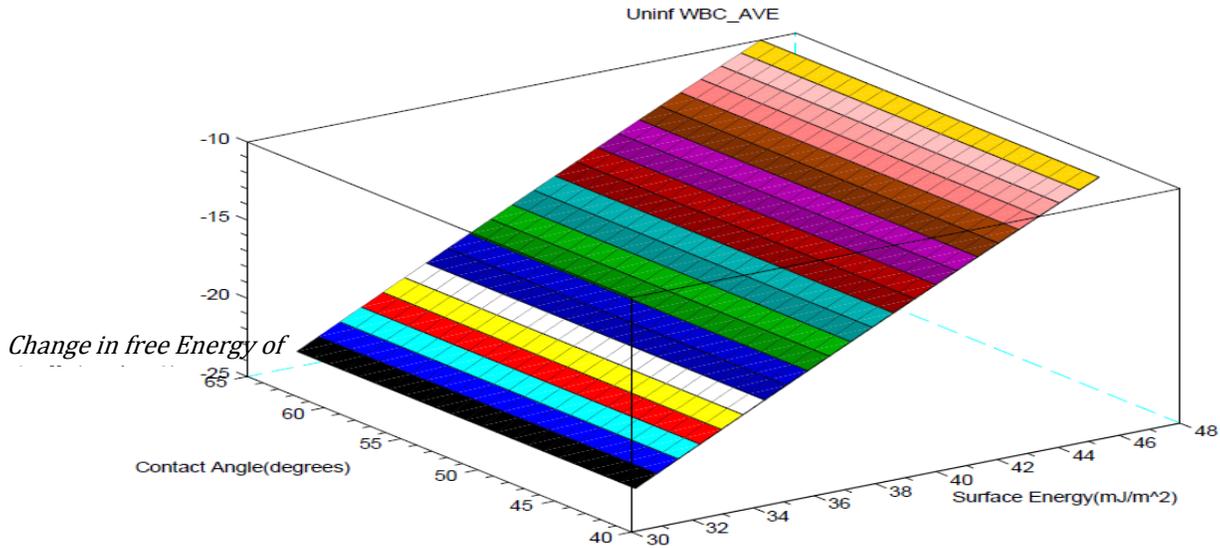


Fig4.7b (i): 3- dimensional plot of Surface Energy, Contact Angle, Change in free energy of adhesion

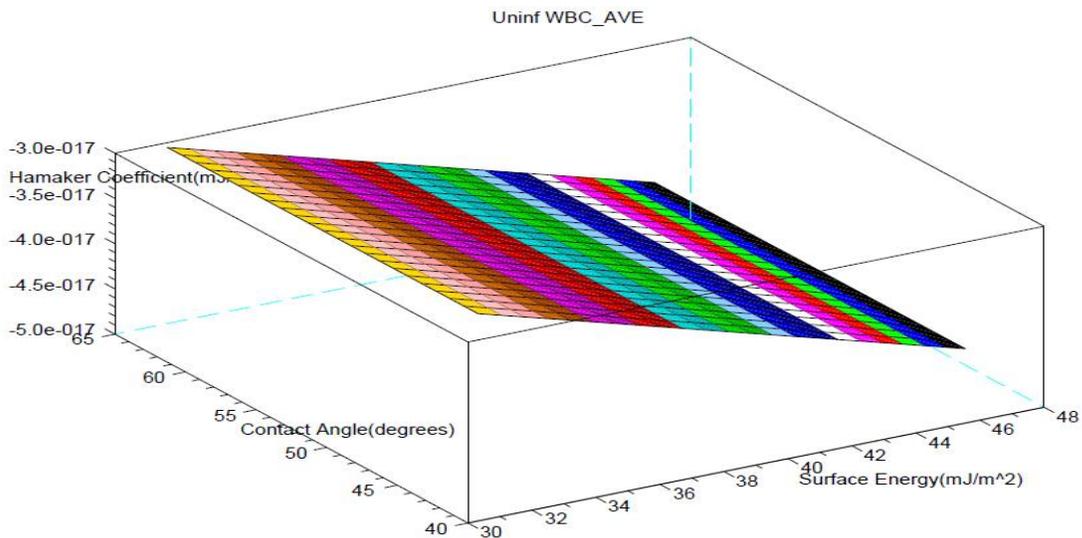


Fig4.7b(ii): 3- dimensional plot of Surface Energy, Contact Angle, Hamaker Coefficient

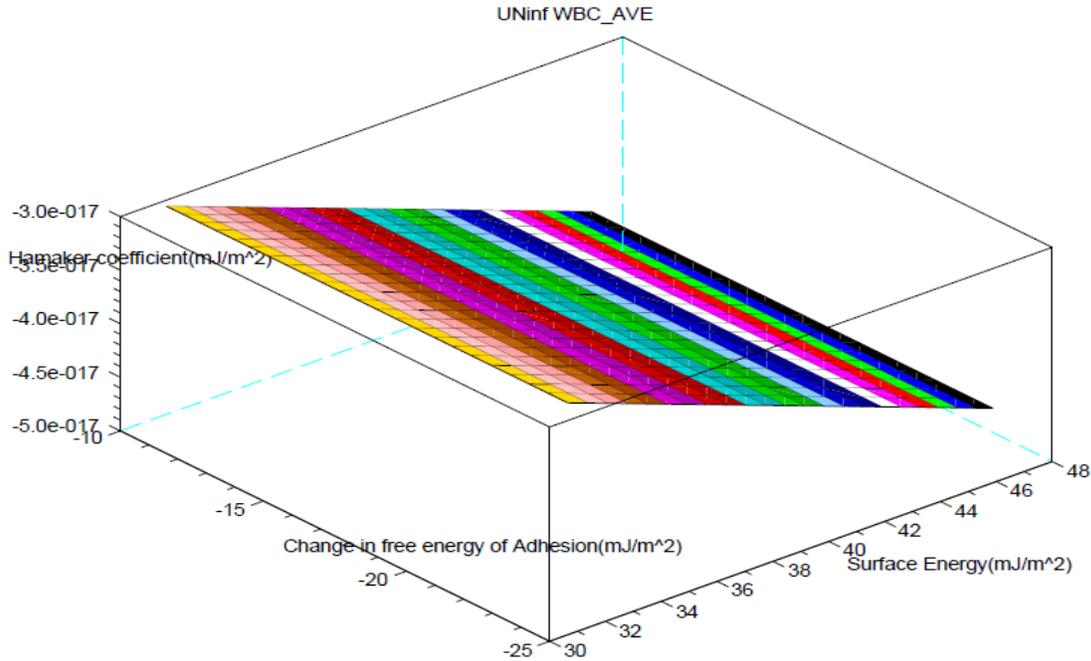


Fig 4.7b (iii): 3- dimensional plot of Surface Energy, Change in free energy of Adh, Hamaker Coefficient (uninf).

The three dimensional plots shown in fig 4.7b is similar to fig 4.7a. When the surface energy increases for uninfected cell, the contact angle and Hamaker coefficient decreases. The 3-D plot can be used to explain the relationships. The surface properties agree with each other indicating variations among the variables.

#### 4.2: Combined Hamaker Coefficient and Components of Blood

Whole blood contains RBCs, WBCs and platelet suspended in Serum (plasma). The serum (plasma) makes up about 55% of the blood volume and in itself contains 90% of water. Besides the WBCs that constitute the blood's security system; other possible cell that can be attacked by the virus is the RBCs but it does not attack it. It does not penetrate the RBC but rather suspends in the films. The virus is able to attack and penetrate the WBCs because the WBCs are phagocytic (fig 1.3) in nature and in the process, synthesize antibodies (enzymes) that are mainly glycoproteins. Such physio-chemical properties of both virus and WBC make them interact with each other. When the interaction leads to a conformational change at the surface of T-

lymphocytes and hinders it from performing the defensive role; infection is assumed to have taken place. This led to the decrease of CD4+ counts and subsequent increase in free energies of adhesion. Now that the HIV is known to interact mainly with WBCs; further confirmation will be based on the negative combined Hamaker coefficient in the study of their interactions. Here the infected WBC represents the virus ( $A_{22}$ ) since it is not possible to separate the virus from the blood. Uninfected WBCs represent lymphocytes ( $A_{11}$ ) while the serum ( $A_{33}$ ) is the intervening medium. Using summary Table 4.7; the energies of interaction are compared. Thus, recall that in all cases where the combined  $A_{132}$  is positive, the net van der Waals attraction exists, and the particles become engulfed. Equally, in all cases where  $A_{132}$  is distinctly negative (so that a van der Waals repulsion prevails), the particles become indeed rejected. Visser [114] had actually shown that  $A_{132}$  is always negative when,

$$A_{11} > A_{33} > A_{22} \quad (2.90)$$

Or when:

$$A_{11} < A_{33} < A_{22} \quad (2.91)$$

Which is the same as stating that  $\Delta F^{\text{adh}}$  will always be negative when

$$\Delta F_{11} > \Delta F_{33} > \Delta F_{22} \quad (2.92)$$

Or when:

$$\Delta F_{11} < \Delta F_{33} < \Delta F_{22} \quad (2.93)$$

It should however be noted Neumann model gave a closer approximation because of the range of values obtained in the previous analysis, though the results of other models appeared in table 4.7 for clarity

**Table 4.7: Summary of absolute values of energies of interactions.**

	<i>Infected Blood</i>						<i>Uninfected Blood</i>					
	<i>WBC</i>		<i>Serum</i>		<i>RBC</i>		<i>WBC</i>		<i>Serum</i>		<i>RBC</i>	
	$\Delta F^{adh}$ <i>mJ/m</i>	$A_{132}$ <i>mJ/m<sup>2</sup></i> <i>x 10<sup>-17</sup></i>	$\Delta F^{adh}$ <i>mJ/m<sup>2</sup></i>	$A_{132}$ <i>mJ/m<sup>2</sup></i> <i>x 10<sup>-17</sup></i>								
<b>Wu</b>	-63.4	6.24	-58.3	5.98	-33.19	6.10	-57.54	5.63	-52.17	5.60	-58.23	5.61
<b>Fowk</b>	-58.4	5.73	-55.5	5.43	-31.60	5.52	-51.26	5.08	-49.62	5.01	-51.75	4.98
<b>Neu</b>	-23.5	2.27	-19.7	1.90	-21.66	1.98	-15.57	1.76	-13.61	1.5	-14.28	1.72
<b>G&amp;G</b>	-36.0	3.49	-35.3	3.43	-30.66	3.15	-25.13	2.21	-20.03	1.92	-26.31	2.56

**4.3: Estimation of  $A_{33}$  for serum to render  $A_{132}$  negative for repulsion:**

Absolute Hamaker constants,  $A_{ij}$  obtained for the various blood components can be employed in the derivation of the negative Hamaker coefficients,  $A_{132}$ . But since HIV has more affinity to WBC, the negative concept of Hamaker coefficient should be verified to check for the possibilities of separating HIV from the lymphocytes as applied to other particulate systems. To define the conditions that will give negative Hamaker Coefficient; cases must arise where

$$A_{132} < 0 \tag{4.1}$$

$$\text{When, } \sqrt{A_{11}} > \sqrt{A_{33}} \text{ and } \sqrt{A_{33}} < \sqrt{A_{22}} \tag{3.41}$$

$$\text{Or } \sqrt{A_{11}} < \sqrt{A_{33}} \text{ and } \sqrt{A_{33}} > \sqrt{A_{22}} \tag{3.42}$$

The mean values of  $A_{11}$  and  $A_{22}$  (table 4.7) are substituted into equation 3.53

$$A_{132} = (\sqrt{A_{11}} - \sqrt{A_{33}})(\sqrt{A_{22}} - \sqrt{A_{33}}) \tag{3.53}$$

The average values of  $A_{11}$  and  $A_{22}$  that would give the absolute values of Hamaker coefficient (Table 4.7) are verified.

$A_{11}$  represents the absolute values for HIV negative lymphocytes (WBC) =  $0.176 \times 10^{-16} \text{mJ/m}^2$

$A_{22}$  represent the absolute values for HIV positive lymphocytes (WBC) =  $0.227 \times 10^{-16} \text{mJ/m}^2$

$A_{33}$  represents the absolute values for HIV positive serum =  $0.190 \times 10^{-16} \text{mJ/m}^2$ .

Then inserting these values into Eq.( 3.53) would yield a negative value for  $A_{132}$  as follows;

$$A_{132} = -0.6637 \times 10^{-19} \text{mJ/m}^2 \text{ (when } A_{33} = 0.190 \times 10^{-16} \text{mJ/m}^2 \text{).}$$

However, for the negative combined Hamaker coefficient  $-A_{132}$  to be achieved,  $A_{33}$  should be of magnitude ;  $A_{33} \geq 0.190 \times 10^{-16} \text{mJ/m}^2$

To obtain a value of the combined Hamaker coefficients  $A_{131}$  (uninfected blood) and  $A_{232}$  (infected blood), the relation of Eq.(3.51) and Eq.(4.2a) are employed.

$$A_{131} = A_{11} + A_{33} - 2A_{13} \quad (3.51)$$

or

$$A_{131} = (\sqrt{A_{11}} - \sqrt{A_{33}})^2 \quad (3.50)$$

$$A_{232} = A_{22} + A_{33} - 2A_{23} \quad (4.2a)$$

or

$$A_{232} = (\sqrt{A_{22}} - \sqrt{A_{33}})^2 \quad (4.2b)$$

**Table 4.8a: Combined Negative Hamaker Coefficient  $A_{132}$  for WBC**

System	Infected WBC ( $\text{mJ/m}^2$ )	Uninfected WBC ( $\text{mJ/m}^2$ )
$A_{11}$	-	$0.176 \times 10^{-16}$
$A_{22}$	$0.227 \times 10^{-16}$	-
$A_{33}$	$0.190 \times 10^{-16}$	$0.15 \times 10^{-16}$
$A_{131}$	$0.2774 \times 10^{-19}$	
$A_{232}$	$0.1648 \times 10^{-18}$	
$A_{132}$	$-0.6637 \times 10^{-19}$	

The combined Hamaker constants for the average of twenty samples of HIV infected and uninfected WBCs are shown in table 4.8a.

- $A_{131}$  and  $A_{232}$  are all positive indicating that van der Waals attraction prevails as individual cell interact with the serum.
- $A_{232}$  (virus) is greater than  $A_{131}$  (lymphocytes) indicating that interactions are more on infected cells than uninfected cells.
- $A_{132}$  is negative indicating possible van der Waals repulsion and a good prove to support the concept of negative Hamaker coefficient.

Similar analysis based on Hamaker combining rule  $A_{132}$  is also applied to interfacial free energy of adhesion,  $\Delta F_{132}^{adh}$  and the result shown in table 4.8b.

**Table 4.8b: Absolute Interactive Energies of WBC**

Systems	$\gamma_{sv}(\text{mJ}/\text{m}^2)$	$\Delta F_{adh} (\text{mJ}/\text{m}^2)$	$A_{132} (\text{mJ}/\text{m}^2)$
$A_{11}$			$0.176 \times 10^{-16}$
$A_{22}$			$0.227 \times 10^{-16}$
$A_{33}$			$0.190 \times 10^{-16}$
$A_{132}$			$A_{11} < A_{33} < A_{22}$ $-0.6637 \times 10^{-19}$
$\Delta F_{11}$		-15.57	
$\Delta F_{22}$		-23.50	
$\Delta F_{33}$		-19.70	
		$\Delta F_{11} > \Delta F_{33} > \Delta F_{22}$	

The results shown in table 4.8a and 4.8b indicated that the negative Hamaker coefficient ( $-0.6637 \times 10^{-19} \text{mJ}/\text{m}^2 \approx -0.6637 \times 10^{-26} \text{J}$ ) together with resent[4] result ( $-0.224 \times 10^{-25} \text{J}$ ) agrees with concept of negative Hamaker coefficient as one of the traditional methods of separation. Thus to ensure the separation, the surface tension/energy of the serum must lowered to the value of  $0.190 \times 10^{-16} \text{mJ}/\text{m}^2$  in order to render  $A_{132}$  negative, up to a value of  $-0.6637 \times 10^{-19} \text{mJ}/\text{m}^2$ . To validate this claim for possible isolation of the virus, the interactive terms for the change in interfacial free energies of adhesion are positive. This proves that van der Waals force is repulsive.

### 4.3 Mathematical model On HIV-Blood interaction.

Using SPSS analytical tools and compared with other software (excel, Scilab), a regression correlation indicates that the measured contact angles and surface energy can be used to model HIV – Blood interaction system.

**Infected WBC:** Fig 4.2a, Table 4.2ai and Table 4.2aii;

The equation:  $y = -1.463x + 110.20$

**Uninfected WBC:** Fig 4.2b, Table 4.2bi and Table 4.2bii

$$y = -1.488x + 111.70$$

The R<sup>2</sup>- square values (0.954 and 0.986) of show a good curve fit; then solving simultaneously,

$$x = 53.57 \text{ when } y = 32.20$$

$$\text{When } y = 0, x = 31.62 \text{mJ/m}^2.$$

The value of  $x$  is equivalent to the surface energy,  $\gamma_{SV}$  found to be  $31.81 \text{mJ/m}^2$  for infected WBCs (HIV).

This means that when  $x$ , the surface energy is zero; then  $y$  (the contact angle =  $110^\circ$ ) is at maximum.

To model an equation that represent the linear behavior of contact angles and surface energy .

Now that  $x = 31.81 \text{mJ/m}^2$ ; the value of  $y$  is approximately equal to the value of the contact angle obtained using the three liquids on average ( $63.28^\circ$ ). Thus;

$$\theta = Ax + B$$

A and B are constants

A = slope -1.5 and

B = intercept obtained by extrapolations ( $110\text{-}112 \text{mJ/m}^2$ )

$x$  = Surface energy,  $\gamma_{SV}$  ( $31.81 \text{mJ/m}^2$ )

$y$  = Contact angle  $\theta$ , ( $63.69^\circ$ )

$$\cos \theta = \phi \left( \frac{-1.5 \gamma_{HV}^V - B}{2 \gamma_{LV}} \right)$$

$$2 \gamma_{LV} \cos \theta = \phi (-1.5 \gamma_{HV}^V - B)$$

Where,

$\gamma_{HV}^V$  = Interfacial energy at HIV- Vapor interface.

B = Constants at intercept(111)

$2 \gamma_{LV}$  = Work of cohesion of the measuring liquid.

$\phi$  = Interaction parameter due to intermolecular interactions between solid and liquid (ie the ratio of work of adhesion to the geometric mean of the work of cohesion of solid and liquid).

(water =0.80-0.10; glycerine =0.60-0.89;diiodomethane=0.50-0.60).

It can be concluded that the intermolecular interactions parameter due the interactions between HIV and blood ranges from 0.50 – 1.0 (See APPENDIX K for details). Then testing the model with the first ten samples on average contact angles and corresponding surface energies.

**Table 4.9:Test for the model:**

S/N	CD4+	Ave( $\theta'$ )	Cos $\theta$	$\gamma_{sv}$ (mJ/m <sup>2</sup> )	Water		Glycerine		Diiodmethane	
					$\varphi$	cos $\theta$	$\varphi$	cos $\theta$	$\varphi$	Cos $\theta$
1	438	67.66	0.380	29.40	0.83	0.381	0.73	0.381	0.58	0.381
2	278	67.33	0.385	29.66	0.83	0.385	0.73	0.385	0.58	0.385
3	282	64.00	0.438	29.86	0.96	0.439	0.85	0.439	0.66	0.430
4	682	64.66	0.427	31.81	0.99	0.428	0.87	0.428	0.69	0.429
5	606	67.00	0.390	29.95	0.86	0.390	0.76	0.392	0.60	0.390
6	20	68.00	0.374	28.94	0.81	0.376	0.71	0.374	0.56	0.374
7	613	66.33	0.40	30.26	0.90	0.40	0.80	0.40	0.65	0.41
8	468	66.53	0.398	29.68	0.86	0.392	0.76	0.394	0.60	0.392
9	853	65.66	0.412	30.38	0.93	0.417	0.81	0.414	0.64	0.412
10	356	66.66	0.396	29.93	0.87	0.395	0.77	0.397	0.60	0.392

# CHAPTER FIVE

## CONCLUSION AND RECOMMENDATION

### 5.1: Conclusion:

Contact angles were conveniently measured on HIV infected and uninfected blood using sessile drop techniques. The results obtained from the contact angle measurements were used to predict interaction that occur between HIV and blood. The prediction was based on the concept of van der Waals attractive forces and absolute Hamaker coefficient whose negative and positive values respectively indicate attraction. But in a case that van der Waals forces become positive and combined absolute Hamaker coefficient becomes negative, the forces repel each other. Therefore, changing the van der Waals attractive force to repulsion becomes a traditional method of separation, which Hamaker and co-workers successfully achieved with particles suspended in a liquid. With this concept in mind, this dissertation was aimed at using the contact angle approach to predict the possible interactions and verification of combined negative Hamaker coefficient. The negative Hamaker coefficient was achieved by pair –wise summation of the geometric means of the combined absolute values. The preliminary calculations of surface free energy and change in free energy of adhesion for both HIV infected and uninfected blood provided a short cut approach to the estimation of Hamaker coefficients for the interacting systems. These energies known as surface physiochemical properties are determined for both infected and uninfected blood from contact angle data. Thus:

- The contact angles measured on different blood components with different liquids are high for infected blood and low for uninfected blood. The WBCs indicated highest contact angle among other component of blood using probe the liquids. The contact angles measured with water are generally higher for both infected and uninfected blood. However, the contact angles tend to increase with a decrease in CD4+ counts for infected blood due to the presence of HIV (Table 4.1).
- The contact angle increases with decrease in surface energy for infected blood and mostly for WBCs. The presence of HIV however, reduces the work done on the surface. The surface of the blood when infected by the virus, becomes a non-wetting surface. This suggest that at lower CD4+ count, the surface energy decreases and thus, HIV has the

capacity to lower the surface energy. The decreases in surface energy for infected blood and mostly for WBCs is a confirmation that WBCs are mostly attacked by the virus (Table 4.2). For uninfected bloods, the contact angles decrease. Absence of HIV however, increases the surface energy and the spreading tension or wetting increases.

- The change in free energy of adhesion increases as contact angle increases (Table 4.3). It then means that presence of HIV increases van der Waals attractive force and mostly for infected WBCs. This justifies the high degree of negative values obtained for the lowest CD4+ count infected patient (20 counts/mm<sup>3</sup>). There is less attraction for other component of infected blood and much less attraction for uninfected blood. The negative values for uninfected blood suggest that attraction is possible due to other precipitates present in the blood like bicarbonates ions.
- The energies of interactions expressed as absolute Hamaker coefficient are all positive and this also validate the claim that attraction occur between HIV and blood during infection (table 4.5).
- The negative combined Hamaker coefficient ( $-0.6637 \times 10^{-19} \text{ mJ/m}^2$ ) show that van der Waals attractive force is be repulsive. Therefore, HIV can be isolated from the lymphocytes by nature of their interaction with the serum (Table 4.8a). This clearly indicate that the van der Waals attractive force can be changed to repulsion.
- Analysis of variance (ANOVA) using SPSS contact angle and HIV infected WBCs have a predictable level of significance.

## **5.2 Implication to HIV Cure**

Contact angle techniques shows that systems (HIV and blood) can interact in such a way that the combined Hamaker coefficient is negative. This means that any additive in form of drugs that would render the energies of interaction negative could create a barrier between the virus and lymphocytes. This research therefore suggests that lowering the surface tension of the serum would cause the virus to be repelled from the lymphocyte. To achieve this, the wettability of the drug with known surface tension, must be increased by adding elutants that will reduce the surface tension of the serum.

### 5.3: Addition to Knowledge

To the best of the available knowledge, no research has studied the HIV-blood interactions from the contact angle approach. This novel idea has therefore increased the possibility of finding a solution to the HIV pandemic through the following research findings:

- The surface energy of HIV infected surfaces are known through the contact angle approach.
- HIV has the energy reducing capacity. It reduces the surface: WBCs from  $39.94\text{mJ/m}^2 - 31.81\text{mJ/m}^2$ ; Serum,  $40.82\text{mJ/m}^2 - 35.47\text{mJ/m}^2$ ; RBCs,  $40.09\text{mJ/m}^2 - 34.10\text{mJ/m}^2$ .
- The positive values of Hamaker coefficient suggest that attraction occur between HIV and blood during infection.
- The concept of negative Hamaker coefficient ( $-A_{132}$ ) was verified with the combining rules and this agreed that changing van der Waals attractive force to repulsion as a traditional method of separation. Thus isolating the virus from is attainable ( $-0.6637 \times 10^{-19}\text{mJ/m}^2$ .)
- Girifalco and Good's intermolecular parameter, due to intermolecular interactions between the HIV and blood ranges from 0.50 – 1.00.

#### **5.4: Recommendation**

In order to ensure that this research has satisfied the objective, the following recommendations therefore are hereby made in furtherance of this research work.

- Further researches which will include finding the actual model that will give a repulsive interfacial energies between particles in a system.
- When properly conducted, such further research should be geared towards seeking for a drug whose surface energies, obtained from the measured contact angle can render the combined Hamaker coefficient negative.
- Neumann model should be given a consideration because of its predictability of separation between HIV and lymphocytes.
- Efforts should be made towards the interpretation of the characteristics and specification of the material that would render the Hamaker coefficient  $A_{132}$  negative as deduced in this research. This should involve a team of medical personnel like pharmacists, pharmacologists, laboratory scientists and doctors in collaboration with engineers and physics.

## REFERENCES

- [1] UNAIDS (2006): Report on the Global AIDS Epidemic/UNAIDS: A UNAIDS 10<sup>th</sup> Anniversary Special Edition, May 2006, pp.1-2
- [2] Fields B.N,2007: Fields` virology. 5<sup>th</sup> edition.Edited by David.M Knipe, Peter M Howley, Diane etal. Philadephia, pa London
- [3] Omenyi S.N etal, 2002. An Estimate of Reasoned Baseline 6n patient Load for Genotype Testing J. American Medical Association.
- [4] Achebe C H: Human immunodeficiency Virus, HIV- blood Interaction:Surface thermodynamic approach. Proceedings of the international MultiConference of Engineers and computer Scientists 2012 Vol 1,IMECS 2012, March 14-16,2012, Hong Kong.
- [5] NAID(2005):Intensifying HIV prevention. Policy position paper: Geneva
- [6] Neumann A W, Good R J, Hope C J and Sejpal M.J, Colloid and Interface Sci,49.29.
- [7] Hough D.H and White L.R,adv Colloida Interface Sci,28(1987)35
- [8] Neumann, A.W., Absolom, D.R., Francis, D.W., et al, (1983): Blood Cell and Protein Surface Tensions, Annals New York Academy of Sciences, p.277
- [9] Viser J,(1981): Advances in interface, Elsevier Scientific publishing company, Armsterdam,Vol. 15,pp.157-169.
- [10] Omenyi, S.N., Smith, R.P., and Neumann, A.W., (1980): Journal of Colloid and Interface Science, Vol.75, No.1, p.117.
- [11.] Paddy J F (1969): Surface and colloid science, Matijevic ed. Vol.1, Wiley-Interscience,New York, p.101.
- [12] Horn R G: Surface forces an their action in ceramic material.Journal of American ceramic society,1990.73(5)p.1117-1135
- [13] Hamaker H C(1936): The London/van der Waals attraction between spherical particles,physics,4.1058.

- [14] Yildirim I (2002): Studies of the interactions between talc and stikies during paper recycling, in SME Annual Meeting and Exhibit.Cincinnati: Society for mining, metallurgy and Exploration.
- [15] Balter M (1997, November 21). How does HIV overcome the body's T-cell bodyguards? Science 278: 1399-1400
- [16] Hraba T and Dolezal J (2009): A Mathematical Model and CD4 lymphocyte dynamics in HIV infection,EID Vol.2,No 4,[http://www.cdc.gov/ncidod/EID/Vol\\_2\\_No\\_4/hraba.htm](http://www.cdc.gov/ncidod/EID/Vol_2_No_4/hraba.htm), Retrieved 24-05-10
- [17] Alderman, L.M,(1988): On the maintenance of T.-cell populations” Technical report,Los Angeles,University of Califonia, Dept. of Computer Science.
- [18] Bragardo,M. Buonfiglio, D(1997):Modulation of lymphocyte interaction with Endothelium and Homing by HI glycoprotein 120, Journal of Immunology, Vol.159,No.4,pp.1619-1627.
- [19] Feeney C, Bryzman S, Kong L, Brazil H, Deutsch R, Fritz LC (1995, Oct). T-lymphocyte subsets in acute illness. Crit Care Med; 23(10):1680-5
- [20] Williams RC, Koster FT, Kilpatrick KA (1983, November). Alterations in lymphocyte cell surface markers in various human infections. Am J Med: Volume 75; 807-816
- [21] Chirenda J (1999). Low CD4 count in HIV-negative malaria cases, and normal CD4 count in HIV-positive and malaria negative patients. Cent Afr J Med; Volume 45(9): page 248
- [22] Carney WP, Rubin RH, Hoffman RA, et al. (1981). Analysis of T lymphocyte subsets in CMV mononucleosis. The Journal of Immunology 126(6); 2114-2116
- [23] Junker AK, Ochs HD, Clark EA et al. (1986, ). Transient immune deficiency in patients with acute Epstein-Barr virus (EBV) infection. Clin Immunol Immunopathol 40(3); 436-446
- [24] Nishijima MK, Takezawa J, Hosotsubo KK et al. (1986). Serial changes in cellular immunity of septic patients with multiple organ-system failure. Critical Care Medicine, Volume 14(2); 87-91

- [25] Beck JS, Potts RC, Kardjito T, and Grange JM (1985). T4 lymphopenia in patients with active pulmonary tuberculosis. *Clin Exp Immunol*, Volume 60, 49-54
- [26] Antonaci S, Jirillo E, Stasi D, De Mitrio V, La Via MF, Bonomo L (1988). Immunoresponsiveness in hemophilia: lymphocyte- and phagocyte-mediated functions. *Diagn Clin Immunol*;5(6):318-25
- [27] Madhok R, Gracie A, Lowe GD, Burnett A, Froebel K, Follett E, Forbes CD (1986, Oct 18). Impaired cell mediated immunity in haemophilia in the absence of infection with human immunodeficiency virus. *Br Med J (Clin Res Ed)*;293(6553):978-80.
- [28] Des Jarlais DC, Friedman SR, Marmor M et al. (1987, July). Development of AIDS, HIV seroconversion, and potential cofactors for CD4 cell loss in a cohort of intravenous drug users. *AIDS* 1(2): 105-111
- [29] Mientjes GH, Miedema F, van Ameijden EJ, Hoek AA, et al. (1991). Frequent injecting impairs lymphocyte reactivity in HIV-positive and HIV-negative drug users. *AIDS*: 5; 35-41
- [30] Mc Donough R. J, Madden J J, Falex A (1980): alteration of T and null lymphocytes frequencies in the peripheral blood of human opiate addicts: In vivo evidence for opiate receptor sites on T lymphocytes. *J Immunol*: 125(6); 2539-43.
- [31] Kotze M (1998). Ability of the total lymphocyte count to accurately predict the CD4+ T-cell count in a group of HIV1-infected South African patients. *Int Conf AIDS - 1998*; 12: 810 (abstract no. 42187.
- [32] Culver KW, Ammann AJ, Partridge JC, Wong DF, Wara DW, Cowan MJ (1987, August). Lymphocyte abnormalities in infants born to drug-abusing mothers. *J Pediatr*;111(2):230-5.
- [33] Burns DN, Nourjah P, Minkoff H, et al. (1996). Changes in CD4 and CD8 cell levels during pregnancy and post partum in women seropositive and seronegative for HIV-1. *Am J Obstet Gyn*; 174(5); 1461-1468
- [34] Castilla JA, Rueda R, Vargas L, et al. (1989). Decreased levels of circulating CD4+ T lymphocytes during normal human pregnancy. *J Reprod Immunol*; 15; 103-11

- [35] Sridama V, Pacini F, Yang S, et al (1882). Decreased levels of helper cells: A possible cause of immunodeficiency in pregnancy. *New Eng J Med*; 15; 103-11.
- [36] Verde TJ, Thomas SG, Moore RW, et al. (1992). Immune responses and increased training of the elite athlete. *J Appl Physiol*; 73(4); 1494-9.
- [37] Hegde HR, Woodman RC, Sankaran K (1999, March). Nutrients as modulators of energy in acquired immune deficiency syndrome. *J Assoc Physicians India*; 47(3): 318-25.
- [38] Beisel WR (1996, October). Nutrition in pediatric HIV infection: setting the research agenda. *Nutrition and immune function: overview. J Nutr*; 126(10 Suppl): 2611S-2615S
- [39] Sapolsky RM, Uno H, Rebert CS, Finch CE (1990 Sep). Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J Neurosci* ; 10(9): 2897-902
- [40] Alberts SC, Sapolsky RM, Altmann J (1992). Behavioral, endocrine and immunological correlates of immigration by an aggressive male into a natural primate group. *Hormones and Behavior* 26; 167-178
- [41] Kennedy S, Kiecolt-Glaser JK, Glaser R (1988 Mar). Immunological consequences of acute and chronic stressors: mediating role of interpersonal relationships. *Br J Med Psychol*; 61(Pt 1): 77-85
- [42] Herbert TB & Cohen S (1993). Stress and immunity in humans: A meta-analytic review. *Psychosomatic Medicine*; 55; 364-379
- [43] Guyton AC & Hall JE (1996). *Textbook of Medical Physiology*. Saunders; New York
- [44] Goodkin K, Feaster DJ, Asthana D, et al. (1998, May). A bereavement support group intervention is longitudinally associated with salutary effects on the CD4 cell count and number of physician visits. *Clin Diagn Lab Immunol*: 5(3); 382-91
- [45] Berkman L & Syme S (1979). Social networks, host resistance, and mortality: a nine year follow up study of Alameda County residents. *Am J Epidemiol*; 109(2): 186-203
- [46] House et al. (1988). Social relationships and health. *Science* ; 241: 540-545

- [47] Ornish D (1997). *Love and Survival: the Scientific Basis for the Healing Power of Intimacy*; Harper Collins; New York
- [48] Hansten P.D 1995: *Drug interactions, the clinical use of drugs*, Vancouver- Applied therapeutics
- [49] Pistelli S and Keith G. 2001, *Interactions amongst drugs for HIV and opportunistic infections*
- [50] Wyles D , 2005. *Antiretroviral drug pharmacokinetics in hepatitis with hepatic*
- [51] Abbas A.K et al, **2003**: *Cellular and Molecular immunology*. 5<sup>th</sup> edition. Philadelphia, Pa, London W.B. Saunders.
- [52] Wang J H,(2009): *Intercellular adhesion molecule(ICAM)-1, but not ICAM 2 and 3, is important for dendritic cell- mediated human immunodeficiency virus type-1 transmission* J virol,2009,83:4195-4204.
- [53] Triboulet R, Mari B, Lin YL, Chable- Bessia C, et al, 2007. *Suppression of microRNA-silencing pathway by HIV-1 during virus replication*. *Science*, 2007.315, 1579 – 1582
- [54] Zheng H 2009. *Reversal of HIV – 1 latency with anti-microRNA inhibitors*. In *J Biochem Cell Biol* 2009, 41.451 – 454
- [55] Leghmari K Bennasser Y, Bahraoui E., 2008. *HIV-1 Tat protein induces IL -10 production by an alternative TNF- alpha- independent pathway in monocytes. role of PKC- delta and p38 MAP kinase*
- [56] Sandanger O, Ryan L, Bohnhorst J, Iversen AC, Husebye H,2009. *IL- 10 enhances MD -2 and CD14 expression in monocytes and protein are increased and correlated in HIV – infected patients*. *J Immunol*, 2009, 182,588-595
- [57] Gruwaldu J,(1996): *Monocyte can phagocytose gram- negative bacteria by a CD14-dependent mechanism*. *J immunol* 1996.
- [58] Moore, K W et al, 2001. *Interleukin -10 and the interleukin – 10 receptor*. *Annu Rev Immunol*

- [59] Foster J. L ,2008:HIV–1Nef at the crossroads.Retrovirology.
- [60] Marini E 2008 HIV- 1 matrix protein p17 binds to monocytes and selectivity stimulates MCP-1 secretion. Role of transcriptional factor AP-1
- [61] Arfi V, 2008: Characterization of the early steps of infection of primary blood monocytes by human immunodeficiency virus type 1. J Virol 1998, 82, 6557-6565
- [62] Boaso A , 2008. PDL – upregulation on monocytes and T cells by HIV via type 1 interferon. Restriction expression of type 1 interferon receptor by CCR5- expressing leukocyte
- [63] Sharpe A H, 2007. The function of programmed cell death 1 and its ligans in regulating autoimmunity and infection. Nat Immunol.
- [64] Kinter A L, 2008.The common gamma – chain cytokines IL – 2,IL-7, IL-15 and IL-21 induces the expression of programmed death-1 and its ligands. J Immunol. 181.6738-6746
- [65] Mc Elrath M J etal, 1991.Latent HIV – 1 infection in enriched population of blood monocytes and t cells from seropositive patients. J Clin Invest,1992,90.1486 – 1491
- [66] Ellery P J etal, 2007: The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo.J Immunol 2007,178,6581- 6589Engulfment and Cell Adhesiveness”, Marcel Dekker, New York
- [67] Dong C, 2009: Transcriptional restriction of human immunodeficiency virus type – 1 gene expression in undifferentiated primary momocytes. J Virol,2009,83.3518 – 3527
- [68] Richman D.D,2009:Th [65]Hart D. N 1997,Dendritic cells,unique leukocytes population which control the primary response Blood 90,3245-3287
- [69] Shortman K, Liu Y.J(2002): Mouse and human dendritic cell subtypes, Nat Rev. Immunol. 2.151.
- [70] Hart D N,1997: Dendritic cells, unique leukocytes population which control the primary response blood 90.3345-3287.

- [71] Merald M, 2008: Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Immunol* 2008, 8935-947
- [72] Wu L, Kewal- Ramani V N.(2006): Dendritic- cell interaction with HIV infection and viral dissemination. *Nat Rev Immunol* 6, 859-868.
- [73] Jonas AM, Dong C, Wang J H, Wu L, 2008. Productive infection of human immunodeficiency virus type 1 in dendritic cells requires fusion-mediated viral entry. *Virology*, 2008, 375, 442-451
- [74] Garcia E, Nikolic D C, Piguet V, 2008: HIV-1 replication in dendritic cells occurs through a tetraspanin-containing compartment enriched in AP – 3
- [75] Groot F, 2006: Opposing roles of blood myeloid and plasmacytoid dendritic cell in HIV-1 infection of T cells: transmission facilitation versus replication inhibition, 108:1957-1964.
- [76] Meyer J H, 2007: Impact of HIV on cell survival and antiviral activity of plasmacytoid dendritic cell. *Microbiol*, Vol. 32, pp. 19-39.
- [77] Chougnet C, Gessani , 2008: Role of gp 120 in dendritic cell dysfunction in HIV infection. *J Leukoc Biol* 2006, 80, 994 – 1000. Comparisons of HIV – 1 viral sequences in brain, choroid plexus and spleen. Potential role in the pathogenesis of HIV encephalitis. *J. Neuroviral* 6, 498 – 506
- [78] Martinson J A, Roman – Gonzalez A, Tenorio AR, 2007. Dendritic cells from HIV-1 infected individual are less responsive to toll- like receptor (TLR) ligands. *Cell Immunol* 2007, 250, 75-84.
- [79] Wu L, Kewal- Ramani V N.(2006): Dendritic- cell interaction with HIV infection and viral dissemination. *Nat Rev Immunol* 6, 859-868
- [80] Wiley K. D, 2006: Immature dendritic cell-derived exosome can mediate HIV-1 trans infection. *Proc Natl Acad Sci USA*, 2006, 103, 738 – 743.
- [81] Izquierdo U N 2006: Capture and transfer of HIV-1 particles by mature dendritic cells converges with the exosome dissemination pathway. *J Biol Chem* 281, 2732-41

- [82] Sowinski S, Jolly C, Berninghausen O et al, 2008. Membrane nonotube physically connect T cells over long distances presenting a novel route for HIV-1 transmission. *Nat Cell Biol*, 2008, 10: 211 – 219
- [83] Shere N.M, Lehmann M J, Jimenez – Soto L F, Horensavitz C, et al, 2007. Retroviruses can establish filopodial bridges for efficient cell- to – cell transmission. *Nat Cell Biol* 2007, 9: 310 – 315.
- [84] Fu, W Scanders-Berand, Ptak, 2009: Human immunodeficiency virus type-1, human protein interaction data base at NCBI. *Nucleic Acids Res* 2009. D417 – 422
- [85] Jeong H, Masson S P, Barabasi A L, Oltval Z N, 2001 . Lethality and centrality in protein networks. *Nature* 2001, 411(6833). 462 – 466
- [86] Subramanian A, 2005, Gene set enrichment analysis, a knowledge- based approach for interpreting genome- wide expression profiles. *Proceeding of the National Academy of Sciences of the United States of America* 2005, 102(43) 15545 – 15550
- [87] Dyer MD, Murali TM, Sobral BW, 2008: The landscape of human proteins interacting with viruses and pathogens. *Plos pathogen*, 2008, 4(2), e32
- [88] Hahn M W, Kern AD, 2005: Comparative genomics of centrality and essentiality in three eukaryotic protein – interaction networks. *Mol Biol Evol* 2005, 22(4). 803 – 806
- [89] Joy M P , Brock A, Ingber DE, Huang S et al, 2005. High - betweenness protein in the yeast protein interaction network. *J Biomed Biotechnol* 2005, (2). 96-103
- [90] Yu H., 2005. The importance of bottlenecks in protein network, Correlation with gene essentiality and expression profile. *Proceeding of the National Academy of Science of the United States of America*, 2005, 102(43) 15545-15550
- [91] Eppig J T , 2007: The mouse genome database (MGD). new features facilitating a model system. *Nucleic acids research* 2007, D630-637
- [92] Achebe C.H, Omenyi S.N, 2013: Mathematical Determination of the Critical Absolute Hamaker Constant of the Serum (As an Intervening Medium) which Favours Repulsion in the Human Immunodeficiency Virus (HIV) - Blood Interaction mechanism. *Proceedings of the world congress on Engineering 2013 Vol II, WCE 2013, July 3 - 5, 2013, London, UK.*

- [93] Achebe C.H,Omenyi SN, 2014:Application of Negative Hamaker Concept to the Human Immunodeficiency Virus(HIV) - Blood Interactions Mechanism.Transaction on Engineering technologies.D01:10,10.07/978 - 94 - 017 - 8832-8-21 @ Springer Science Business Media
- [94] Achebe C.H,2014: Surface thermodynamics Approach to HIV- Blood Interactions.LAP Lambert Academic Publishing.ISBN-13:978-3-659.55486-5.
- [95] Absolom, D.R., van Oss, C.J., Genco, R.J. and Neumann, A.W., (1980): Surface thermodynamics of normal and Pathological human granulocytes, Cell Biophys.Vol.2, pp.113-126
- [96] Neumann, A W, Absolom, D R, Francis D W et al(1983):Blood Cell and protein Surface Tension,Annal New York Academy of Sciences,p.277.
- [97] Sherman, I.A., Grayson, J. and Zingg, W. (1980): The Critical closing
- [98] van Oss, C.J., Good, R.J. and Neumann, A.W., (1972): J. Electroanal. Chem., Vol.37, p.387
- [99] Capo, C., Bondgrand, P., Benoliel, A.M., and Depieds, R. (1978): Non-Specific Recognition in phagocytosis: Ingestion of aldehyde-treated erythrocytes by rat Peritoneal macrophages, Immunology, Vol.36, pp.501-508
- [100] Good R J and Stromberg R.R,1979:Surface and Colloidal Science, Plenum,Vol 11,p.1
- [101] Girifalco L.A and Good R.J,J.Phys.Chem,61(1957)904;J.Phys.Chem,64(1960)561.
- [102] Fowkes F.M,Ind Eng .Chem,56(1964)40.
- [103] Owens D.K andWendt R.C, J.Appl Polym.Sci,13(1969)1741.
- [104] Kaeible D.H. J.Adhesion,2,(1970),66.
- [105] Wu.S, Polymer Interface and Adhesion,Marcel Dekker, New York, 1982.
- [106] Gibbs J .W,Collected Works,Vol 1, Longman Green - Villars, Paris,1869,p.369.
- [107] Dupre A,Theorie Mechanique de la Chaleur, Gauthier - Villars,paris, 1869,p.369.

- [108] Berthelot D, Compt, Rend, 126(1898)1703
- [109] McLachlan, Proc. Roy. Soc. London, A271(1963)387
- [110] Casimir H.P.G and Polder D, Phys Rev, 73(1948)360
- [111] Lifshitz E.M., Adv Phys, 10(1961)165
- [112] Fowkes F.M., in K.L Mittal(ed), Physicochemical Aspects of Polymeric Surfaces, Plenum, New York, 1983, Vol 2, p.583.
- [113] Isrealachvili J, Quart, Rev Biophys, 6(1974)387.
- [114] Hough D.H and White L.R, Adv Colloid Interface Sci, 28(1987)35.
- [115] van Oss CJ; Chaudhury M.K and Good R. J, Chem Rev, 88(1988)927; Adv Colloidal Interface sci, 28(1987) 35.
- [116] Chaudhury M.K, Ph.D Thesis, State University of New York at Buffalo, 1984.
- [117] Neumann AW, van Oss C.J, Omenyi S.N, Absolom D. R, Viser J; Adv Colloid Interface Sci, 18(1983)133.
- [118] Bolger J.C and Micheals A.S, in P.Weiss and D.Cheevers(eds), Interface Conversion, Chapt 1, Elsevier, New York, 1969.
- [119] Drago R.S., Vogel G.C, Needham T.E; J. Am Chem Soc 93(1971)6014
- [120] London, F.(1930). Physics, Vol.63, p.245
- [121] Hamaker H C(1936): The London/van der Waals attraction between spherical particles, physics, 4.1058.
- [122] Lifshitz, E M (1961): advanced Physics. Vol.10, p.165
- [123] Viser J (1981): Advances in interface, Elsevier scientific publishing company, Amsterdam, vol.15, pp.157-169.
- [124] Omenyi, S N, Smith, R P and Neuman, A W(1980) Journal of Colloidal and Interface science, vol.75, No.1, p.117.

- [125] van Oss C J , Absolom D.R , Grossberg A.L and Neuman,AW Immunol. Commun, (in press)
- [126] van Oss CJ , Gillman C F and Neuman A W: Phagocytic Engulfment and Cell Adhesiveness” Marcel Dekker, New York 1975
- [127] Omenyi, S.N:“Attraction and Repulsion of particles by Solidification Melts” PhD Dissertation, University of Toronto, 1978.
- [128] Kua V.H.S and Wilcox W.R,Separ. Sci, 8,375;1973
- [129] van der Esker M.W.J and Vrij,A J Polymer Sci,14 1943(19760
- [130] van Oss CJ, Absolom D R and AW Neuman Separ, Sci. and Technol, 14 (1979)
- [131] Doellgast G J and W H.Fishman, Biochem. J.141,103 (1974)
- [132] Stjernstrom I, Magnusson K E, O. Stendahi and C. Tagesson, Infect.Immun, 18,261(1977)
- [133] van Oss C J and Grossberg A L in “Principles of Immunology”, NR. Rose, F. Milgram and CJ. Van Oss, eds, Macmillan, New York, 1979, chaper 5.
- [134] Grossberg A L and D Pressman, BIOchem,1, 391 (1962)
- [135] van Oss C J and Neuman,A W Immunol.Commun,6,341 (1977)
- [136] Neuman,A W ,Good,R J, Hope C J and Sejpal,M J. Colloid and Interface Sci, 49.291 (1974)
- [137] van Oss, C J ,Absolom, D R Andres G A, B Albin B and Neuman, A W .unpublished result
- [138] Dingles, N M and Harris M.T(2005): A robust algorithm for the simultaneous parameter estimation of interfacial tension and contact angle from sessile drop profile.Journal of Colloid and interface Science, Vol.286,pp.565-569
- [139] Dreu R;Sircab, J;Pintye-Hodi,K;Burjan, T;Planinsek, O & Srcic, S(2005):Physiochemical properties of granulating liquids and their influence on microcrystalline cellulose pellets obtained by extrusion-spheronisation technology.International Journal of Pharmaceutics, Vol. 291,pp.99-111

- [140] Ortiz,S E M;Sanchez,C.C;Rodriguez Nino,M R; Anon,M .C&RODriguez Patino,J M.(2003).Structural characteristics and surface activity of spread and adsorbed soyglobulin filma at equilibrium.colloid and Surfaes B: Bointerfaces,Vol.32,pp.57-67.
- [141] Vejnovic,I;Simmer,L & Betz,G(2010). Investigation of different formulations for drug delivery through the nail plate, International Journal of Pharmaceutics, Vol.386,pp.185-194.
- [142] Vasir,J.K; Tambwekar, K & Garg,S(2003). Bioadhesives microsperes as a controlled drug delivery system.International Journal of pharmaceutics,Vol.255,pp.13-32.
- [143] Bodour, A.A and Miller-Maier,RM(1998).Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms.journal of Microbiological methods,Vol.32,pp.273-280.
- [144] Thiesen, D.B and Man, K.F(1999).Surface tension measurements,CRC press, Retrieved from <<http://www.engnetbase.com>>
- [145] Norris,D.A; Puri, N;Labib, M.E & Sinko,P.J(1999).Determining the absolute surface hydrophobicity of microparticulates using thin layer wicking. Journal of controlled Release,Vol.59,pp.173-185.
- [146] Ramirez-Flores,J.C; Bachmann, J and Marmur,A.(2010).Direct determination of contact angle of model soils in comparison with wettability characterization by capillary rise.Journal of Hydrology, Vol.382,pp.10-19.
- [147] Allen J.S(2003).An analytical solution for determination of small contact angles from sessile drops of arbitrary size. Journal of colloid and Interface science,Vol.261,pp.481-489.
- [148] Xu H,2001: the role of ICAM-1 molecule in the migration of langerhans cells in the skin and regional lymph node.Er J. Immunol 31,3085-3093
- [149] Venkatramen S,(2002):Elastic contact between randomly rough surfaces: Comparison theory with numerical results, physical review B,65(18),P.184106.

- [150] Al-saidan,1983; On two methods of calculation of the force of sticking of an elastic sphere to a rigid plane, *colloids and surfaces*,7(3),pp.251-259.
- [151] Buhse L,Gibson,R D and Thomas,T R,1975: The elastic contact of a rough surface, *wear*,35(1),pp.87-111.
- [152] Hendrik J, Anton H, Weekamp, Henry C, van der Mel, Anton W J:Applied and Environmental Microbiology, Nov 1984 p.980 – 983.denial Sch, University of Groningen, Antonius Deusinglam 1,9713 AV Groningen, Nertherland.
- [153] Neumann AW ,Smith R.P, Omenyi S.N(1983)Physiochemical Aspect of polymer surfaces,vol.1,155 – 171,ed Mittal, K.L(Plenum press New York)
- [154] Tavakol M, Mohagheghi M A,Dennick R(2008):Assessing the skills of surgical residents using simulation. *J. Surg Educ*.65(2):77-83.
- [155] Cronbach L:(1951).Coefficient alpha and the internal structure test.*Psychomerika*;16:297-334.
- [155] Streiner D.(2003).Starting at the beginning: an introduction to coefficient alpha and internal consistency.*Journal of personality assessment*;80:99-103

**APPENDIX A1**

<b>Table 3.4ai Wu, Whole Blood infected using water</b>					<b>Table 3.4ii Wu :Whole Blood uninfected using water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	75	-103.18	9.96E-17	36	4500	52	-96.75	9.34E-17
51	278	66	-100.01	9.65E-17	30	6000	55	-97.31	9.39E-17
56	282	73	-102.3	9.88E-17	30	8000	60	-98.4	9.50E-17
64	682	79	-104.91	1.01E-16	35	4900	65	-99.72	9.62E-17
62	606	74	-102.78	9.92E-17	36	5000	59	-98.16	9.47E-17
72	20	80	-105.37	1.02E-16	33	4500	57	-97.72	9.43E-17
56	613	67	-100.32	9.68E-17	39	4000	58	97.94	9.45E-17
52	468	76	-103.59	1.00E-16	48	6200	59	-98.16	9.47E-17
51	853	65	-99.72	9.62E-17	48	4900	50	-96.42	9.31E-17
50	356	80	-105.37	1.02E-16	40	4800	50	-96.42	9.31E-17
52	268	68	-100.64	9.71E-17	38	4000	55	-97.31	9.39E-17
60	625	70	-101.3	9.78E-17	40	5000	53	-96.93	9.35E-17
47	230	72	-102.02	9.85E-17	41	4000	51	-96.58	9.32E-17
41	246	70	-101.3	9.78E-17	34	4000	58	-97.94	9.45E-17
56	339	67	-100.32	9.68E-17	44	6000	60	-98.4	9.50E-17
50	316	78	-104.46	1.01E-16	39	4400	55	-97.31	9.39E-17
52	220	73	-102.39	9.88E-17	36	4700	53	-96.93	9.35E-17
64	374	68	-100.64	9.71E-17	42	4800	56	-97.51	9.41E-17
63	593	82	-106.34	1.03E-16	40	4300	55	-97.31	9.39E-17
53	372	75	-103.18	9.98E-17	30	6000	52	-96.75	9.34E-17
<b>AVE</b>	<b>408.95</b>	<b>72.9</b>	<b>-102.507</b>	<b>9.899E-17</b>		<b>5000</b>	<b>55.65</b>	<b>-87.7045</b>	<b>9.409E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>5.190071493</b>	<b>2.00754393</b>	<b>2.02923E-18</b>		<b>999.474</b>	<b>3.897029908</b>	<b>43.70385825</b>	<b>7.87334E-19</b>

**APPENDIX A2**

<b>Table 3.42ai.Wu: Whole Blood infected with diiodomethane.</b>					<b>Table 3.42aiiWu: Whole Blood Uninfected with diiodometehane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	35	-11.94	1.15E-17	36	4500	30	-9.49	9.16E-18
51	278	40	-14.77	1.43E-17	30	6000	34	-11.42	1.10E-17
56	282	50	-21.61	2.09E-17	30	8000	33	-10.91	1.05E-17
64	682	31	-9.95	9.60E-18	35	4900	31	-9.95	9.60E-18
62	606	32	-10.42	1.01E-17	36	5000	32	-10.42	1.01E-17
72	20	38	-13.59	1.31E-17	33	4500	38	-13.59	1.31E-17
56	613	42	-16.01	1.55E-17	39	4000	35	-11.94	1.15E-17
52	468	33	-10.91	1.05E-17	48	6200	38	-13.59	1.31E-17
51	853	45	-17.99	1.74E-17	48	4900	39	-14.17	1.37E-17
50	356	25	-7.44	7.18E-17	40	4800	40	-14.77	1.43E-17
52	268	30	-9.49	9.16E-17	38	4000	36	-12.47	1.20E-17
60	625	37	-13.02	1.26E-17	40	5000	32	-10.42	1.02E-17
47	230	41	-15.38	1.48E-17	41	4000	36	-12.47	1.20E-17
41	246	37	-13.02	1.20E-17	34	4000	36	-12.47	1.20E-17
56	339	34	-11.42	1.10E-17	44	6000	40	-14.77	1.43E-17
50	316	32	-10.42	1.01E-17	39	4400	45	-17.99	1.74E-17
52	220	29	-9.05	8.74E-17	36	4700	38	-13.59	1.31E-17
64	374	50	-21.61	2.09E-17	42	4800	43	-16.66	1.61E-17
63	593	46	-18.69	1.80E-17	40	4300	46	-18.69	1.80E-17
53	372	43	-16.66	1.61E-17	30	6000	45	-17.99	1.74E-17
<b>AVE</b>	<b>408.95</b>	<b>37.5</b>	<b>-13.6695</b>	<b>2.446E-17</b>		<b>5000</b>	<b>37.35</b>	<b>-13.3885</b>	<b>1.2928E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>7.022520165</b>	<b>4.088606087</b>	<b>2.59312E-17</b>		<b>999.474</b>	<b>4.782368935</b>	<b>2.76251654</b>	<b>2.66737E-18</b>

**APPENDIX A3**

<b>Table 3.43aiWu: Whole Blood Infected with Glycerine</b>					<b>Table 3.43aiiWu: Whole Blood Uninfected with Glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	62	-64.04	6.18E-17	36	4500	47	-56.08	5.41E-17
51	278	60	-62.81	6.06E-17	30	6000	38	-52.63	5.08E-17
56	282	58	-61.63	5.95E-17	30	8000	47	-56.08	5.41E-17
64	682	59	-62.21	6.00E-17	35	4900	49	-56.97	5.50E-17
62	606	60	-62.81	6.06E-17	36	5000	55	-59.96	5.79E-17
72	20	54	-59.43	5.74E-17	33	4500	54	-59.43	5.74E-17
56	613	63	-64.68	6.24E-17	39	4000	46	-55.65	5.37E-17
52	468	66	-66.68	6.44E-17	48	6200	55	-59.96	5.79E-17
51	853	63	-64.68	6.24E-17	48	4900	40	-53.32	5.15E-17
50	356	63	-64.68	6.24E-17	40	4800	45	-55.23	5.33E-17
52	268	55	-59.96	5.79E-17	38	4000	48	-56.52	5.45E-17
60	625	53	-58.91	5.69E-17	40	5000	42	-54.05	5.22E-17
47	230	54	-59.43	5.74E-17	41	4000	40	-53.32	5.15E-17
41	246	55	-59.96	5.79E-17	34	4000	50	-57.44	5.54E-17
56	339	52	-58.41	5.64E-17	44	6000	50	-57.44	5.54E-17
50	316	64	-65.33	6.31E-17	39	4400	50	-57.44	5.54E-17
52	220	60	-62.81	6.06E-17	36	4700	40	-53.32	5.15E-17
64	374	62	-64.04	6.18E-17	42	4800	47	-56.08	5.41E-17
63	593	59	-62.21	6.00E-17	40	4300	50	-57.44	5.54E-17
53	372	57	-61.06	5.89E-17	30	6000	51	-57.92	5.59E-17
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-62.2885</b>	<b>6.012E-17</b>		<b>5000</b>			
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>2.386841725</b>	<b>2.29292E-18</b>		<b>999.474</b>			

**APPENDIX A4**

<b>Table 3.4ai Fowkes: Whole Blood infected with water</b>					<b>Table 3.4aii Fowkes Model: Whole Blood uninfected using water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>
54	438	75	-95.46	9.21E-17	36	4500	52	-81.24	7.84E-17
51	278	66	-89.56	8.64E-17	30	6000	55	-82.91	8.00E-17
56	282	73	-94.11	9.08E-17	30	8000	60	-85.84	8.28E-17
64	682	79	-98.16	9.47E-17	35	4900	65	-88.93	8.58E-17
62	606	74	-94.78	9.15E-17	36	5000	59	-85.24	8.23E-17
72	20	80	-98.84	9.54E-17	33	4500	57	-84.07	8.11E-17
56	613	67	-90.2	8.70E-17	39	4000	58	-84.65	8.17E-17
52	468	76	-96.12	9.28E-17	48	6200	59	-85.24	8.28E-17
51	853	65	-88.93	8.58E-17	48	4900	50	-80.16	7.74E-17
50	356	80	-98.84	9.54E-17	40	4800	50	-80.16	7.74E-17
52	268	68	-90.84	8.77E-17	38	4000	55	-82.91	8.00E-17
60	625	70	-92.14	8.89E-17	40	5000	53	-81.79	7.89E-17
47	230	72	-93.45	9.02E-17	41	4000	51	-80.69	7.79E-17
41	246	70	-92.14	8.89E-17	34	4000	58	-84.65	8.17E-17
56	339	67	-90.2	8.70E-17	44	6000	60	-85.84	8.28E-17
50	316	78	-97.48	9.41E-17	39	4400	55	-82.91	8.00E-17
52	220	73	-94.11	9.08E-17	36	4700	53	-81.79	7.89E-17
64	374	68	-90.84	8.77E-17	42	4800	56	-83.49	8.06E-17
63	593	82	-100.22	9.53E-17	40	4300	55	-82.91	8.00E-17
53	372	75	-95.45	9.21E-17	30	6000	52	-96.75	7.84E-17
<b>AVE</b>	<b>408.95</b>	<b>72.9</b>	<b>-94.0935</b>	<b>9.05E-17</b>		<b>5000</b>	<b>55.65</b>	<b>-84.1085</b>	<b>8.0445E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>5.190071493</b>	<b>3.446075228</b>	<b>3.11357E-18</b>		<b>999.474</b>	<b>3.897029908</b>	<b>3.700454133</b>	<b>2.19221E-18</b>

**APPENDIX A5**

<b>Table 3.45aiFowkes: Whole Blood infected with diiodomethane.</b>					<b>Table 3.45aiiFowkes: Whole Blood Uninfected with diiodomethane.</b>				
<i>Netropil</i> (counts/ $mm^3$ of blood)	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ ( $mJ/m^2$ )	$A_{132}$ ( $mJ/m^2$ )	<i>PcV</i> ( $mm^3$ )	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ ( $mJ/m^2$ )	$A_{132}$ ( $mJ/m^2$ )
54	438	35	-11.46	9.21E-17	36	4500	30	-8.98	8.66E-18
51	278	40	-13.94	8.64E-17	30	6000	34	-10.81	1.04E-17
56	282	50	-24.11	9.08E-17	30	8000	33	-10.33	9.97E-18
64	682	31	-9.42	9.47E-17	35	4900	31	-9.42	9.09E-18
62	606	32	-9.87	9.15E-17	36	5000	32	-9.87	9.52E-18
72	20	38	-12.85	9.54E-17	33	4500	38	-12.85	1.24E-17
56	613	42	-15.2	8.70E-17	39	4000	35	-11.3	1.09E-17
52	468	33	-10.33	9.28E-17	48	6200	38	-12.85	1.24E-17
51	853	45	-16.93	8.58E-17	48	4900	39	-13.39	1.29E-17
50	356	25	-6.98	9.54E-17	40	4800	40	-13.94	1.35E-17
52	268	30	8.84	8.77E-17	38	4000	36	-11.81	1.14E-17
60	625	37	-12.14	8.89E-17	40	5000	32	-9.87	9.52E-18
47	230	41	-13.45	9.02E-17	41	4000	36	-11.81	1.14E-17
41	246	37	-12.14	8.89E-17	34	4000	36	-11.81	1.14E-17
56	339	34	-10.81	8.70E-17	44	6000	40	-13.94	1.35E-17
50	316	32	-9.87	1.60E-10	39	4400	45	-16.86	1.63E-17
52	220	29	-8.11	9.08E-17	36	4700	38	-12.85	1.24E-17
64	374	50	-24.11	8.77E-17	42	4800	43	-15.66	1.51E-17
63	593	46	-17.22	9.53E-17	40	4300	46	17.48	1.69E-17
53	372	43	-15.66	9.21E-17	30	6000	45	-16.93	1.63E-17
<b>AVE</b>	<b>408.95</b>	<b>37.5</b>	<b>-12.288</b>	<b>8.00009E-12</b>		<b>5000</b>	<b>37.35</b>	<b>-10.89</b>	<b>1.2198E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>7.022520165</b>	<b>6.760282305</b>	<b>3.57771E-11</b>		<b>999.474</b>	<b>4.782368935</b>	<b>7.060002982</b>	<b>2.47622E-18</b>

**APPENDIX A6**

<b>Table 3.46aiFowkes :Whole Blood Infected with Glycerine</b>					<b>Table 3.46aiiFowkes :Whole Blood Uninfected with Glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	62	-59.45	5.74E-17	36	4500	47	-49.64	4.78E-17
51	278	60	-58.03	5.60E-17	30	6000	38	-44.59	4.30E-17
56	282	58	-56.63	5.47E-17	30	8000	47	-49.54	4.78E-17
64	682	59	-57.33	5.53E-17	35	4900	49	-50.75	4.90E-17
62	606	60	-58.03	5.60E-17	36	5000	55	-54.6	5.27E-17
72	20	54	-53.93	5.21E-17	33	4500	54	-53.93	5.21E-17
56	613	63	-60.17	5.81E-17	39	4000	46	-48.95	4.72E-17
52	468	66	-62.38	6.02E-17	48	6200	55	-54.6	5.27E-17
51	853	63	-60.17	5.81E-17	48	4900	40	-45.62	4.40E-17
50	356	63	-60.17	5.81E-17	40	4800	45	-48.37	4.67E-17
52	268	55	-54.6	5.27E-17	38	4000	48	-50.14	4.84E-17
60	625	53	-53.28	5.14E-17	40	5000	42	46.69	4.51E-17
47	230	54	-53.93	5.21E-17	41	4000	40	-45.62	4.40E-17
41	246	55	-54.6	5.27E-17	34	4000	50	-51.37	4.96E-17
56	339	52	-52.63	5.08E-17	44	6000	50	-51.37	4.96E-17
50	316	64	-60.9	5.88E-17	39	4400	50	-51.37	4.96E-17
52	220	60	-58.03	5.60E-17	36	4700	40	-45.62	4.40E-17
64	374	62	-59.45	5.74E-17	42	4800	47	-49.54	4.78E-17
63	593	59	-57.33	5.53E-17	40	4300	50	-51.37	4.96E-17
53	372	57	-55.95	5.40E-17	30	6000	51	-51.96	5.02E-17
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-57.3495</b>	<b>5.536E-17</b>		<b>5000</b>	<b>47.2</b>	<b>-45.113</b>	<b>4.8045E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>2.82664107</b>	<b>2.72906E-18</b>		<b>999.474</b>	<b>5.084548316</b>	<b>21.80310265</b>	<b>2.91141E-18</b>

**APPENDIX A7**

<b>Table 3.47ai Owens &amp; Wendt Model: Whole Blood Uninfected with Water</b>					<b>Table 3.47aiiOwens &amp; Wendt :Whole Blood infected with Water.</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	52	-27.98	2.70E-17	36	4500	75	-53.96	5.21E-17
51	278	55	-31.04	3.00E-17	30	6000	66	-43.19	4.17E-17
56	282	60	-36.4	3.51E-17	30	8000	73	-51.52	4.97E-17
64	682	65	-42.03	4.06E-17	35	4900	79	-58.91	5.69E-17
62	606	59	-35.31	3.41E-17	36	5000	74	52.73	5.09E-17
72	20	57	-33.15	3.20E-17	33	4500	80	-60.16	5.81E-17
56	613	58	-34.22	3.30E-17	39	4000	67	-44.35	4.28E-17
52	468	59	-35.19	3.41E-17	48	6200	76	-55.19	5.33E-17
51	853	50	-26.01	2.51E-17	48	4900	65	-42.03	4.06E-17
50	356	50	-26.01	2.51E-17	40	4800	80	-60.16	5.81E-17
52	268	55	-31.04	3.00E-17	38	4000	68	-45.53	4.39E-17
60	625	53	-28.99	2.80E-17	40	5000	70	-47.9	4.62E-17
47	230	51	-26.99	2.60E-17	41	4000	72	-50.3	4.85E-17
41	246	58	-34.22	3.30E-17	34	4000	70	-47.9	4.62E-17
56	339	60	-36.4	3.51E-17	44	6000	67	-44.35	4.28E-17
50	316	55	-31.04	3.00E-17	39	4400	78	-57.66	5.57E-17
52	220	53	-28.99	2.80E-17	36	4700	73	-51.52	4.97E-17
64	374	56	-32.09	3.10E-17	42	4800	68	-45.53	4.39E-17
63	593	55	-31.04	3.00E-17	40	4300	82	-62.67	6.05E-17
53	372	52	-27.98	2.70E-17	30	6000	75	-53.96	5.21E-17
<b>AVE</b>	<b>408.95</b>	<b>55.65</b>	<b>-31.806</b>	<b>3.071E-17</b>		<b>5000</b>	<b>72.9</b>	<b>-46.203</b>	<b>4.9685E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>3.897029908</b>	<b>4.1159221</b>	<b>3.9798E-18</b>		<b>999.474</b>	<b>5.190071493</b>	<b>24.12215232</b>	<b>6.09903E-18</b>

**APPENDIX A8**

<b>Table 3.48aiOwen&amp;Wendt: Whole Blood Uninfected with diiodomethane.</b>					<b>Table 3.48iiOwen&amp;Wendt: Whole Blood infected with diiodomethane.</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	30	-6.81	6.57E-18	36	4500	35	-9.19	8.87E-18
51	278	34	-8.68	8.38E-18	30	6000	40	-11.88	1.15E-17
56	282	33	-8.2	7.91E-18	30	8000	50	-18.15	1.75E-17
64	682	31	-7.26	7.00E-18	35	4900	31	-7.26	7.00E-18
62	606	32	-7.72	7.45E-18	36	5000	32	-7.72	7.45E-18
72	20	38	10.77	1.04E-17	33	4500	38	-10.77	1.04E-17
56	613	35	-9.19	8.87E-18	39	4000	42	-13.05	1.26E-17
52	468	38	-10.77	1.04E-17	48	6200	33	-8.2	7.91E-18
51	853	39	-11.32	1.09E-17	48	4900	45	-14.88	1.44E-17
50	356	40	-11.88	1.15E-17	40	4800	25	-4.76	4.59E-18
52	268	36	-9.7	9.36E-18	38	4000	30	-6.81	6.57E-18
60	625	32	-7.72	7.45E-18	40	5000	37	-10.23	9.87E-18
47	230	36	-9.7	9.36E-18	41	4000	41	-12.48	1.20E-17
41	246	36	9.7	9.36E-18	34	4000	37	-10.23	9.87E-18
56	339	40	-11.88	1.15E-17	44	6000	34	-8.68	8.38E-18
50	316	45	-14.88	1.44E-17	39	4400	32	-7.72	7.45E-18
52	220	38	-10.77	1.04E-17	36	4700	29	-6.37	6.15E-18
64	374	43	13.65	1.32E-17	42	4800	50	-18.15	1.75E-17
63	593	46	-15.51	1.50E-17	40	4300	46	-15.51	1.50E-17
53	372	45	-14.88	1.44E-17	30	6000	43	-13.65	1.32E-17
<b>AVE</b>	<b>408.95</b>	<b>37.35</b>	<b>-7.1375</b>	<b>1.01905E-17</b>		<b>5000</b>	<b>37.5</b>	<b>-10.7845</b>	<b>1.04105E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>4.782368935</b>	<b>8.387999495</b>	<b>2.53701E-18</b>		<b>999.474</b>	<b>7.022520165</b>	<b>3.853041826</b>	<b>3.72095E-18</b>

**APPENDIX A9**

<b>Table 3.49ai Owen&amp; Wendt: Whole Blood infected with glycerine.</b>					<b>Table 3.49aii Owen&amp; Wendt: Whole Blood Uninfected with glycerine.</b>					
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	
54	438	62	-33.95	3.28E-17	36	4500	47	-20.35	1.96E-17	
51	278	60	-32	3.09E-17	30	6000	38	-13.57	1.31E-17	
56	282	58	-30.09	2.90E-17	30	8000	47	-20.35	2.90E-17	
64	682	59	-31.04	3.00E-17	35	4900	49	-22.01	2.12E-17	
62	606	60	-32	3.09E-17	36	5000	55	-27.29	2.63E-17	
72	20	54	-26.38	2.55E-17	33	4500	54	-26.38	2.55E-17	
56	613	63	-34.94	3.37E-17	39	4000	46	-19.54	1.89E-17	
52	468	66	-37.97	3.66E-17	48	6200	55	-27.29	2.63E-17	
51	853	63	-34.94	3.37E-17	48	4900	40	-14.97	1.45E-17	
50	356	63	-34.94	3.37E-17	40	4800	45	-18.75	1.81E-17	
52	268	55	-27.29	2.63E-17	38	4000	48	-21.18	2.04E-17	
60	625	53	-25.48	2.46E-17	40	5000	42	-16.44	1.59E-17	
47	230	54	-26.38	2.55E-17	41	4000	40	-14.97	1.45E-17	
41	246	55	27.29	2.63E-17	34	4000	50	22.86	2.21E-17	
56	339	52	-24.6	2.37E-17	44	6000	50	-22.86	2.21E-17	
50	316	64	-35.94	3.47E-17	39	4400	50	-22.86	2.21E-17	
52	220	60	-32	3.09E-17	36	4700	40	-14.97	1.45E-17	
64	374	62	-33.95	3.28E-17	42	4800	47	-20.35	1.96E-17	
63	593	59	-31.04	3.00E-17	40	4300	50	-22.86	2.21E-17	
53	372	57	-29.14	2.81E-17	30	6000	51	-23.72	2.29E-17	
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-28.339</b>	<b>2.9985E-17</b>		<b>5000</b>	<b>47.2</b>	<b>-18.3925</b>	<b>2.02389E-17</b>	
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>13.6270632</b>	<b>3.74282E-18</b>		<b>999.474</b>	<b>5.084548316</b>	<b>10.53356434</b>	<b>4.51209E-18</b>	

**APPENDIX A10**

<b>Table 3.50ai Zisman: Whole Blood infected with water</b>						<b>Table 3.50aii Zisman Model: Whole Blood Uninfected with Water</b>					
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>		
54	438	75	-53.96	5.21E-17	36	4500	52	-27.98	2.70E-17		
51	278	66	-43.19	4.17E-17	30	6000	55	-31.04	3.00E-17		
56	282	73	-51.52	4.97E-17	30	8000	60	-36.4	3.51E-17		
64	682	79	-58.91	5.69E-17	35	4900	65	-42.03	4.06E-17		
62	606	74	-52.73	5.09E-17	36	5000	59	-35.31	3.41E-17		
72	20	80	-60.16	5.81E-17	33	4500	57	-33.15	3.20E-17		
56	613	67	-44.35	4.28E-17	39	4000	58	-34.22	3.30E-17		
52	468	76	-55.19	5.33E-17	48	6200	59	-35.19	3.41E-17		
51	853	65	-42.03	4.06E-17	48	4900	50	-26.01	2.51E-17		
50	356	80	-60.16	5.81E-17	40	4800	50	-26.01	2.51E-17		
52	268	68	-45.53	4.39E-17	38	4000	55	-31.04	3.00E-17		
60	625	70	-47.9	4.62E-17	40	5000	53	-28.99	2.80E-17		
47	230	72	-50.3	4.85E-17	41	4000	51	-26.99	2.60E-17		
41	246	70	-47.9	4.62E-17	34	4000	58	-34.22	3.30E-17		
56	339	67	-44.35	4.28E-17	44	6000	60	-36.4	3.51E-17		
50	316	78	-57.66	5.57E-17	39	4400	55	-31.04	3.00E-17		
52	220	73	-51.52	4.97E-17	36	4700	53	-28.99	2.80E-17		
64	374	68	-45.53	4.39E-17	42	4800	56	-32.09	3.10E-17		
63	593	82	-62.67	6.05E-17	40	4300	55	-31.04	3.00E-17		
53	372	75	-53.96	5.21E-17	30	6000	52	-27.98	2.70E-17		
<b>AVE</b>	<b>408.95</b>	<b>72.9</b>	<b>-51.476</b>	<b>4.9685E-17</b>		<b>5000</b>	<b>55.65</b>	<b>-31.806</b>	<b>3.071E-17</b>		
<b>SD</b>	<b>199.3012729</b>	<b>5.190071493</b>	<b>6.301457977</b>	<b>6.09903E-18</b>		<b>999.474</b>	<b>3.897029908</b>	<b>4.1159221</b>	<b>3.9798E-18</b>		

**APPENDIX A11**

<b>Table 3.51ai Zisman: Whole Blood infected with diiodomethane</b>					<b>Table 3.51aii Zisman: Whole Blood Uninfected with diiodomethane</b>					
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	
54	438	35	-9.19	8.87E-18	36	4500	30	-6.81	6.57E-18	
51	278	40	-11.88	1.15E-17	30	6000	34	-8.68	8.38E-18	
56	282	50	-18.15	1.75E-17	30	8000	33	-8.2	7.91E-18	
64	682	31	-7.26	7.00E-18	35	4900	31	-7.26	7.00E-18	
62	606	32	-7.72	7.45E-18	36	5000	32	-7.72	7.45E-18	
72	20	38	10.77	1.04E-17	33	4500	38	-10.77	1.04E-17	
56	613	42	-13.05	1.26E-17	39	4000	35	-9.19	8.87E-18	
52	468	33	-8.2	7.91E-18	48	6200	38	-10.77	1.04E-17	
51	853	45	-14.88	1.44E-17	48	4900	39	-11.32	1.09E-17	
50	356	25	-4.76	4.59E-18	40	4800	40	-11.88	1.15E-17	
52	268	30	-6.81	6.57E-18	38	4000	36	-9.7	9.36E-18	
60	625	37	-10.23	9.87E-18	40	5000	32	-7.72	7.45E-18	
47	230	41	-12.46	1.20E-17	41	4000	36	-9.7	9.36E-18	
41	246	37	-10.23	9.87E-17	34	4000	36	-9.7	9.36E-18	
56	339	34	-8.68	8.38E-18	44	6000	40	-11.88	1.15E-17	
50	316	32	-7.72	7.45E-18	39	4400	45	-14.88	1.44E-17	
52	220	29	-6.37	6.15E-18	36	4700	38	-10.77	1.04E-17	
64	374	50	-18.15	1.75E-17	42	4800	43	-13.65	1.32E-17	
63	593	46	-15.51	1.50E-17	40	4300	46	-15.51	1.50E-17	
53	372	43	13.65	1.32E-17	30	6000	45	-14.88	1.44E-17	
<b>AVE</b>	<b>408.95</b>	<b>37.5</b>	<b>-8.3415</b>	<b>1.4852E-17</b>		<b>5000</b>	<b>37.35</b>	<b>-10.5495</b>	<b>1.01905E-17</b>	
<b>SD</b>	<b>199.3012729</b>	<b>7.022520165</b>	<b>8.000231099</b>	<b>2.00831E-17</b>		<b>999.474</b>	<b>4.782368935</b>	<b>2.614245337</b>	<b>2.53701E-18</b>	

**APPENDIX A12**

<b>Table 3.52ai Zisman: Whole Blood infected with glycerine</b>					<b>Table 3.52aii Zisman: Whole Blood Un infected with glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	62	-33.95	3.28E-17	36	4500	47	-20.35	1.96E-17
51	278	60	-32	3.09E-17	30	6000	38	-13.57	1.31E-17
56	282	58	-30.09	2.90E-17	30	8000	47	-20.35	1.96E-17
64	682	59	31.04	3.00E-17	35	4900	49	-22.01	2.12E-17
62	606	60	-32	3.09E-17	36	5000	55	-27.29	2.63E-17
72	20	54	-26.38	2.55E-17	33	4500	54	-26.38	2.55E-17
56	613	63	-34.94	3.37E-17	39	4000	46	-19.54	1.89E-17
52	468	66	-37.97	3.66E-17	48	6200	55	-27.29	2.63E-17
51	853	63	-34.94	3.37E-17	48	4900	40	-14.97	1.45E-17
50	356	63	-34.94	3.37E-17	40	4800	45	-18.75	1.81E-17
52	268	55	-27.29	2.63E-17	38	4000	48	-21.18	2.04E-17
60	625	53	-25.48	2.46E-17	40	5000	42	-16.44	1.59E-17
47	230	54	-26.38	2.55E-17	41	4000	40	-14.97	1.45E-17
41	246	55	-27.29	2.63E-17	34	4000	50	-22.86	2.21E-17
56	339	52	-24.6	2.37E-17	44	6000	50	-22.86	2.21E-17
50	316	64	-35.94	3.47E-17	39	4400	50	-22.86	2.21E-17
52	220	60	-32	3.09E-17	36	4700	40	-14.97	1.45E-17
64	374	62	-33.95	3.28E-17	42	4800	47	-20.35	1.96E-17
63	593	59	-31.04	3.00E-17	40	4300	50	-22.86	2.21E-17
53	372	57	-29.14	2.81E-17	30	6000	51	-23.72	2.29E-17
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-27.964</b>	<b>2.9985E-17</b>		<b>5000</b>	<b>47.2</b>	<b>-20.6785</b>	<b>1.9965E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>14.41949098</b>	<b>3.74282E-18</b>		<b>999.474</b>	<b>5.084548316</b>	<b>4.115678974</b>	<b>3.95851E-18</b>

**APPENDIX A13**

<b>Table 3.53aiNeuman: Whole Blood infected with water</b>					<b>Table 3.53aiiNeuman Model: Whole Blood Uninfected with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	75	-33.85	3.27E-17	36	4500	52	-11.38	1.10E-17
51	278	66	-25.87	2.50E-17	30	6000	55	-14.82	1.43E-17
56	282	73	-32.15	3.10E-17	30	8000	60	-20.07	1.94E-17
64	682	79	-37.12	3.58E-17	35	4900	65	-24.93	2.41E-17
62	606	74	-33	3.19E-17	36	5000	59	-19.05	1.84E-17
72	20	80	-37.92	3.66E-17	33	4500	57	-16.97	1.64E-17
56	613	67	-26.8	2.59E-17	39	4000	58	-18.02	1.74E-17
52	468	76	-34.68	3.35E-17	48	6200	59	-19.05	1.84E-17
51	853	65	-24.93	2.41E-17	48	4900	50	-8.89	8.58E-18
50	356	80	-37.92	3.66E-17	40	4800	50	-8.89	8.58E-18
52	268	68	-27.72	2.67E-17	38	4000	55	-14.82	1.43E-17
60	625	70	-29.52	2.85E-17	40	5000	53	-12.56	1.21E-17
47	230	72	-31.28	3.02E-17	41	4000	51	-10.16	9.80E-18
41	246	70	-29.52	2.85E-17	34	4000	58	-18.02	1.74E-17
56	339	67	-26.8	2.59E-17	44	6000	60	-20.07	1.94E-17
50	316	78	-36.32	3.51E-17	39	4400	55	-14.82	1.43E-17
52	220	73	-32.15	3.10E-17	36	4700	53	-12.56	1.21E-17
64	374	68	-27.72	2.67E-17	42	4800	56	-15.91	1.54E-17
63	593	82	-39.47	3.81E-17	40	4300	55	-14.82	1.43E-17
53	372	75	-33.85	3.27E-17	30	6000	52	-11.38	1.10E-17
<b>AVE</b>	<b>408.95</b>	<b>72.9</b>	<b>-31.9295</b>	<b>3.0825E-17</b>		<b>5000</b>	<b>55.65</b>	<b>-15.3595</b>	<b>1.4833E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>5.190071493</b>	<b>4.453510118</b>	<b>4.29784E-18</b>		<b>999.474</b>	<b>3.897029908</b>	<b>4.227230616</b>	<b>4.09097E-18</b>

**APPENDIX A14**

<b>Table 3.54ai Neuman: Whole Blood infected with diiodomethane</b>					<b>Table 3.54ii Neuman: Whole Blood Uninfected with diiodomethane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	35	-6.67	6.44E-18	36	4500	30	-4.93	4.75E-18
51	278	40	-8.64	8.34E-18	30	6000	34	-6.3	6.08E-18
56	282	50	-13.12	1.27E-17	30	8000	33	-5.95	5.74E-18
64	682	31	-5.26	5.07E-18	35	4900	31	-5.26	5.07E-18
62	606	32	-5.6	5.40E-18	36	5000	32	-5.6	5.40E-18
72	20	38	-7.83	7.56E-18	33	4500	38	-7.83	7.56E-18
56	613	42	-9.48	9.15E-18	39	4000	35	-6.67	6.44E-18
52	468	33	-5.96	5.74E-18	48	6200	38	-7.83	7.56E-18
51	853	45	-10.8	1.04E-17	48	4900	39	-8.23	7.94E-18
50	356	25	-3.43	3.31E-18	40	4800	40	-8.64	8.34E-18
52	268	30	-4.93	4.75E-18	38	4000	36	-7.05	6.80E-18
60	625	37	-7.43	7.17E-18	40	5000	32	-5.6	5.40E-18
47	230	41	-9.06	8.74E-18	41	4000	36	-7.05	6.80E+00
41	246	37	-7.43	7.17E-18	34	4000	36	-7.05	6.80E-18
56	339	34	-6.3	6.08E-18	44	6000	40	-8.64	8.34E-18
50	316	32	-5.6	5.40E-18	39	4400	45	-10.8	1.04E-17
52	220	29	-4.61	4.45E-18	36	4700	38	-7.83	7.56E-18
64	374	50	-13.12	1.27E-17	42	4800	43	-9.92	9.57E-18
63	593	46	-11.26	1.09E-17	40	4300	46	-11.26	1.09E-17
53	372	43	-9.92	9.57E-18	30	6000	45	-10.8	1.04E-17
<b>AVE</b>	<b>408.95</b>	<b>37.5</b>	<b>-7.8225</b>	<b>7.552E-18</b>		<b>5000</b>	<b>37.35</b>	<b>-7.662</b>	<b>0.34</b>
<b>SD</b>	<b>199.3012729</b>	<b>7.022520165</b>	<b>2.791009768</b>	<b>2.70333E-18</b>		<b>999.474</b>	<b>4.782368935</b>	<b>1.901375126</b>	<b>1.520526225</b>

**APPENDIX A15**

<b>Table 3.55aiNeuman: Whole Blood infected with glycerine</b>					<b>Table 3.55aiiNeuman: Whole BloodUninfected with glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	62	-22.25	2.15E-17	36	4500	47	-12.13	1.17E-17
51	278	60	-20.86	2.01E-17	30	6000	38	-6.87	6.63E-18
56	282	58	-19.47	1.88E-17	30	8000	47	-12.13	1.17E-17
64	682	59	-20.16	1.95E-17	35	4900	49	-13.41	1.29E-17
62	606	60	-20.86	2.01E-17	36	5000	55	-17.41	1.68E-17
72	20	54	-16.73	1.61E-17	33	4500	54	-16.73	1.61E-17
56	613	63	-22.94	2.21E-17	39	4000	46	-11.5	1.11E-17
52	468	66	-25.03	2.42E-17	48	6200	55	-17.41	1.68E-17
51	853	63	-22.94	2.21E-17	48	4900	40	-7.95	7.68E-18
50	356	63	-22.94	2.21E-17	40	4800	45	-10.89	1.05E-17
52	268	55	-17.41	1.68E-17	38	4000	48	-12.77	1.23E-17
60	625	53	-16.06	1.55E-17	40	5000	42	-9.09	8.77E-18
47	230	54	-16.73	1.61E-17	41	4000	40	-7.95	7.68E-18
41	246	55	-17.41	1.68E-17	34	4000	50	-14.06	1.36E-17
56	339	52	-15.39	1.48E-17	44	6000	50	-14.06	1.36E-17
50	316	64	-23.64	2.28E-17	39	4400	50	-14.06	1.36E-17
52	220	60	-20.88	2.01E-17	36	4700	40	-7.95	7.68E-18
64	374	62	-22.25	2.15E-17	42	4800	47	-12.13	1.17E-17
63	593	59	-20.16	1.95E-17	40	4300	50	-14.06	1.36E-17
53	372	57	-18.78	1.81E-17	30	6000	51	-14.72	1.42E-17
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-20.1445</b>	<b>1.943E-17</b>		<b>5000</b>	<b>47.2</b>	<b>-12.364</b>	<b>1.1932E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>2.808263136</b>	<b>2.71915E-18</b>		<b>999.474</b>	<b>5.084548316</b>	<b>3.16841551</b>	<b>3.05532E-18</b>

**APPENDIX A16**

<b>Table 3.56aiGirifalco&amp; Good: Whole Blood infected with water</b>					<b>Table 3.56aiiGirifalco&amp;Good Model: Whole Blood Uninfected with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	75	-53.96	5.21E-17	36	4500	52	-27.98	2.70E-17
51	278	66	-32	4.17E-17	30	6000	55	-31.04	3.00E-17
56	282	73	-51.52	4.97E-17	30	8000	60	-36.4	3.51E-17
64	682	79	-58.91	5.69E-17	35	4900	65	-42.03	4.06E-17
62	606	74	-52.73	5.09E-17	36	5000	59	-35.31	3.41E-17
72	20	80	-60.11	5.81E-17	33	4500	57	-33.15	3.20E-17
56	613	67	-44.35	4.28E-17	39	4000	58	-34.22	3.30E-17
52	468	76	-55.19	5.33E-17	48	6200	59	-35.31	3.41E-17
51	853	65	-42.03	4.06E-17	48	4900	50	-26.01	2.51E-17
50	356	80	60.16	5.81E-17	40	4800	50	-26.01	2.51E-17
52	268	68	-45.53	4.39E-17	38	4000	55	-31.04	3.00E-17
60	625	70	-47.9	4.62E-17	40	5000	53	-28.99	2.80E-17
47	230	72	-50.3	4.85E-17	41	4000	51	-26.99	2.60E-17
41	246	70	-47.9	4.62E-17	34	4000	58	-34.22	3.30E-17
56	339	67	-44.35	4.28E-17	44	6000	60	-36.4	3.51E-17
50	316	78	-57.66	5.57E-17	39	4400	55	-31.04	3.00E-17
52	220	73	-51.52	4.97E-17	36	4700	53	-28.99	2.80E-17
64	374	68	-45.53	4.39E-17	42	4800	56	-32.09	3.10E-17
63	593	82	-62.67	6.05E-17	40	4300	55	-31.04	3.00E-17
53	372	75	-53.96	5.21E-17	30	6000	52	-27.98	2.70E-17
<b>AVE</b>	<b>408.95</b>	<b>72.9</b>	<b>-44.898</b>	<b>4.9685E-17</b>		<b>5000</b>	<b>55.65</b>	<b>-31.812</b>	<b>3.071E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>5.190071493</b>	<b>25.73757536</b>	<b>6.09903E-18</b>		<b>999.474</b>	<b>3.897029908</b>	<b>4.121198855</b>	<b>3.9798E-18</b>

**APPENDIX A17**

<b>Table 3.57ai.Girifalco&amp;Good: Whole Blood infected with diiodomethane</b>					<b>Table 3.57aii.Girifalco &amp;Good: Whole Blood Uninfected with diiodomethane</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	35	-9.19	8.87E-18	36	4500	30	-6.81	6.57E-18
51	278	40	-11.88	1.15E-17	30	6000	34	-8.68	8.38E-18
56	282	50	-18.15	1.75E-17	30	8000	33	-8.2	7.91E-18
64	682	31	-7.26	7.00E-18	35	4900	31	-7.26	7.00E-18
62	606	32	-7.72	7.45E-18	36	5000	32	-7.72	7.45E-18
72	20	38	-10.77	1.04E-17	33	4500	38	-10.77	1.04E-17
56	613	42	-13.05	1.26E-17	39	4000	35	-9.19	8.87E-18
52	468	33	-8.2	7.91E-18	48	6200	38	-10.77	1.04E-17
51	853	45	-14.88	1.44E-17	48	4900	39	-11.32	1.09E-17
50	356	25	-4.76	4.59E-18	40	4800	40	-11.88	1.15E-17
52	268	30	-6.81	6.57E-18	38	4000	36	-9.7	9.36E-18
60	625	37	-10.23	9.87E-18	40	5000	32	-7.72	7.45E-18
47	230	41	-12.46	1.20E-17	41	4000	36	-9.7	9.36E-18
41	246	37	-10.23	9.87E-18	34	4000	36	-9.7	9.36E-18
56	339	34	-8.68	8.38E-18	44	6000	40	-11.88	1.15E-17
50	316	32	-7.72	7.45E-18	39	4400	45	-14.88	1.44E-17
52	220	29	-6.37	6.15E-18	36	4700	38	-10.77	1.04E-17
64	374	50	-18.15	1.75E-17	42	4800	43	-13.65	1.32E-17
63	593	46	-15.51	1.50E-17	40	4300	46	-15.51	1.50E-17
53	372	43	-13.65	1.32E-17	30	6000	45	-14.88	1.44E-17
<b>AVE</b>	<b>408.95</b>	<b>37.5</b>	<b>-10.7835</b>	<b>1.04105E-17</b>		<b>5000</b>	<b>37.35</b>	<b>-10.5495</b>	<b>1.01905E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>7.022520165</b>	<b>3.852581192</b>	<b>3.72095E-18</b>		<b>999.474</b>	<b>4.782368935</b>	<b>2.614245337</b>	<b>2.53701E-18</b>

**APPENDIX A18**

<b>Table 3.58ai.Girifalco&amp;Good: Whole Blood infected with glycerine</b>					<b>Table 3.58aii.Girifalco&amp;Good: Whole Blood Unfected with glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	62	-33.95	3.28E-17	36	4500	47	-6.81	6.57E-18
51	278	60	-32	3.09E-17	30	6000	38	-8.68	8.38E-18
56	282	58	-30.09	2.90E-17	30	8000	47	-8.2	7.91E-18
64	682	59	-31.04	3.00E-17	35	4900	49	-7.26	7.00E-18
62	606	60	-32	3.09E-17	36	5000	55	-7.72	7.45E-18
72	20	54	-26.38	2.55E-17	33	4500	54	-10.77	1.04E-17
56	613	63	-34.94	3.37E-17	39	4000	46	-9.19	8.87E-18
52	468	66	-37.97	3.66E-17	48	6200	55	-10.77	1.04E-17
51	853	63	-34.94	3.37E-17	48	4900	40	-11.32	1.09E-17
50	356	63	-34.94	3.37E-17	40	4800	45	-11.88	1.15E-17
52	268	55	-27.29	2.63E-17	38	4000	48	-9.7	9.36E-18
60	625	53	-25.48	2.46E-17	40	5000	42	-7.72	7.45E-18
47	230	54	-26.38	2.55E-17	41	4000	40	-9.7	9.36E-18
41	246	55	-27.29	2.63E-17	34	4000	50	9.7	9.36E-18
56	339	52	-24.6	2.37E-17	44	6000	50	-11.88	1.15E-17
50	316	64	-35.94	3.47E-17	39	4400	50	-14.88	1.44E-17
52	220	60	-32	3.09E-17	36	4700	40	-10.77	1.04E-17
64	374	62	-33.95	3.28E-17	42	4800	47	-13.65	1.32E-17
63	593	59	-31.04	3.00E-17	40	4300	50	-15.51	1.50E-17
53	372	57	-29.14	2.81E-17	30	6000	51	-14.88	1.44E-17
<b>AVE</b>	<b>408.95</b>	<b>58.95</b>	<b>-31.068</b>	<b>2.9985E-17</b>		<b>5000</b>	<b>47.2</b>	<b>-9.5795</b>	<b>1.01905E-17</b>
<b>SD</b>	<b>199.3012729</b>	<b>4.07140219</b>	<b>3.878456828</b>	<b>3.74282E-18</b>		<b>999.474</b>	<b>5.084548316</b>	<b>5.233263523</b>	<b>2.53701E-18</b>

## APPENDIX B1

<b>Table 3.41bi. Wu Model: Separated infected White Cell using water</b>					<b>Table 3.41bii. Model: Separated uninfected White Cell using water.</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	80	-105.37	1.02E-16	36	4500	59	-98.16	9.47E-17
51	278	78	-104.46	1.01E-16	30	6000	78	-104.46	1.01E-16
56	282	76	102.39	9.88E-17	30	8000	50	-96.42	9.31E-17
64	682	64	99.43	1.01E-16	35	4900	57	-97.72	9.43E-17
62	606	70	101.3	9.92E-17	36	5000	63	-99.16	9.57E-17
72	20	85	-107.89	1.02E-16	33	4500	64	-99.43	9.60E-17
56	613	71	-101.65	9.81E-17	39	4000	60	-98.4	9.50E-17
52	468	76	-103.59	1.00E-16	48	6200	63	-99.16	9.57E-17
51	853	74	102.78	9.92E-17	48	4900	58	-97.94	9.45E-17
50	356	75	-103.18	9.96E-17	40	4800	60	-98.4	9.50E-17
52	268	64	-99.43	1.01E-16	38	4000	64	-99.43	9.60E-17
60	625	75	-103.18	9.78E-17	40	5000	60	-98.4	9.50E-17
47	230	69	100.96	9.85E-17	41	4000	63	-99.16	9.57E-17
41	246	70	101.3	9.78E-17	34	4000	64	-99.43	9.60E-17
56	339	71	-101.65	9.81E-17	44	6000	55	-97.31	9.39E-17
50	316	66	-100.01	9.61E-17	39	4400	65	-99.72	9.62E-17
52	220	73	-102.39	9.88E-17	36	4700	58	-97.94	9.45E-17
64	374	69	-100.96	9.74E-17	42	4800	59	-98.16	9.47E-17
63	593	65	99.72	9.62E-17	40	4300	58	-97.94	9.45E-17
53	372	75	-103.18	9.96E-17	30	6000	60	-98.4	9.50E-17
<b>AVE</b>	<b>408.95</b>	<b>72.3</b>	<b>-31.453</b>	<b>9.92E-17</b>		<b>5000</b>	<b>60.9</b>	<b>-98.757</b>	<b>9.5E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.48778</b>	<b>99.83221</b>	<b>1.70163E-18</b>		<b>999.47</b>	<b>5.40857</b>	<b>1.57536</b>	<b>1.6E-18</b>

**APPENDIX B2**

<b>Table 3.42bi.Wu: Separated infected White Cell with diiodomethane.</b>					<b>Table 3.42bii.Wu: Separated uninfected White Cell with diiodomethane.</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	65	-34.66	3.35E-17	36	4500	42	-16.01	1.55E-17
51	278	65	-34.66	3.35E-17	30	6000	65	-34.66	3.35E-17
56	282	50	-21.61	2.09E-17	30	8000	32	-10.42	1.01E-17
64	682	63	-32.75	3.16E-17	35	4900	43	-16.66	1.61E-17
62	606	63	-32.75	3.16E-17	36	5000	53	-23.96	2.31E-17
72	20	60	-29.97	2.89E-17	33	4500	50	-21.61	2.09E-17
56	613	60	-29.97	2.89E-17	39	4000	43	-16.66	1.61E-17
52	468	50	-21.61	2.09E-17	48	6200	48	-20.12	1.94E-17
51	853	54	-24.78	2.39E-17	48	4900	54	-24.78	2.39E-17
50	356	60	-29.97	2.89E-17	40	4800	47	-19.39	1.87E-17
52	268	52	-23.16	2.24E-17	38	4000	48	-20.12	1.94E-17
60	625	41	-15.38	1.48E-17	40	5000	40	-14.77	1.43E-17
47	230	42	-16.01	1.55E-17	41	4000	35	-11.94	1.15E-17
41	246	57	-27.31	2.64E-17	34	4000	49	-20.86	2.01E-17
56	339	63	-32.75	3.16E-17	44	6000	48	-20.12	1.94E-17
50	316	50	-21.61	2.09E-17	39	4400	45	-17.99	1.74E-17
52	220	46	-18.69	1.80E-17	36	4700	47	-19.39	1.87E-17
64	374	50	-21.61	2.09E-17	42	4800	38	-13.59	1.31E-17
63	593	52	-23.16	2.24E-17	40	4300	45	-17.99	1.74E-17
53	372	55	-25.6	2.47E-17	30	6000	40	-14.77	1.43E-17
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-25.9005</b>	<b>2.501E-17</b>		<b>5000</b>	<b>45.6</b>	<b>-18.7905</b>	<b>1.81E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.38348</b>	<b>6.054003</b>	<b>5.8405E-18</b>		<b>999.47</b>	<b>7.2504</b>	<b>5.26734</b>	<b>5.1E-18</b>

**APPENDIX B3**

<b>Table 3.43bi. Wu: Separated Infected White Cell with Glycerine</b>					<b>Table 3.43bii. Wu: Separated Uninfected White Cell with Glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	58	-61.63	5.95E-17	36	4500	45	-55.23	5.33E-17
51	278	59	-62.21	6.00E-17	30	6000	51	-57.92	5.59E-17
56	282	66	-66.68	6.44E-17	30	8000	47	-56.08	5.41E-17
64	682	67	-67.37	6.50E-17	35	4900	48	-56.52	5.45E-17
62	606	68	-68.08	6.57E-17	36	5000	48	-56.52	5.45E-17
72	20	59	-62.21	6.00E-17	33	4500	50	-57.44	5.54E-17
56	613	68	-68.08	6.57E-17	39	4000	48	-56.52	5.45E-17
52	468	73	-71.83	6.93E-17	48	6200	51	-57.92	5.59E-17
51	853	69	-68.8	6.64E-17	48	4900	55	-59.96	5.79E-17
50	356	65	-66	6.37E-17	40	4800	50	-57.44	5.54E-17
52	268	61	-63.42	6.12E-17	38	4000	50	-57.44	5.54E-17
60	625	60	-62.81	6.06E-17	40	5000	61	-63.42	6.12E-17
47	230	63	-64.68	6.24E-17	41	4000	50	-57.44	5.54E-17
41	246	60	-62.81	6.06E-17	34	4000	53	-58.91	5.69E-17
56	339	65	-66	6.37E-17	44	6000	48	-56.52	5.45E-17
50	316	63	-64.68	6.24E-17	39	4400	51	-57.92	5.59E-17
52	220	60	-62.81	6.06E-17	36	4700	52	-58.41	5.64E-17
64	374	66	-66.68	6.44E-17	42	4800	46	-55.65	5.37E-17
63	593	60	-62.81	6.06E-17	40	4300	50	-57.44	5.54E-17
53	372	65	-66	6.37E-17	30	6000	49	-56.97	5.50E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-65.2795</b>	<b>6.2995E-17</b>		<b>5000</b>	<b>50.15</b>	<b>-57.5835</b>	<b>5.56E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.08946</b>	<b>2.729678</b>	<b>2.64047E-18</b>		<b>999.47</b>	<b>3.46828</b>	<b>1.76272</b>	<b>1.7E-18</b>

**APPENDIX B4**

<b>Table 3.44bi.Fowkes Model :Separated Infected white cell using water</b>					<b>Table 3.44bii.FowkesModel:Separated uninfected White Cell using water</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	80	-98.84	9.54E-17	36	4500	59	-85.24	8.23E-17
51	278	78	-97.48	9.41E-17	30	6000	78	-97.48	9.41E-17
56	282	76	-96.12	9.28E-17	30	8000	50	-80.16	7.74E-17
64	682	64	-88.3	8.52E-17	35	4900	57	-84.07	8.11E-17
62	606	70	-92.14	8.89E-17	36	5000	63	-87.68	8.46E-17
72	20	85	-102.29	9.87E-17	33	4500	64	-88.3	8.52E-17
56	613	71	-92.79	8.96E-17	39	4000	60	-85.84	8.28E-17
52	468	76	-96.12	9.28E-17	48	6200	63	-87.68	8.46E-17
51	853	74	-94.78	9.15E-17	48	4900	58	-84.65	8.17E-17
50	356	75	-95.45	9.21E-17	40	4800	60	-85.84	8.28E-17
52	268	64	-88.3	8.52E-17	38	4000	64	-88.3	8.52E-17
60	625	75	-95.45	9.21E-17	40	5000	60	-85.84	8.28E-17
47	230	69	-91.49	8.83E-17	41	4000	63	-87.68	8.46E-17
41	246	70	-92.14	8.89E-17	34	4000	64	-88.3	8.52E-17
56	339	71	-92.79	8.96E-17	44	6000	55	-82.91	8.00E-17
50	316	66	-89.56	8.64E-17	39	4400	65	-88.93	8.58E-17
52	220	73	-94.11	9.08E-17	36	4700	58	-84.65	8.17E-17
64	374	69	-91.49	8.83E-17	42	4800	59	-85.24	8.23E-17
63	593	65	-88.93	8.58E-17	40	4300	58	-84.65	8.17E-17
53	372	75	-97.48	9.41E-17	30	6000	60	-85.84	8.28E-17
<b>AVE</b>	<b>408.95</b>	<b>72.3</b>	<b>-93.8025</b>	<b>9.053E-17</b>		<b>5000</b>	<b>60.9</b>	<b>-86.464</b>	<b>8.34E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.487785</b>	<b>3.715195</b>	<b>3.59416E-18</b>		<b>999.47</b>	<b>5.4085</b>	<b>3.360062</b>	<b>3.24E-18</b>

**APPENDIX B5**

**Table 3.45bi.Fowkes Model: Separated infected White Cell with diiodomethane.**

Netropil (counts/ mm <sup>3</sup> of blood)	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	65	-30.99	2.99E-17
51	278	65	-30.99	2.99E-17
56	282	50	-20.06	1.94E-17
64	682	63	-29.43	2.84E-17
62	606	63	-29.43	2.84E-17
72	20	60	-27.14	2.62E+17
56	613	60	-27.14	2.62E+17
52	468	50	-20.06	1.94E-17
51	853	54	-22.79	2.20E-17
50	356	60	-27.14	2.62E-17
52	268	52	-21.4	2.07E-17
60	625	41	-14.5	1.40E-17
47	230	42	-15.08	1.45E-17
41	246	57	-24.93	2.41E-17
56	339	63	-29.43	2.84E-17
50	316	50	-20.06	1.94E-17
52	220	46	-17.48	1.69E-17
64	374	50	-20.08	1.94E-17
63	593	52	-21.4	2.07E-17
53	372	55	-23.49	2.27E-17
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-23.651</b>	<b>2.75789E+16</b>
<b>SD</b>	<b>199.3013</b>	<b>7.383481</b>	<b>5.14879</b>	<b>8.26091E+16</b>

**Table 3.45bii.Fowkes Model: Separated Uninfected White with diiodometehane.**

PcV (mm <sup>3</sup> )	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
36	4500	42	-15.08	1.45E-17
30	6000	65	-30.99	2.99E-17
30	8000	32	-9.87	9.52E-18
35	4900	43	-15.66	1.51E-17
36	5000	53	-22.09	2.13E-17
33	4500	50	-20.06	1.94E-17
39	4000	43	-15.66	1.51E-17
48	6200	48	-18.75	1.81E-17
48	4900	54	-22.79	2.20E-17
40	4800	47	-18.11	1.75E-17
38	4000	48	-18.75	1.81E-17
40	5000	40	-13.94	1.35E-17
41	4000	35	-11.3	1.09E-17
34	4000	49	-19.4	1.87E-17
44	6000	48	-18.75	1.81E-17
39	4400	45	-16.86	1.63E-17
36	4700	47	-18.11	1.75E-17
42	4800	38	12.85	1.24E-17
40	4300	45	-16.66	1.63E-17
30	6000	40		1.35E-17
	<b>5000</b>	<b>45.6</b>	<b>-16.3147</b>	<b>1.69E-17</b>
	<b>999.47</b>	<b>7.2504</b>	<b>8.380305</b>	<b>4.45E-18</b>

**APPENDIX B6**

<b>Table 3.46bi.Fowkes Model: Separated Infected White Cell with Glycerine</b>					<b>Table 3.46bii.Fowkes Model: Separated Uninfected White Cell with Glycerine</b>				
<i>Netropil</i> (counts/ $mm^3$ of blood)	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> ( $mm^3$ )	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	58	-56.63	5.47E-17	36	4500	45	-48.37	4.67E-17
51	278	59	-57.33	5.53E-17	30	6000	51	-52	5.02E-17
56	282	66	-62.38	6.02E-17	30	8000	47	-49.54	4.78E-17
64	682	67	-63.13	6.09E-17	35	4900	48	-50.14	4.84E-17
62	606	68	-63.88	6.16E-17	36	5000	48	-50.14	4.84E-17
72	20	59	-57.33	5.53E-17	33	4500	50	-51.37	4.96E-17
56	613	68	-63.88	6.16E-17	39	4000	48	50.14	4.84E-17
52	468	73	-67.71	6.54E-17	48	6200	51	-52	5.02E-17
51	853	69	-64.64	6.24E-17	48	4900	55	-54.6	5.27E-17
50	356	65	-61.64	5.95E-17	40	4800	50	-51.37	4.96E-17
52	268	61	-58.74	5.67E-17	38	4000	50	-51.37	4.96E-17
60	625	60	-58.03	5.60E-17	40	5000	61	58.74	5.67E-17
47	230	63	-60.17	5.81E-17	41	4000	50	-51.37	4.96E-17
41	246	60	-58.03	5.60E-17	34	4000	53	-53.28	5.14E-17
56	339	65	-61.64	5.95E-17	44	6000	48	-50.14	4.84E-17
50	316	63	-60.17	5.81E-17	39	4400	51	52	5.02E-17
52	220	60	-58.03	5.60E-17	36	4700	52	52.63	5.08E-17
64	374	66	-62.38	6.02E-17	42	4800	46	-48.95	4.72E-17
63	593	60	-58.03	5.60E-17	40	4300	50	-51.37	4.96E-17
53	372	65	-61.64	5.95E-17	30	6000	49	50.75	4.90E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-60.7705</b>	<b>5.865E-17</b>		<b>5000</b>	<b>50.15</b>	<b>-25.0875</b>	<b>4.97E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.08946</b>	<b>3.001642</b>	<b>2.89837E-18</b>		<b>999.47</b>	<b>3.46828</b>	<b>46.2149</b>	<b>2.2E-18</b>

**APPENDIX B7**

<b>Table 3.47bi.Owens &amp;Wendt I:Separated infected White Cell with Water.</b>					<b>Table 3.47bii.Owens &amp;Wendt SeparatedUninfected White Cell with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	80	-60.18	5.81E-17	36	4500	59	-35.31	3.41E-17
51	278	78	-57.66	5.57E-17	30	6000	78	-57.66	5.57E-17
56	282	76	-55.19	5.33E-17	30	8000	50	-26.01	2.51E-17
64	682	64	-40.89	3.95E-17	35	4900	57	-33.15	3.20E-17
62	606	70	-47.9	4.62E-17	36	5000	63	-39.75	3.84E-17
72	20	85	-66.48	6.41E-17	33	4500	64	-40.89	3.95E-17
56	613	71	-49.1	4.74E-17	39	4000	60	-36.4	3.51E-17
52	468	76	-55.19	5.33E-17	48	6200	63	-39.75	3.84E-17
51	853	74	-52.73	5.09E-17	48	4900	58	-34.22	3.30E-17
50	356	75	-53.96	5.21E-17	40	4800	60	-36.4	3.51E-17
52	268	64	-40.89	3.95E-17	38	4000	64	-40.89	3.95E-17
60	625	75	-53.96	5.21E-17	40	5000	60	-36.4	3.51E-17
47	230	69	-46.71	4.51E-17	41	4000	63	-39.75	3.84E-17
41	246	70	-47.9	4.62E-17	34	4000	64	-40.89	3.95E-17
56	339	71	49.1	4.74E-17	44	6000	55	-31.04	3.00E-17
50	316	66	-43.19	4.17E-17	39	4400	65	-42.03	4.06E-17
52	220	73	-51.52	4.97E-17	36	4700	58	-34.22	3.30E-17
64	374	69	-46.71	4.51E-17	42	4800	59	-35.31	3.41E-17
63	593	65	-42.03	4.06E-17	40	4300	58	-34.22	3.30E-17
53	372	75	-57.66	5.57E-17	30	6000	60	-36.4	3.51E-17
<b>AVE</b>	<b>408.95</b>	<b>72.3</b>	<b>-46.0375</b>	<b>4.9185E-17</b>		<b>5000</b>	<b>60.9</b>	<b>-37.5345</b>	<b>3.62E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.487785</b>	<b>23.39761</b>	<b>6.55256E-18</b>		<b>999.47</b>	<b>5.40857</b>	<b>6.13861</b>	<b>5.9E-18</b>

**APPENDIX B8**

<i>Table 3.48bi.Owen&amp;Wendt:Separated infected White Cell with diiodomethane.</i>					<i>Table 3.48bii.Owen&amp;Wendt:SeparatedUninfected White Cell with diiodomethane.</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	65	-29.33	2.83E-17	36	4500	42	-13.05	1.26E-17
51	278	65	-29.33	2.83E-17	30	6000	65	-29.33	2.83E-17
56	282	50	-18.15	1.75E-17	30	8000	32	-7.72	7.45E-18
64	682	63	-27.74	2.68E-17	35	4900	43	-13.65	1.32E-17
62	606	63	-27.74	2.68E-17	36	5000	53	-20.23	1.95E-17
72	20	60	-25.4	2.45E-17	33	4500	50	-18.15	1.75E-17
56	613	60	-25.4	2.45E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	1.75E-17	48	6200	48	-16.81	1.62E-17
51	853	54	-20.94	2.02E-17	48	4900	54	-20.94	2.02E-17
50	356	60	-25.4	2.45E-17	40	4800	47	-16.15	1.56E-17
52	268	52	-19.52	1.88E-17	38	4000	48	-16.81	1.62E-17
60	625	41	-12.48	1.20E-17	40	5000	40	-11.88	1.15E-17
47	230	42	-13.05	1.26E-17	41	4000	35	-9.19	8.97E-18
41	246	57	-23.13	2.23E-17	34	4000	49	-17.47	1.69E-17
56	339	63	-27.74	2.68E-17	44	6000	48	-16.81	1.62E-17
50	316	50	-18.15	1.75E-17	39	4400	45	-14.88	1.44E-17
52	220	46	-15.51	1.50E-17	36	4700	47	-16.15	1.56E-17
64	374	50	-18.15	1.75E-17	42	4800	38	-10.77	1.04E-17
63	593	52	-19.52	1.88E-17	40	4300	45	-14.88	1.44E-17
53	372	55	-21.66	2.09E-17	30	6000	40	-11.88	1.15E-17
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-21.8245</b>	<b>2.1055E-17</b>		<b>5000</b>	<b>45.6</b>	<b>-15.52</b>	<b>1.5E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.383481</b>	<b>5.268199</b>	<b>5.09267E-18</b>		<b>999.47</b>	<b>7.25041</b>	<b>4.71653</b>	<b>4.5E-18</b>

**APPENDIX B9**

<i>Table 3.49bi. Owen &amp; Wendt: Separated infected White Cell with glycerine.</i>					<i>Table 3.49bii. Owen &amp; Wendt: Separated uninfected White Cell with glycerine</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>
54	438	58	-30.09	2.90E-17	36	4500	45	-18.75	1.81E-17
51	278	59	-31.04	3.00E-17	30	6000	51	-23.72	2.29E-17
56	282	66	-37.97	3.66E-17	30	8000	47	-20.35	1.96E-17
64	682	67	-38.99	3.76E-17	35	4900	48	-21.18	2.04E-17
62	606	68	-40.03	3.86E-17	36	5000	48	-21.18	2.04E-17
72	20	59	-31.04	3.00E-17	33	4500	50	-22.86	2.21E-17
56	613	68	-40.03	3.86E-17	39	4000	48	-21.18	2.04E-17
52	468	73	-45.29	4.37E-17	48	6200	51	-23.72	2.29E-17
51	853	69	-41.06	3.96E-17	48	4900	55	-27.29	2.63E-17
50	356	65	-36.95	3.57E-17	40	4800	50	-22.86	2.21E-17
52	268	61	-32.97	3.18E-17	38	4000	50	-22.86	2.21E-17
60	625	60	-32	3.09E-17	40	5000	61	-32.97	3.18E-17
47	230	63	-34.94	3.37E-17	41	4000	50	-22.86	2.21E-17
41	246	60	-32	3.09E-17	34	4000	53	-25.48	2.46E-17
56	339	65	-35.95	3.57E-17	44	6000	48	-21.18	2.04E-17
50	316	63	-34.94	3.37E-17	39	4400	51	-23.72	2.29E-17
52	220	60	-32	3.09E-17	36	4700	52	-24.6	2.37E-17
64	374	66	-37.97	3.66E-17	42	4800	46	-19.54	1.89E-17
63	593	60	-32	3.09E-17	40	4300	50	-22.88	2.21E-17
53	372	65	-36.95	3.57E-17	30	6000	49	-22.01	2.12E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-35.7105</b>	<b>3.451E-17</b>		<b>5000</b>	<b>50.15</b>	<b>-23.0595</b>	<b>2.23E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.08946</b>	<b>4.108925</b>	<b>3.96151E-18</b>		<b>999.47</b>		<b>199.301</b>	<b>3.46828</b>

**APPENDIX B10**

<b>Table 3.50bi.Zisman: Separated infected White Cell with water</b>					<b>Table 3.50bii.Zisman Model: Separated Uninfected White Cell with Water</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	80	-60.16	5.81E-17	36	4500	59	-35.31	3.41E-17
51	278	78	-57.66	5.57E-17	30	6000	78	-57.66	5.57E-17
56	282	76	-55.19	5.33E-17	30	8000	50	-26.01	2.51E-17
64	682	64	-40.89	3.95E-17	35	4900	57	-33.15	3.20E-17
62	606	70	-47.9	4.62E-17	36	5000	63	-39.75	3.84E-17
72	20	85	-66.46	6.41E-17	33	4500	64	-40.89	3.95E-17
56	613	67	-49.1	4.74E-17	39	4000	60	-36.4	3.51E-17
52	468	76	-55.19	5.33E-17	48	6200	63	-39.75	3.84E-17
51	853	74	-52.73	5.09E-17	48	4900	58	-34.22	3.30E-17
50	356	75	-53.96	5.21E-17	40	4800	60	-36.4	3.51E-17
52	268	64	-40.89	3.95E-17	38	4000	64	-40.89	3.95E-17
60	625	70	-53.96	5.21E-17	40	5000	60	-36.4	3.51E-17
47	230	69	-46.71	4.51E-17	41	4000	63	-39.75	3.84E-17
41	246	70	-47.9	4.62E-17	34	4000	64	-40.89	3.95E-17
56	339	67	-49.1	4.74E-17	44	6000	55	-31.04	3.00E-17
50	316	78	-43.19	4.17E-17	39	4400	65	-42.03	4.06E-17
52	220	73	-51.52	4.97E-17	36	4700	58	-34.22	3.30E-17
64	374	68	-46.71	4.51E-17	42	4800	59	-35.31	3.41E-17
63	593	82	-42.03	4.06E-17	40	4300	58	-34.22	3.30E-17
53	372	75	-57.66	5.57E-17	30	6000	58	-36.4	3.51E-17
<b>AVE</b>	<b>408.95</b>	<b>73.05</b>	<b>-50.9455</b>	<b>4.9185E-17</b>		<b>5000</b>	<b>60.8</b>	<b>-37.535</b>	<b>3.6E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.86223</b>	<b>6.792399</b>	<b>6.55256E-18</b>		<b>999.47</b>	<b>199.301</b>	<b>5.444456</b>	<b>6.13861</b>

**APPENDIX B11**

<b>Table 3.51bi. Zisman: Separated infected White Cell with diiodomethane</b>					<b>Table 3.51bii. Zisman: Separated Uninfected White Cell with diiodomethane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	65	-29.33	2.83E-17	36	4500	42	-13.05	1.26E-17
51	278	65	-29.33	2.83E-17	30	6000	65	-29.33	2.83E-17
56	282	50	-18.15	1.75E-17	30	8000	32	-7.72	7.45E-18
64	682	63	-27.74	2.68E-17	35	4900	43	-13.65	1.32E-17
62	606	63	-27.74	2.68E-17	36	5000	53	-20.23	1.95E-17
72	20	60	-25.4	2.45E-17	33	4500	50	-18.15	1.75E-17
56	613	60	-25.4	2.45E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	1.75E-17	48	6200	48	-16.81	1.62E-17
51	853	54	-20.94	2.02E-17	48	4900	54	-20.94	2.02E-17
50	356	60	-25.4	2.45E-17	40	4800	47	-16.15	1.56E-17
52	268	52	-19.52	1.88E-17	38	4000	48	-16.81	1.62E-17
60	625	41	-12.48	1.20E-17	40	5000	40	-11.88	1.15E-17
47	230	42	-13.05	1.26E-17	41	4000	35	-9.19	8.97E-18
41	246	57	-23.13	2.23E-17	34	4000	49	-17.47	1.69E-17
56	339	63	-27.74	2.68E-17	44	6000	48	-16.81	1.62E-17
50	316	50	-18.15	1.75E-17	39	4400	45	-14.88	1.44E-17
52	220	46	-15.51	1.50E-17	36	4700	47	-16.15	1.56E-17
64	374	50	-18.15	1.75E-17	42	4800	38	-10.77	1.04E-17
63	593	52	-19.52	1.88E-17	40	4300	45	-14.88	1.44E-17
53	372	55	-21.66	2.09E-17	30	6000	40	-11.88	1.15E-17
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-21.8245</b>	<b>2.1055E-17</b>		<b>5000</b>	<b>45.6</b>	<b>-15.52</b>	<b>1.5E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.383481</b>	<b>5.268199</b>	<b>5.09267E-18</b>		<b>999.47</b>	<b>7.25040</b>	<b>4.716525</b>	<b>4.54E-18</b>

**APPENDIX B12**

<b>Table 3.52bi. Zisman: Separated infected White Cell with glycerine</b>					<b>Table 3.52bii. Zisman: Separated Uninfected White Cell with glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	58	-30.09	2.90E-17	36	4500	45	-18.75	1.81E-17
51	278	59	-31.04	3.00E-17	30	6000	51	-23.72	2.29E-17
56	282	66	-37.97	3.66E-17	30	8000	47	-20.35	1.96E-17
64	682	67	-38.99	3.76E-17	35	4900	48	-21.18	2.04E-17
62	606	68	-40.03	3.86E-17	36	5000	48	-21.18	2.04E-17
72	20	59	-31.04	3.00E-17	33	4500	50	-22.86	2.21E-17
56	613	68	-40.03	3.86E-17	39	4000	48	-21.18	2.04E-17
52	468	73	-45.29	4.37E-17	48	6200	51	-23.72	2.29E-17
51	853	69	-41.06	3.96E-17	48	4900	55	-27.29	2.63E-17
50	356	65	-36.95	3.57E-17	40	4800	50	-22.86	2.21E-17
52	268	61	-32.97	3.18E-17	38	4000	50	-22.86	2.21E-17
60	625	60	-32	3.09E-17	40	5000	61	-32.97	3.18E-17
47	230	63	-34.94	3.37E-17	41	4000	50	-22.86	2.21E-17
41	246	60	-32	3.09E-17	34	4000	53	-25.48	2.46E-17
56	339	65	-35.95	3.57E-17	44	6000	48	-21.18	2.04E-17
50	316	63	-34.94	3.37E-17	39	4400	51	-23.72	2.29E-17
52	220	60	-32	3.09E-17	36	4700	52	-24.6	2.37E-17
64	374	66	-37.97	3.66E-17	42	4800	46	-19.54	1.89E-17
63	593	60	-32	3.09E-17	40	4300	50	-22.88	2.21E-17
53	372	65	-36.95	3.57E-17	30	6000	49	-22.01	2.12E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-35.7105</b>	<b>3.451E-17</b>		<b>5000</b>	<b>50.15</b>	<b>-23.0595</b>	<b>2.23E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.08946</b>	<b>4.108925</b>	<b>3.96151E-18</b>		<b>999.47</b>	<b>3.46827</b>	<b>3.07262</b>	<b>2.96E-18</b>

**APPENDIX B13**

<b>Table 3.53bi.Neuman: Separated infected White Cell with water</b>					<b>Table 3.53bii.Neuman Model: Separated Uninfected White Cell with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	80	-37.92	3.66E-17	36	4500	59	-19.05	1.84E-17
51	278	78	-36.32	3.51E-17	30	6000	78	-36.32	3.51E-17
56	282	76	-34.68	3.35E-17	30	8000	50	-8.89	8.58E-17
64	682	64	-23.98	2.31E-17	35	4900	57	-16.97	1.64E-17
62	606	70	-29.52	2.85E-17	36	5000	63	-23.02	2.22E-17
72	20	85	-41.71	4.03E-17	33	4500	64	-23.98	2.31E+17
56	613	71	-30.4	2.93E-17	39	4000	60	-20.07	1.94E-17
52	468	76	-34.68	3.35E-17	48	6200	63	-23.02	2.22E-17
51	853	74	-33	3.19E-17	48	4900	58	-18.02	1.74E-17
50	356	75	-33.85	3.27E-17	40	4800	60	-20.07	1.94E-17
52	268	64	-23.98	2.31E-17	38	4000	64	-23.98	2.31E-17
60	625	75	-33.85	3.27E-17	40	5000	60	-20.07	1.94E-17
47	230	69	-28.62	2.76E-17	41	4000	63	-23.02	2.22E-17
41	246	70	-29.52	2.85E-17	34	4000	64	-23.98	2.31E-17
56	339	71	-30.4	2.93E-17	44	6000	55	-14.82	1.43E-17
50	316	66	-25.87	2.50E-17	39	4400	65	-24.93	2.41E-17
52	220	73	-32.15	3.10E-17	36	4700	58	-18.02	1.74E-17
64	374	69	-28.62	2.76E-17	42	4800	59	-19.05	1.84E-17
63	593	65	-24.93	2.41E-17	40	4300	58	-18.02	1.74E-17
53	372	78	-36.32	3.51E-17	30	6000	60	-20.07	1.94E-17
<b>AVE</b>	<b>408.95</b>	<b>72.45</b>	<b>-31.516</b>	<b>3.0425E-17</b>		<b>5000</b>	<b>60.9</b>	<b>-20.7685</b>	<b>1.16E+16</b>
<b>SD</b>	<b>199.301</b>	<b>5.60521</b>	<b>4.81595</b>	<b>4.66667E-18</b>		<b>999.47</b>	<b>5.4085</b>	<b>5.26532</b>	<b>5.2E+16</b>

**APPENDIX B14**

<b>Table 3.54bi.Neuman: Separated infected White Cell with diiodomethane.</b>					<b>Table 3.54bii.Neuman Model: Separated Uninfected White Cell with diiodomethane.</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{I32}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{I32}$ (mJ/m <sup>2</sup> )
54	438	65	-20.60	1.99E-17	36	4500	42	-9.48	9.15E-18
51	278	65	-20.60	1.99E-17	30	6000	65	-20.60	1.99E-17
56	282	50	-13.12	1.27E-17	30	8000	32	-5.60	5.40E-18
64	682	63	-19.59	1.89E-17	35	4900	43	-9.92	9.57E-18
62	606	63	-19.59	1.89E-17	36	5000	53	-14.57	1.41E-17
72	20	60	-18.07	1.74E-17	33	4500	50	-13.12	1.27E-17
56	613	60	-18.07	1.74E-17	39	4000	43	-9.92	9.57E-18
52	468	50	-13.12	1.27E-17	48	6200	48	-12.18	1.18E-17
51	853	54	-15.06	1.45E-17	48	4900	54	-15.05	1.45E-17
50	356	60	-18.07	1.74E-17	40	4800	47	-11.71	1.13E-17
52	268	52	-14.09	1.36E-17	38	4000	48	-12.18	1.18E-17
60	625	41	-9.06	8.74E-18	40	5000	40	-8.64	8.34E-18
47	230	42	-9.48	9.15E-18	41	4000	35	-6.67	6.44E-18
41	246	57	-16.56	1.60E-17	34	4000	49	-12.65	1.22E-17
56	339	63	-19.59	1.89E-17	44	6000	48	-12.18	1.18E-17
50	316	50	-13.12	1.27E-17	39	4400	45	10.80	1.04E-17
52	220	46	-11.26	1.09E-17	36	4700	47	-11.71	1.13E-17
64	374	50	-13.12	1.27E-17	42	4800	38	-7.83	7.55E-18
63	593	52	-14.09	1.36E-17	40	4300	45	-10.8	1.04E-17
53	372	55	-15.56	1.50E-17	30	6000	40	-8.64	8.34E-18
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-15.59</b>	<b>1.5E-17</b>		<b>5000</b>	<b>45.6</b>	<b>-10.1325</b>	<b>1.08E-17</b>
<b>SD</b>	<b>199.301</b>	<b>7.383</b>	<b>3.594</b>	<b>3.46E-18</b>		<b>999.47</b>	<b>7.250</b>	<b>5.927</b>	<b>3.19E-18</b>

**APPENDIX B15**

<b>Table 3.55bi.Neuman: Separated infected White Cell with glycerine</b>					<b>Table 3.55bii.Neuman: Separated Uninfected White Cell with glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	58	-19.47	1.88E-15	36	4500	45	-10.89	1.05E-17
51	278	59	-20.16	1.95E-17	30	6000	51	-14.72	1.42E-17
56	282	66	-25.03	2.42E-17	30	8000	47	-12.13	1.17E-17
64	682	67	-25.72	2.48E-17	35	4900	48	-12.77	1.23E-17
62	606	68	-26.41	2.55E-17	36	5000	48	-12.77	1.23E-17
72	20	59	-20.16	1.95E-17	33	4500	50	-14.06	1.36E-17
56	613	68	-26.41	2.55E-17	39	4000	48	-12.77	1.23E-17
52	468	73	-29.82	2.88E-17	48	6200	51	-14.72	1.42E-17
51	853	69	-27.1	2.62E-17	48	4900	55	-17.41	1.68E-17
50	356	65	-24.34	2.35E-17	40	4800	50	-14.06	1.36E-17
52	268	61	-21.55	2.08E-17	38	4000	50	-14.06	1.36E-17
60	625	60	-20.86	2.01E-17	40	5000	61	-21.55	2.08E-17
47	230	63	-22.94	2.21E-17	41	4000	50	-14.06	1.36E-17
41	246	60	-20.86	2.01E-17	34	4000	53	-16.06	1.55E-17
56	339	65	-24.34	2.35E-17	44	6000	48	-12.77	1.23E-17
50	316	63	-22.94	2.21E-17	39	4400	51	-14.72	1.42E-17
52	220	60	-20.86	2.01E-17	36	4700	52	-15.39	1.48E-17
64	374	66	-25.03	2.42E-17	42	4800	46	-11.5	1.11E-17
63	593	60	-20.86	2.01E-17	40	4300	50	-14.06	1.36E-17
53	372	65	-24.34	2.35E-17	30	6000	49	-13.41	1.29E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-23.46</b>	<b>1.15705E-16</b>		<b>5000</b>	<b>50.15</b>	<b>-14.194</b>	<b>1.37E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.0894</b>	<b>2.83209</b>	<b>4.1528E-16</b>		<b>999.47</b>	<b>3.46827</b>	<b>2.310679</b>	<b>2.23E-18</b>

**APPENDIX B16**

<b>Table 3.56bi.Girifalco&amp; Good: Separated infected White Cell with water</b>						<b>Table 3.56bii.Girifalco&amp;Good Model: Separated Uninfected White Cell with Water</b>					
<i>Netropil (counts/mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>		
54	438	80	-60.16	5.81E-17	36	4500	59	-35.31	3.41E-17		
51	278	78	-57.66	5.57E-17	30	6000	78	-57.66	5.57E-17		
56	282	76	-55.19	5.33E-17	30	8000	50	-26.01	2.51E-17		
64	682	64	-40.89	3.95E-17	35	4900	57	-33.15	3.20E-17		
62	606	70	-47.9	4.62E-17	36	5000	63	-39.75	3.84E-17		
72	20	85	-66.46	6.41E-17	33	4500	64	-40.89	3.95E-17		
56	613	67	-49.1	4.74E-17	39	4000	60	-36.4	3.51E-17		
52	468	76	-55.19	5.33E-17	48	6200	63	-39.75	3.84E-17		
51	853	74	-52.73	5.09E-17	48	4900	58	-34.22	3.30E-17		
50	356	75	-53.96	5.21E-17	40	4800	60	-36.4	3.51E-17		
52	268	64	-40.89	3.95E-17	38	4000	64	-40.89	3.95E-17		
60	625	75	-53.96	5.21E-17	40	5000	60	-36.4	3.51E-17		
47	230	69	-46.71	4.51E-17	41	4000	63	-39.75	3.84E-17		
41	246	70	-47.9	4.62E-17	34	4000	64	-40.89	3.95E-17		
56	339	67	-49.1	4.74E-17	44	6000	55	-31.04	3.00E-17		
50	316	78	-43.19	4.17E-17	39	4400	65	-42.03	4.06E-17		
52	220	73	-51.52	4.97E-17	36	4700	58	-34.22	3.30E-17		
64	374	68	-46.71	4.51E-17	42	4800	59	-35.31	3.41E-17		
63	593	82	-42.03	4.06E-17	40	4300	58	-34.22	3.30E-17		
53	372	75	-57.66	5.57E-17	30	6000	60	-36.4	3.51E-17		
<b>AVE</b>	<b>408.95</b>	<b>73.3</b>	<b>-50.9455</b>	<b>4.9185E-17</b>		<b>5000</b>	<b>60.9</b>	<b>-37.5345</b>	<b>3.64E-17</b>		
<b>SD</b>	<b>199.3013</b>	<b>5.83185</b>	<b>6.792399</b>	<b>6.55256E-18</b>		<b>999.47</b>	<b>5.4085</b>	<b>6.13861</b>	<b>6.05E-18</b>		

**APPENDIX B17**

<b>Table 3.57bi.Girifalco&amp;Good: Separated infected White Cell with diiodomethane</b>					<b>Table 3.57bii.Girifalco &amp;Good: Separated Uninfected White Cell with diiodomethane</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	65	-29.33	2.83E-17	36	4500	42	-13.05	1.26E-17
51	278	65	-29.33	2.83E-17	30	6000	65	-29.33	2.83E-17
56	282	50	-18.15	1.75E-17	30	8000	32	-7.72	7.45E-18
64	682	63	-27.74	2.68E-17	35	4900	43	-13.65	1.32E-17
62	606	63	-27.74	2.68E-17	36	5000	53	-20.23	1.95E-17
72	20	60	-25.4	2.45E-17	33	4500	50	-18.15	1.75E-17
56	613	60	-25.4	2.45E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	1.75E-17	48	6200	48	-16.81	1.62E-17
51	853	54	-20.94	2.02E-17	48	4900	54	-20.94	2.02E-17
50	356	60	-25.4	2.45E-17	40	4800	47	-16.15	1.56E-17
52	268	52	-19.52	1.88E-17	38	4000	48	-16.81	1.62E-17
60	625	41	-12.48	1.20E-17	40	5000	40	-11.88	1.15E-17
47	230	42	-13.05	1.26E-17	41	4000	35	-9.19	8.97E-18
41	246	57	-23.13	2.23E-17	34	4000	49	-17.47	1.69E-17
56	339	63	-27.74	2.68E-17	44	6000	48	-16.81	1.62E-17
50	316	50	-18.15	1.75E-17	39	4400	45	-14.88	1.44E-17
52	220	46	-15.51	1.50E-17	36	4700	47	-16.15	1.56E-17
64	374	50	-18.15	1.75E-17	42	4800	38	-10.77	1.04E-17
63	593	52	-19.52	1.88E-17	40	4300	45	-14.88	1.44E-17
53	372	55	-21.66	2.09E-17	30	6000	40	-11.88	1.15E-17
<b>AVE</b>	<b>408.95</b>	<b>54.9</b>	<b>-21.8245</b>	<b>2.1055E-17</b>		<b>5000</b>	<b>45.6</b>	<b>-15.52</b>	<b>1.5E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.3834</b>	<b>5.268199</b>	<b>5.09267E-18</b>		<b>999.47</b>	<b>7.250408</b>	<b>4.716525</b>	<b>4.54E-18</b>

**APPENDIX B18**

<b>Table 3.58bi. Girifalco &amp; Good: Separated infected White Cell with glycerine</b>					<b>Table 3.58bii. Girifalco &amp; Good: Separated Uninfected White with glycerine</b>				
<i>Netropil (counts/mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup> (mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub> (mJ/m<sup>2</sup>)</i>
54	438	58	-30.09	2.90E-17	36	4500	45	-13.05	1.26E-17
51	278	59	-31.04	3.00E-17	30	6000	51	-29.33	2.83E-17
56	282	66	-37.97	3.66E-17	30	8000	47	-7.72	7.45E-18
64	682	67	-38.99	3.76E-17	35	4900	48	-13.65	1.32E-17
62	606	68	-40.03	3.86E-17	36	5000	48	-20.23	1.95E-17
72	20	59	-31.04	3.00E-17	33	4500	50	-18.15	1.75E-17
56	613	68	-40.03	3.86E-17	39	4000	48	-13.65	1.32E-17
52	468	73	-45.29	4.37E-17	48	6200	51	-16.81	1.62E-17
51	853	69	-41.06	3.96E-17	48	4900	55	-20.94	2.02E-17
50	356	65	-36.95	3.57E-17	40	4800	50	-16.15	1.56E-17
52	268	61	-32.97	3.18E-17	38	4000	50	-16.81	1.62E-17
60	625	60	-32	3.09E-17	40	5000	61	-11.88	1.15E-17
47	230	63	-34.94	3.37E-17	41	4000	50	-9.19	8.97E-18
41	246	60	-32	3.09E-17	34	4000	53	-17.47	1.69E-17
56	339	65	-35.95	3.57E-17	44	6000	48	-16.81	1.62E-17
50	316	63	-34.94	3.37E-17	39	4400	51	-14.88	1.44E-17
52	220	60	-32	3.09E-17	36	4700	52	-16.15	1.56E-17
64	374	66	-37.97	3.66E-17	42	4800	46	-10.77	1.04E-17
63	593	60	-32	3.09E-17	40	4300	50	-14.88	1.44E-17
53	372	65	-36.95	3.57E-17	30	6000	49	-11.88	1.15E-17
<b>AVE</b>	<b>408.95</b>	<b>63.75</b>	<b>-35.7105</b>	<b>3.451E-17</b>		<b>5000</b>	<b>50.15</b>	<b>-15.52</b>	<b>1.5E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.08946</b>	<b>4.10893</b>	<b>3.96151E-18</b>		<b>999.47</b>	<b>3.46828</b>	<b>4.71653</b>	<b>4.5E-18</b>

## APPENDIX C1

<b>Table 3.41ci. Wu: Separated Infected Plasma with water</b>					<b>Table 3.41cii. Wu: Separated uninfected Plasma with water</b>				
Netropil (counts/ mm <sup>3</sup> of blood)	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	PcV (mm <sup>3</sup> )	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	69	-100.96	9.74E-17	36	4500	54	-97.12	9.37E-17
51	278	69	-100.96	9.74E-17	30	6000	60	-98.4	9.50E-17
56	282	75	-103.18	9.96E-17	30	8000	50	-96.42	9.31E-17
64	682	72	-102.02	9.85E-17	35	4900	58	-97.94	9.45E-17
62	606	70	-101.3	9.78E-17	36	5000	58	-97.94	9.45E-17
72	20	72	-102.02	9.85E-17	33	4500	61	-98.64	9.52E-17
56	613	60	-98.4	9.50E-17	39	4000	50	-96.42	9.31E-17
52	468	60	-98.4	9.50E-17	48	6200	58	-97.94	9.45E-17
51	853	59	-98.16	9.47E-17	48	4900	63	-99.16	9.57E-17
50	356	70	-101.3	9.78E-17	40	4800	58	-97.94	9.45E-17
52	268	65	-99.72	9.62E-17	38	4000	58	-97.94	9.45E-17
60	625	69	100.96	9.74E-17	40	5000	59	-98.16	9.47E-17
47	230	70	-101.3	9.78E-17	41	4000	57	-97.72	9.43E-17
41	246	57	-97.72	9.43E-17	34	4000	62	-98.9	9.54E-17
56	339	66	-100.01	9.65E-17	44	6000	58	-97.94	9.45E-17
50	316	60	98.4	9.50E-17	39	4400	62	-98.9	9.54E-17
52	220	65	-99.72	9.62E-17	36	4700	54	-97.12	9.37E-17
64	374	50	-96.42	9.31E-17	42	4800	50	-96.42	9.31E-17
63	593	60	98.4	9.50E-17	40	4300	50	-96.42	9.31E-17
53	372	70	-101.3	9.78E-17	30	6000	50	-96.42	9.31E-17
<b>AVE</b>	<b>408.95</b>	<b>65.4</b>	<b>-70.2565</b>	<b>9.64E-17</b>		<b>5000</b>	<b>56.5</b>	<b>-97.693</b>	<b>9.43E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>6.361066</b>	<b>73.07893</b>	<b>1.59E-18</b>		<b>999.4735</b>	<b>4.466248</b>	<b>0.912101</b>	<b>8.58E-19</b>

**APPENDIX C2**

<b>Table 3.42ci. Wu: Separated infected Plasma with diiodomethane.</b>					<b>Table 3.42cii. Wu: Separated Uninfected Plasma with diiodomethane.</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	54	-24.78	2.39E-17	36	4500	50	-21.61	2.09E-17
51	278	60	-29.97	2.89E-17	30	6000	50	-21.61	2.09E-17
56	282	62	-31.81	3.07E-17	30	8000	45	-17.99	1.74E-17
64	682	60	-29.97	2.89E-17	35	4900	48	-20.12	1.94E-17
62	606	54	-24.78	2.39E-17	36	5000	38	-13.59	1.31E-17
72	20	48	-20.12	1.94E-17	33	4500	47	-19.39	1.87E-17
56	613	47	-19.39	1.87E-17	39	4000	45	-17.99	1.74E-17
52	468	49	-20.86	2.01E-17	48	6200	44	-17.32	1.67E-17
51	853	50	-21.61	2.09E-17	48	4900	52	-23.16	2.24E-17
50	356	65	-34.66	3.35E-17	40	4800	50	-21.61	2.09E-17
52	268	50	-21.61	2.09E-17	38	4000	42	-16.01	1.55E-17
60	625	57	-27.31	2.64E-17	40	5000	57	-27.31	2.64E-17
47	230	65	-34.66	3.35E-17	41	4000	50	-21.61	2.09E-17
41	246	56	-26.45	2.55E-17	34	4000	50	-21.61	2.09E-17
56	339	50	-21.61	2.09E-17	44	6000	51	-22.38	2.16E-17
50	316	58	-28.18	2.72E-17	39	4400	47	-19.39	1.87E-17
52	220	41	-15.38	1.48E-17	36	4700	40	-14.77	1.43E-17
64	374	48	-20.12	1.94E-17	42	4800	43	-16.66	1.61E-17
63	593	42	-16.01	1.55E-17	40	4300	42	-16.01	1.55E-17
53	372	44	-17.32	1.67E-17	30	6000	44	-17.32	1.67E-17
<b>AVE</b>	<b>408.95</b>	<b>53</b>	<b>-24.33</b>	<b>2.35E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-19.373</b>	<b>1.87E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.232965</b>	<b>5.867585</b>	<b>5.67E-18</b>		<b>999.4735</b>	<b>4.6325</b>	<b>3.315231</b>	<b>3.21E-18</b>

**APPENDIX C3**

<b>Table 3.43ci. Wu: Separated Infected Plasma with Glycerine</b>					<b>Table 3.43cii. Wu: Separated Uninfected Plasma with Glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	59	-62.21	6.00E-17	36	4500	45	-55.23	5.33E-17
51	278	58	-61.63	5.95E-17	30	6000	53	-58.91	5.69E-17
56	282	55	-59.96	5.79E-17	30	8000	40	-53.32	5.15E-17
64	682	65	-66	6.37E-17	35	4900	55	-59.96	5.79E-17
62	606	62	-64.04	6.18E-17	36	5000	50	-57.44	5.54E-17
72	20	60	-62.81	6.06E-17	33	4500	49	-56.97	5.50E-17
56	613	70	-69.54	6.71E-17	39	4000	50	-57.44	5.54E-17
52	468	56	-60.5	5.84E-17	48	6200	53	-58.91	5.69E-17
51	853	54	-59.43	5.74E-17	48	4900	50	-57.44	5.54E-17
50	356	58	-61.63	5.95E-17	40	4800	52	-58.41	5.64E-17
52	268	50	-57.44	5.54E-17	38	4000	51	-57.92	5.59E-17
60	625	63	-64.68	6.24E-17	40	5000	58	-61.63	5.95E-17
47	230	63	-64.68	6.24E-17	41	4000	53	-58.91	5.69E-17
41	246	50	-57.44	5.54E-17	34	4000	52	-58.41	5.64E-17
56	339	58	-61.63	5.95E-17	44	6000	48	-56.52	5.45E-17
50	316	59	-62.21	6.00E-17	39	4400	51	-57.92	5.59E-17
52	220	54	-59.43	5.74E-17	36	4700	54	-59.43	5.74E-17
64	374	49	-56.97	5.50E-17	42	4800	49	-56.97	5.50E-17
63	593	58	-61.63	5.95E-17	40	4300	48	-56.52	5.45E-17
53	372	63	-64.68	6.24E-17	30	6000	53	-58.91	5.69E-17
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-61.927</b>	<b>5.98E-17</b>		<b>5000</b>	<b>50.7</b>	<b>-57.8585</b>	<b>5.59E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.3469</b>	<b>3.130959</b>	<b>3.01E-18</b>		<b>999.4735</b>	<b>3.81272</b>	<b>1.767291</b>	<b>1.72E-18</b>

**APPENDIX C4**

<b>Table 3.44ci.Fowkes: Separated infected Plasma with water</b>				<b>Table 3.44cii.Fowkes: Separated uninfected Plasma using water</b>				
<i>CD4+</i> (counts/ <i>mm</i> <sup>3</sup> of blood)	<i>θ</i> (°C)	<i>F</i> <sup>adh</sup> (mJ/m <sup>2</sup> )	<i>A</i> <sub>132</sub> (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ <i>mm</i> <sup>3</sup> of blood)	<i>θ</i> (°C)	<i>F</i> <sup>adh</sup> (mJ/m <sup>2</sup> )	<i>A</i> <sub>132</sub> (mJ/m <sup>2</sup> )
438	69	-91.49	8.83E-17	36	4500	54	-82.36	7.95E-17
278	69	-91.49	8.83E-17	30	6000	60	-85.84	8.28E-17
282	75	-95.45	9.21E-17	30	8000	50	-80.16	7.74E-17
682	72	-93.45	9.02E-17	35	4900	58	-84.65	8.17E-17
606	70	-92.14	8.89E-17	36	5000	58	-84.65	8.17E-17
20	72	-93.45	9.02E-17	33	4500	61	-86.45	8.34E-17
613	60	-85.84	8.28E-17	39	4000	50	-80.16	7.74E-17
468	60	-85.84	8.28E-17	48	6200	58	-84.65	8.17E-17
853	59	-85.24	8.23E+00	48	4900	63	-87.68	8.46E-17
356	70	-92.14	8.89E-17	40	4800	58	-84.65	8.17E-17
268	65	-88.93	8.58E-17	38	4000	58	-84.65	8.17E-17
625	69	-91.49	8.83E-17	40	5000	59	-85.24	8.23E-17
230	70	-92.14	8.89E-17	41	4000	57	-84.07	8.11E-17
246	57	-84.07	8.11E-17	34	4000	62	-87.06	8.40E-17
339	66	-89.56	8.64E-17	44	6000	58	-84.65	8.17E-17
316	60	-85.84	8.28E-17	39	4400	62	-87.06	8.40E-17
220	65	-88.93	8.58E-17	36	4700	54	-82.36	7.95E-17
374	50	-80.16	7.74E-17	42	4800	50	-80.16	7.74E-17
593	60	-85.84	8.28E-17	40	4300	50	-80.16	7.74E-17
372	70	-92.14	8.89E-17	30	6000	50	-80.16	7.74E-17
<b>408.95</b>	<b>65.4</b>	<b>-89.2815</b>	<b>0.4115</b>		<b>5000</b>	<b>56.5</b>	<b>-83.841</b>	<b>8.09E-17</b>
<b>199.3013</b>	<b>6.361066</b>	<b>3.937106</b>	<b>1.840284</b>		<b>999.4735</b>	<b>4.466248</b>	<b>2.558132</b>	<b>2.45E-18</b>

**APPENDIX C5**

<b>Table 3.45ci.Fowkes:Separated infected Plasma with diiodomethane.</b>					<b>Table 3.45cii.Fowkes:Separated Uninfected Plasma with diiodomethane.</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	54	-22.79	2.20E-17	36	4500	50	-20.06	1.94E-17
51	278	60	-27.14	2.62E-17	30	6000	50	-20.06	1.94E-17
56	282	62	-28.66	2.77E-17	30	8000	45	-16.86	1.63E-17
64	682	60	-27.14	2.62E-17	35	4900	48	-18.75	1.81E-17
62	606	54	-22.79	2.20E-17	36	5000	38	12.85	1.24E-17
72	20	48	-18.75	1.81E-17	33	4500	47	18.11	1.75E-17
56	613	47	18.11	1.75E-17	39	4000	45	-16.86	1.63E-17
52	468	49	-19.4	1.87E-17	48	6200	44	-16.26	1.57E-17
51	853	50	-20.06	1.94E-17	48	4900	52	-21.4	2.07E-17
50	356	65	-30.99	2.99E-17	40	4800	50	-20.06	1.94E-17
52	268	50	-20.06	1.94E-17	38	4000	42	-15.08	1.45E-17
60	625	57	-24.93	2.41E-17	40	5000	57	-24.93	2.41E-17
47	230	65	-30.99	2.99E-17	41	4000	50	-20.06	1.94E-17
41	246	56	-24.21	2.34E-17	34	4000	50	-20.06	1.94E-17
56	339	50	-20.06	1.94E-17	44	6000	51	-20.73	2.00E-17
50	316	58	-25.66	2.48E-17	39	4400	47	18.11	1.75E-17
52	220	41	-14.5	1.40E-17	36	4700	40	-13.94	1.35E-17
64	374	48	-18.75	1.81E-17	42	4800	43	-15.66	1.51E-17
63	593	42	-15.08	1.45E-17	40	4300	42	-15.08	1.45E-17
53	372	44	-16.26	1.57E-17	30	6000	44	-16.26	1.57E-17
<b>AVE</b>	<b>408.95</b>	<b>53</b>	<b>-20.5055</b>	<b>2.16E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-13.152</b>	<b>1.74E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.2329</b>	<b>10.32969</b>	<b>4.84E-18</b>		<b>999.4735</b>	<b>4.632551</b>	<b>13.03035</b>	<b>2.84E-18</b>

**APPENDIX C6**

<b>Table 3.46ci.Fowkes: Separated Infected Plasma with Glycerine.</b>					<b>Table 3.46cii.Fowkes :Separated Uninfected Plasma with Glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	59	-57.33	5.53E-17	36	4500	45	-48.37	4.67E-17
51	278	58	-56.63	5.47E-17	30	6000	53	-53.28	5.14E-17
56	282	55	-54.6	5.27E-17	30	8000	40	-45.62	4.40E-17
64	682	65	-61.64	5.95E-17	35	4900	55	-54.6	5.27E-17
62	606	62	-59.45	5.74E-17	36	5000	50	-51.37	4.96E-17
72	20	60	-58.03	5.60E-17	33	4500	49	-50.75	4.90E-17
56	613	70	-65.4	6.31E-17	39	4000	50	-51.37	4.96E-17
52	468	56	-55.27	5.33E-17	48	6200	53	-53.28	5.14E-17
51	853	54	-53.93	5.21E-17	48	4900	50	-51.37	4.96E-17
50	356	58	-56.63	5.47E-17	40	4800	52	-52.63	5.08E-17
52	268	50	-51.37	4.96E-17	38	4000	51	-52	5.02E-17
60	625	63	-60.17	5.81E-17	40	5000	58	-56.63	5.47E-17
47	230	63	-60.17	5.81E-17	41	4000	53	-53.28	5.14E-17
41	246	50	-51.37	4.96E-17	34	4000	52	-52.63	5.08E-17
56	339	58	-56.63	5.47E-17	44	6000	48	-50.14	4.84E-17
50	316	59	-57.33	5.53E-17	39	4400	51	-52	5.02E-17
52	220	54	-53.93	5.21E-17	36	4700	54	-53.93	5.21E-17
64	374	49	-50.75	4.90E-17	42	4800	49	-50.75	4.90E-17
63	593	58	-56.63	5.47E-17	40	4300	48	-50.14	4.84E-17
53	372	63	-60.17	5.81E-17	30	6000	53	-53.28	5.14E-17
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-56.8715</b>	<b>5.49E-17</b>		<b>5000</b>	<b>50.7</b>	<b>-51.871</b>	<b>5.01E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.3469</b>	<b>3.697751</b>	<b>3.56E-18</b>		<b>999.4735</b>	<b>3.81272</b>	<b>2.340182</b>	<b>2.26E-18</b>

**APPENDIX C7**

<b>Table 3.47ci.Owens &amp; Wendt : Separated infected Plasma with Water</b>					<b>Table 3.47cii.Owens &amp; Wendt : Separated Uninfected Plasma with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	69	-46.71	4.51E-17	36	4500	54	-30.01	2.90E-17
51	278	69	-46.71	4.51E-17	30	6000	60	-36.4	3.51E-17
56	282	75	-53.96	5.21E-17	30	8000	50	-26.01	2.51E-17
64	682	72	-50.3	4.85E-17	35	4900	58	-34.22	3.30E-17
62	606	70	-47.9	4.62E-17	36	5000	58	-34.22	3.30E-17
72	20	72	-50.3	4.85E-17	33	4500	61	-37.51	3.62E-17
56	613	60	-36.4	3.51E-17	39	4000	50	-26.01	2.51E-17
52	468	60	-36.4	3.51E-17	48	6200	58	-34.22	3.30E-17
51	853	59	-35.31	3.41E-17	48	4900	63	-39.75	3.84E-17
50	356	70	-47.9	4.62E-17	40	4800	58	-34.22	3.30E-17
52	268	65	-42.03	4.05E-17	38	4000	58	-34.22	3.30E-17
60	625	69	-46.71	4.51E-17	40	5000	59	-35.31	3.41E-17
47	230	70	-47.9	4.62E-17	41	4000	57	-33.15	3.20E-17
41	246	57	-33.15	3.20E-17	34	4000	62	-38.62	3.73E-17
56	339	66	-43.19	4.17E-17	44	6000	58	-34.22	3.30E-17
50	316	60	-38.4	3.51E-17	39	4400	62	-38.62	3.73E-17
52	220	65	-42.03	4.05E-17	36	4700	54	-30.01	2.90E-17
64	374	50	-26.01	2.51E-17	42	4800	50	-26.01	2.51E-17
63	593	60	-36.4	3.51E-17	40	4300	50	-26.01	2.51E-17
53	372	70	-47.9	4.62E-17	30	6000	50	-26.01	2.51E-17
<b>AVE</b>	<b>408.95</b>	<b>65.4</b>	<b>-42.7805</b>	<b>4.12E-17</b>		<b>5000</b>	<b>56.5</b>	<b>-32.7375</b>	<b>3.16E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>6.3610</b>	<b>7.114281</b>	<b>6.94E-18</b>		<b>999.4735</b>	<b>4.466248</b>	<b>4.677623</b>	<b>4.52E-18</b>

**APPENDIX C8**

<b>Table 3.48ci. Owen &amp; Wendt: Separated infected Plasma with diiodomethane.</b>					<b>Table 3.48cii. Owen &amp; Wendt: Separated Uninfected Plasma with diiodomethane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	54	-20.94	2.02E-17	36	4500	50	-18.15	1.75E-17
51	278	60	-25.4	2.45E-17	30	6000	50	-18.15	1.75E-17
56	282	62	-26.95	2.60E-17	30	8000	45	-14.88	1.44E-17
64	682	60	-25.4	2.45E-17	35	4900	48	-16.81	1.62E-17
62	606	54	-20.94	2.02E-17	36	5000	38	-10.77	1.04E-17
72	20	48	-16.81	1.62E-17	33	4500	47	-16.15	1.56E-17
56	613	47	-16.15	1.56E-17	39	4000	45	-14.88	1.44E-17
52	468	49	-17.47	1.69E-17	48	6200	44	-14.26	1.38E-17
51	853	50	-18.15	1.75E-17	48	4900	52	-19.52	1.88E-17
50	356	65	-29.33	2.83E-17	40	4800	50	-18.15	1.75E-17
52	268	50	-18.15	1.75E-17	38	4000	42	-13.05	1.26E-17
60	625	57	-23.13	2.23E-17	40	5000	57	-23.13	2.23E-17
47	230	65	-29.33	2.83E-17	41	4000	50	-18.15	1.75E-17
41	246	56	-22.39	2.16E-17	34	4000	50	-18.15	1.75E-17
56	339	50	-18.15	1.75E-17	44	6000	51	-18.83	1.82E-17
50	316	58	-23.88	2.30E-17	39	4400	47	-16.15	1.56E-17
52	220	41	-12.46	1.20E-17	36	4700	40	-11.88	1.15E-17
64	374	48	-16.81	1.62E-17	42	4800	43	-13.65	1.32E-17
63	593	42	-13.05	1.26E-17	40	4300	42	-13.05	1.26E-17
53	372	44	-14.26	1.38E-17	30	6000	44	-14.26	1.38E-17
<b>AVE</b>	<b>408.95</b>	<b>53</b>	<b>-20.458</b>	<b>2E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-16.101</b>	<b>1.55E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.232965</b>	<b>5.123781</b>	<b>4.94E-18</b>		<b>999.4735</b>	<b>4.632551</b>	<b>2.997056</b>	<b>2.88E-18</b>

**APPENDIX C9**

<i>Table 3.49ci.Owen&amp;Wendt:Separated infected Plasma with glycerine.</i>					<i>Table 3.49cii.Owen&amp;Wendt:Separated Uninfected Plasma with glycerine.</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	59	-31.04	3.00E-17	36	4500	45	-18.75	1.81E-17
51	278	58	-30.09	2.90E-17	30	6000	53	-25.48	2.46E-17
56	282	55	-27.29	2.63E-17	30	8000	40	-14.97	1.45E-17
64	682	65	-38.95	3.57E-17	35	4900	55	-27.29	2.63E-17
62	606	62	-33.95	3.28E-17	36	5000	50	-22.86	2.21E-17
72	20	60	-32	3.09E-17	33	4500	49	-22.01	2.12E-17
56	613	70	-42.11	4.06E-17	39	4000	50	-22.86	2.21E-17
52	468	56	-28.21	2.72E-17	48	6200	53	-25.48	2.46E-17
51	853	54	-26.38	2.55E-17	48	4900	50	-22.86	2.21E-17
50	356	58	-30.09	2.90E-17	40	4800	52	-24.6	2.37E-17
52	268	50	-22.86	2.21E-17	38	4000	51	-23.72	2.29E-17
60	625	63	-34.94	3.37E-17	40	5000	58	-30.09	2.90E-17
47	230	63	-34.94	3.37E-17	41	4000	53	-25.48	2.46E-17
41	246	50	-22.86	2.21E-17	34	4000	52	-24.6	2.37E-17
56	339	58	-30.09	2.90E-17	44	6000	48	-21.18	2.04E-17
50	316	59	-31.04	3.00E-17	39	4400	51	-23.72	2.29E-17
52	220	54	-26.38	2.55E-17	36	4700	54	-26.38	2.55E-17
64	374	49	-22.01	2.12E-17	42	4800	49	-22.01	2.12E-17
63	593	58	-30.09	2.90E-17	40	4300	48	-21.18	2.04E-17
53	372	63	-34.94	3.37E-17	30	6000	53	-25.48	2.46E-17
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-30.513</b>	<b>2.9E-17</b>		<b>5000</b>	<b>50.7</b>	<b>-23.55</b>	<b>2.25E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.3469</b>	<b>5.226735</b>	<b>4.89E-18</b>		<b>999.4735</b>	<b>3.81272</b>	<b>3.211756</b>	<b>3.06E-18</b>

**APPENDIX C10**

<b>Table 3.50ci.Zisman: Separated infected Plasma with water</b>					<b>Table 3.50cii.Zisman : Separated Uninfected Plasma with Water</b>					
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	
54	438	69	-46.71	4.51E-17	36	4500	54	-30.01	2.90E-17	
51	278	69	-46.71	4.51E-17	30	6000	60	-36.4	3.51E-17	
56	282	75	-53.96	5.21E-17	30	8000	50	-26.01	2.51E-17	
64	682	72	-50.3	4.85E-17	35	4900	58	-34.22	3.30E-17	
62	606	70	-47.9	4.62E-17	36	5000	58	-34.22	3.30E-17	
72	20	72	-50.3	4.85E-17	33	4500	61	-37.51	3.62E-17	
56	613	60	-36.4	3.51E-17	39	4000	50	-26.01	2.51E-17	
52	468	60	-36.4	3.51E-17	48	6200	58	-34.22	3.30E-17	
51	853	59	-35.31	3.41E-17	48	4900	63	-39.75	3.84E-17	
50	356	70	-47.9	4.62E-17	40	4800	58	-34.22	3.30E-17	
52	268	65	-42.03	4.05E-17	38	4000	58	-34.22	3.30E-17	
60	625	69	-46.71	4.51E-17	40	5000	59	-35.31	3.41E-17	
47	230	70	-47.9	4.62E-17	41	4000	57	-33.15	3.20E-17	
41	246	57	-33.15	3.20E-17	34	4000	62	-38.62	3.73E-17	
56	339	66	-43.19	4.17E-17	44	6000	58	-34.22	3.30E-17	
50	316	60	-38.4	3.51E-17	39	4400	62	-38.62	3.73E-17	
52	220	65	-42.03	4.05E-17	36	4700	54	-30.01	2.90E-17	
64	374	50	-26.01	2.51E-17	42	4800	50	-26.01	2.51E-17	
63	593	60	-36.4	3.51E-17	40	4300	50	-26.01	2.51E-17	
53	372	70	-47.9	4.62E-17	30	6000	50	-26.01	2.51E-17	
<b>AVE</b>	<b>408.95</b>	<b>65.4</b>	<b>-42.7805</b>	<b>4.12E-17</b>		<b>5000</b>	<b>56.5</b>	<b>-32.7375</b>	<b>3.16E-17</b>	
<b>SD</b>	<b>199.3013</b>	<b>6.361066</b>	<b>7.114281</b>	<b>6.94E-18</b>		<b>999.4735</b>	<b>4.46624</b>	<b>4.677623</b>	<b>4.52E-18</b>	

**APPENDIX C11**

<b>Table 3.51ci.Zisman: Separated infected Plasma with diiodomethane</b>					<b>Table 3.51cii.Zisman: Separated Uninfected Plasma with diiodomethane</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	54	-20.94	2.02E-17	36	4500	50	-18.15	1.75E-17
51	278	60	-25.4	2.45E-17	30	6000	50	-18.15	1.75E-17
56	282	62	-26.95	2.60E-17	30	8000	45	-14.88	1.44E-17
64	682	60	-25.4	2.45E-17	35	4900	48	-16.81	1.62E-17
62	606	54	-20.94	2.02E-17	36	5000	38	-10.77	1.04E-17
72	20	48	-16.81	1.62E-17	33	4500	47	-16.15	1.56E-17
56	613	47	-16.15	1.56E-17	39	4000	45	-14.88	1.44E-17
52	468	49	-17.47	1.69E-17	48	6200	44	-14.26	1.38E-17
51	853	50	-18.15	1.75E-17	48	4900	52	-19.52	1.88E-17
50	356	65	-29.33	2.83E-17	40	4800	50	-18.15	1.75E-17
52	268	50	-18.15	1.75E-17	38	4000	42	-13.05	1.26E-17
60	625	57	-23.13	2.23E-17	40	5000	57	-23.13	2.23E-17
47	230	65	-29.33	2.83E-17	41	4000	50	-18.15	1.75E-17
41	246	56	-22.39	2.16E-17	34	4000	50	-18.15	1.75E-17
56	339	50	-18.15	1.75E-17	44	6000	51	-18.83	1.82E-17
50	316	58	-23.88	2.30E-17	39	4400	47	-16.15	1.56E-17
52	220	41	-12.46	1.20E-17	36	4700	40	-11.88	1.15E-17
64	374	48	-16.81	1.62E-17	42	4800	43	-13.65	1.32E-17
63	593	42	-13.05	1.26E-17	40	4300	42	-13.05	1.26E-17
53	372	44	-14.26	1.38E-17	30	6000	44	-14.26	1.38E-17
<b>AVE</b>	<b>408.95</b>	<b>53</b>	<b>-20.4575</b>	<b>1.97E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-16.101</b>	<b>1.55E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>7.232</b>	<b>5.123781</b>	<b>4.94E-18</b>		<b>999.4735</b>	<b>4.632551</b>	<b>2.997056</b>	<b>2.88E-18</b>

**APPENDIX C12**

Netropil (counts/ mm <sup>3</sup> of blood)	<b>Table 3.52ci. Zisman: Separated infected Plasma with glycerine</b>				PcV (mm <sup>3</sup> )	<b>Table3.52cii. Zisman: Separated Uninfected Plasma with glycerine</b>			
	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )		CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	59	-31.04	3.00E-17	36	4500	45	-18.75	1.81E-17
51	278	58	-30.09	2.90E-17	30	6000	53	-25.48	2.46E-17
56	282	55	-27.29	2.63E-17	30	8000	40	-14.97	1.45E-17
64	682	65	-38.95	3.57E-17	35	4900	55	-27.29	2.63E-17
62	606	62	-33.95	3.28E-17	36	5000	50	-22.86	2.21E-17
72	20	60	-32	3.09E-17	33	4500	49	-22.01	2.12E-17
56	613	70	-42.11	4.06E-17	39	4000	50	-22.86	2.21E-17
52	468	56	-28.21	2.72E-17	48	6200	53	-25.48	2.46E-17
51	853	54	-26.38	2.55E-17	48	4900	50	-22.86	2.21E-17
50	356	58	-30.09	2.90E-17	40	4800	52	-24.6	2.37E-17
52	268	50	-22.86	2.21E-17	38	4000	51	-23.72	2.29E-17
60	625	63	-34.94	3.37E-17	40	5000	58	-30.09	2.90E-17
47	230	63	-34.94	3.37E-17	41	4000	53	-25.48	2.46E-17
41	246	50	-22.86	2.21E-17	34	4000	52	-24.6	2.37E-17
56	339	58	-30.09	2.90E-17	44	6000	48	-21.18	2.04E-17
50	316	59	-31.04	3.00E-17	39	4400	51	-23.72	2.29E-17
52	220	54	-26.38	2.55E-17	36	4700	54	-26.38	2.55E-17
64	374	49	-22.01	2.12E-17	42	4800	49	-22.01	2.12E-17
63	593	58	-30.09	2.90E-17	40	4300	48	-21.18	2.04E-17
53	372	63	-34.94	3.37E-17	30	6000	53	-25.48	2.46E-17
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-30.513</b>	<b>2.94E-17</b>		<b>5000</b>	<b>50.7</b>	<b>-23.55</b>	<b>2.25E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.346913</b>	<b>5.226735</b>	<b>4.89E-18</b>		<b>999.4735</b>	<b>3.812721</b>	<b>3.211756</b>	<b>3.06E-18</b>

**APPENDIX C13**

<b>Table 3.53ci. Neuman: Separated infected Plasma with water</b>					<b>Table 3.53cii. Neuman: Separated Uninfected Plasma with water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	69	-28.62	2.76E-17	36	4500	54	-13.7	1.32E-17
51	278	69	-28.62	2.76E-17	30	6000	60	-20.07	1.94E-17
56	282	75	-33.85	3.27E-17	30	8000	50	-8.89	8.58E-18
64	682	72	-31.28	3.02E-17	35	4900	58	-18.02	1.74E-17
62	606	70	-29.52	2.85E-17	36	5000	58	-18.02	1.74E-17
72	20	72	-31.28	3.02E-17	33	4500	61	-21.06	2.03E-17
56	613	60	-20.07	1.94E-17	39	4000	50	-8.89	8.58E-18
52	468	60	-20.07	1.94E-17	48	6200	58	-18.02	1.74E-17
51	853	59	-19.05	1.84E-17	48	4900	63	-23.02	2.22E-17
50	356	70	-29.52	2.85E-17	40	4800	58	-18.02	1.74E-17
52	268	65	-24.93	2.41E-17	38	4000	58	-18.02	1.74E-17
60	625	69	-28.62	2.76E-17	40	5000	59	-19.05	1.84E-17
47	230	70	-29.52	2.85E-17	41	4000	57	-16.97	1.64E-17
41	246	57	-16.97	1.64E-17	34	4000	62	-22.05	2.13E-17
56	339	66	-25.87	2.50E-17	44	6000	58	-18.02	1.74E-17
50	316	60	-20.07	1.94E-17	39	4400	62	-22.05	2.13E-17
52	220	65	-24.93	2.41E-17	36	4700	54	-13.7	1.32E-17
64	374	50	-8.89	8.58E-18	42	4800	50	-8.89	8.58E-18
63	593	60	-20.07	1.94E-17	40	4300	50	-8.89	8.58E-18
53	372	70	-29.52	2.85E-17	30	6000	50	-8.89	8.58E-18
<b>AVE</b>	<b>408.95</b>	<b>65.4</b>	<b>-25.064</b>	<b>2.4E-17</b>		<b>5000</b>	<b>56.5</b>	<b>-16.212</b>	<b>1.57E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>6.361066</b>	<b>6.222012</b>	<b>6E-18</b>		<b>999.4735</b>	<b>4.466248</b>	<b>4.930151</b>	<b>4.76E-18</b>

**APPENDIX C14**

<b>Table 3.54ci.Neuman: Separated infected Plasma with diiodomethane</b>					<b>Table 3.54cii.Neuman: Separated Uninfected Plasma with diiodomethane</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	54	-15.06	1.45E-17	36	4500	50	-13.12	1.27E-17
51	278	60	-18.07	1.74E-17	30	6000	50	-13.12	1.27E-17
56	282	62	-19.08	1.84E-17	30	8000	45	-10.8	1.04E-17
64	682	60	-18.07	1.74E-17	35	4900	48	-12.18	1.18E-17
62	606	54	-15.06	1.45E-17	36	5000	38	-7.83	7.56E-18
72	20	48	-12.18	1.18E-17	33	4500	47	-11.71	1.13E-17
56	613	47	-11.71	1.13E-17	39	4000	45	-10.8	1.04E-17
52	468	49	-12.65	1.22E-17	48	6200	44	-10.36	1.00E-17
51	853	50	-13.12	1.27E-17	48	4900	52	-14.09	1.36E-17
50	356	65	-20.6	1.99E-17	40	4800	50	-13.12	1.27E-17
52	268	50	-13.12	1.27E-17	38	4000	42	-9.48	9.15E-17
60	625	57	-16.56	1.60E-17	40	5000	57	-16.56	1.60E-17
47	230	65	-20.6	1.99E-17	41	4000	50	-13.12	1.27E-17
41	246	56	-16.06	1.55E-17	34	4000	50	-13.12	1.27E-17
56	339	50	-13.12	1.27E-17	44	6000	51	-13.6	1.31E-17
50	316	58	-17.06	1.65E-17	39	4400	47	-11.71	1.13E-17
52	220	41	-9.06	8.74E-18	36	4700	40	-8.64	8.34E-18
64	374	48	-12.18	1.18E-17	42	4800	43	-9.92	9.57E-18
63	593	42	-9.48	9.15E-18	40	4300	42	-9.48	9.15E-18
53	372	44	-10.36	1.00E-17	30	6000	44	-10.36	1.00E-17
<b>AVE</b>	<b>408.95</b>	<b>53</b>	<b>-14.66</b>	<b>1.42E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-11.656</b>	<b>1.54E-17</b>
<b>SD</b>	<b>189.4992</b>	<b>13.53249</b>	<b>22.89719</b>	<b>0.524089</b>		<b>999.4735</b>	<b>4.632551</b>	<b>2.124946</b>	<b>1.8E-17</b>

**APPENDIX C15**

<b>Table 3.55ci.Neuman: Separated infected Plasma with glycerine</b>					<b>Table 3.55cii.Neuman: Separated Uninfected Plasma with glycerine</b>				
<i>Netropil (counts/mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	59	-20.16	1.95E-17	36	4500	45	-10.89	1.05E-17
51	278	58	-19.47	1.88E-17	30	6000	53	45	-10.89
56	282	55	-17.41	1.66E-17	30	8000	40	53	16.06
64	682	65	-24.34	2.35E-17	35	4900	55	40	-7.95
62	606	62	-22.25	2.15E-17	36	5000	50	55	-17.41
72	20	60	-20.86	2.01E-17	33	4500	49	50	-14.06
56	613	70	-27.79	2.66E-17	39	4000	50	49	-13.41
52	468	56	-18.09	1.75E-17	48	6200	53	50	-14.06
51	853	54	-16.73	1.61E-17	48	4900	50	53	-16.06
50	356	58	-19.47	1.88E-17	40	4800	52	50	-14.06
52	268	50	-14.06	1.36E-17	38	4000	51	52	-15.39
60	625	63	-22.94	2.21E-17	40	5000	58	51	-14.72
47	230	63	-22.94	2.21E-17	41	4000	53	58	-19.47
41	246	50	-14.06	1.36E-17	34	4000	52	53	-16.06
56	339	58	-19.47	1.88E-17	44	6000	48	52	-15.39
50	316	59	-20.16	1.95E-17	39	4400	51	48	-12.77
52	220	54	-16.73	1.61E-17	36	4700	54	51	-14.72
64	374	49	-13.41	1.29E-17	42	4800	49	54	-16.73
63	593	58	-19.47	1.88E-17	40	4300	48	49	-13.41
53	372	63	-22.94	2.21E-17	30	6000	53	48	-12.77
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-19.6375</b>	<b>1.89E-17</b>		<b>5000</b>	<b>50.7</b>	<b>47.5055</b>	<b>-12.1635</b>
<b>SD</b>	<b>188.3373</b>	<b>13.52833</b>	<b>23.16305</b>	<b>0.529033</b>		<b>999.4735</b>	<b>3.812721</b>	<b>14.25361</b>	<b>7.761397</b>

**APPENDIX C16**

<b>Table 3.56ci.Girifalco&amp; Good: Separated infected Plasma with water</b>					<b>Table 3.56cii.Girifalco&amp;GoodI:SeparatedUninfected Plasma with Water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts /mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	69	-46.71	4.51E-17	36	4500	54	-20.94	2.02E-17
51	278	69	-46.71	4.51E-17	30	6000	60	-25.4	2.45E-17
56	282	75	-53.96	5.21E-17	30	8000	62	-26.95	2.60E-17
64	682	72	-50.3	4.85E-17	35	4900	60	-25.4	2.45E-17
62	606	70	-47.9	4.62E-17	36	5000	54	-20.94	2.02E-17
72	20	72	-50.3	4.85E-17	33	4500	48	-16.81	1.62E-17
56	613	60	-36.4	3.51E-17	39	4000	47	-16.15	1.56E-17
52	468	60	-36.4	3.51E-17	48	6200	49	-17.47	1.69E-17
51	853	59	-35.31	3.41E-17	48	4900	50	-18.15	1.75E-17
50	356	70	-47.9	4.62E-17	40	4800	65	-29.33	2.83E-17
52	268	65	-42.03	4.05E-17	38	4000	50	-18.15	1.75E-17
60	625	69	-46.71	4.51E-17	40	5000	57	-23.13	2.23E-17
47	230	70	-47.9	4.62E-17	41	4000	65	-29.33	2.83E-17
41	246	57	-33.15	3.20E-17	34	4000	56	-22.39	2.16E-17
56	339	66	-43.19	4.17E-17	44	6000	50	-18.15	1.75E-17
50	316	60	-38.4	3.51E-17	39	4400	58	-23.88	2.30E-17
52	220	65	-42.03	4.05E-17	36	4700	41	-12.46	1.20E-17
64	374	50	-26.01	2.51E-17	42	4800	48	-16.81	1.62E-17
63	593	60	-36.4	3.51E-17	40	4300	42	-13.05	1.26E-17
53	372	70	-47.9	4.62E-17	30	6000	44	-14.26	1.38E-17
<b>AVE</b>	<b>408.95</b>	<b>65.4</b>	<b>-42.7805</b>	<b>4.12E-17</b>		<b>5000</b>	<b>53</b>	<b>-20.4575</b>	<b>1.97E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>6.3610</b>	<b>7.114281</b>	<b>6.94E-18</b>		<b>999.4735</b>	<b>7.232965</b>	<b>5.123781</b>	<b>4.94E-18</b>

**APPENDIX C17**

<b>Table 3.57ci. Girifalco &amp; Good: Separated infected Plasma with diiodomethane</b>					<b>Table 3.57cii. Girifalco &amp; Good: Separated Uninfected Plasma with diiodomethane</b>				
Netropil (counts/ mm <sup>3</sup> of blood)	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	PcV (mm <sup>3</sup> )	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	54	-30.01	2.90E-17	36	4500	50	-18.15	1.75E-17
51	278	60	-36.4	3.51E-17	30	6000	50	-18.15	1.75E-17
56	282	50	-26.01	2.51E-17	30	8000	45	-14.88	1.44E-17
64	682	58	-34.22	3.30E-17	35	4900	48	-16.81	1.62E-17
62	606	58	-34.22	3.30E-17	36	5000	38	-10.77	1.04E-17
72	20	61	-37.51	3.62E-17	33	4500	47	-16.15	1.56E-17
56	613	50	-26.01	2.51E-17	39	4000	45	-14.88	1.44E-17
52	468	58	-34.22	3.30E-17	48	6200	44	-14.26	1.38E-17
51	853	63	-39.75	3.84E-17	48	4900	52	-19.52	1.88E-17
50	356	58	-34.22	3.30E-17	40	4800	50	-18.15	1.75E-17
52	268	58	-34.22	3.30E-17	38	4000	42	-13.05	1.26E-17
60	625	59	-35.31	3.41E-17	40	5000	57	-23.13	2.23E-17
47	230	57	-33.15	3.20E-17	41	4000	50	-18.15	1.75E-17
41	246	62	-38.62	3.73E-17	34	4000	50	-18.15	1.75E-17
56	339	58	-34.22	3.30E-17	44	6000	51	-18.83	1.82E-17
50	316	62	-38.62	3.73E-17	39	4400	47	-16.15	1.56E-17
52	220	54	-30.01	2.90E-17	36	4700	40	-11.88	1.15E-17
64	374	50	-26.01	2.51E-17	42	4800	43	-13.65	1.32E-17
63	593	50	-26.01	2.51E-17	40	4300	42	-13.05	1.26E-17
53	372	50	-26.01	2.51E-17	30	6000	44	-14.26	1.38E-17
<b>AVE</b>	<b>408.95</b>	<b>56.5</b>	<b>-32.7375</b>	<b>3.16E-17</b>		<b>5000</b>	<b>46.75</b>	<b>-16.101</b>	<b>1.55E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.466248</b>	<b>4.677623</b>	<b>4.52E-18</b>		<b>999.4735</b>	<b>4.632551</b>	<b>2.997056</b>	<b>2.88E-18</b>

**APPENDIX C18**

<b>Table 3.58ci.Girifalco&amp;Good: Separated infected Plasma glycerine</b>					<b>Table 3.58cii.Girifalco &amp;Good: Separated Uninfected Plasmawith glycerine</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	59	-31.04	3.00E-17	36	4500	45	-18.75	1.81E-17
51	278	58	-30.09	2.90E-17	30	6000	53	-25.48	2.46E-17
56	282	55	-27.29	2.63E-17	30	8000	40	-14.97	1.45E-17
64	682	65	-38.95	3.57E-17	35	4900	55	-27.29	2.63E-17
62	606	62	-33.95	3.28E-17	36	5000	50	-22.86	2.21E-17
72	20	60	-32	3.09E-17	33	4500	49	-22.01	2.12E-17
56	613	70	-42.11	4.06E-17	39	4000	50	-22.86	2.21E-17
52	468	56	-28.21	2.72E-17	48	6200	53	-25.48	2.46E-17
51	853	54	-26.38	2.55E-17	48	4900	50	-22.86	2.21E-17
50	356	58	-30.09	2.90E-17	40	4800	52	-24.6	2.37E-17
52	268	50	-22.86	2.21E-17	38	4000	51	-23.72	2.29E-17
60	625	63	-34.94	3.37E-17	40	5000	58	-30.09	2.90E-17
47	230	63	-34.94	3.37E-17	41	4000	53	-25.48	2.46E-17
41	246	50	-22.86	2.21E-17	34	4000	52	-24.6	2.37E-17
56	339	58	-30.09	2.90E-17	44	6000	48	-21.18	2.04E-17
50	316	59	-31.04	3.00E-17	39	4400	51	-23.72	2.29E-17
52	220	54	-26.38	2.55E-17	36	4700	54	-26.38	2.55E-17
64	374	49	-22.01	2.12E-17	42	4800	49	-22.01	2.12E-17
63	593	58	-30.09	2.90E-17	40	4300	48	-21.18	2.04E-17
53	372	63	-34.94	3.37E-17	30	6000	53	-25.48	2.46E-17
<b>AVE</b>	<b>408.95</b>	<b>58.2</b>	<b>-30.513</b>	<b>2.9E-17</b>		<b>5000</b>	<b>50.7</b>	<b>-23.55</b>	<b>2.25E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.346913</b>	<b>5.226735</b>	<b>4.89E-18</b>		<b>999.4735</b>	<b>3.812721</b>	<b>3.211756</b>	<b>3.06E-18</b>

## APPENDIX D1

<b>Table 3.41di..Wu: Separated infected Red Cell Using water</b>					<b>Table 3.41dii.. Wu: Separated uninfected Red Cell Using water</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	78	-104	1.01E-16	36	4500	64	-99.43	9.60E-17
51	278	65	-99.72	9.62E-17	30	6000	65	-99.72	9.62E-17
56	282	69	-100.96	9.74E-17	30	8000	70	-101.3	9.78E-17
64	682	69	-100.96	9.74E-17	35	4900	69	-100.96	9.74E-17
62	606	63	-99.16	9.57E-17	36	5000	63	-99.16	9.57E-17
72	20	76	-103.59	1.00E-16	33	4500	76	-103.59	1.00E-16
56	613	67	-100.32	9.68E-17	39	4000	60	-98.4	9.50E-17
52	468	60	-98.4	9.50E-17	48	6200	60	-98.4	9.50E-17
51	853	71	-101.65	9.81E-17	48	4900	63	-99.16	9.57E-17
50	356	68	-100.64	9.71E-17	40	4800	50	-96.42	9.31E-17
52	268	68	-100.64	9.71E-17	38	4000	73	-102.39	9.88E-17
60	625	60	-98.4	9.50E-17	40	5000	58	-97.94	9.45E-17
47	230	68	-100.64	9.71E-17	41	4000	69	-100.96	9.74E-17
41	246	64	-99.43	9.60E-17	34	4000	60	-98.4	9.50E-17
56	339	62	-98.9	9.54E-17	44	6000	50	-96.42	9.31E-17
50	316	60	-98.4	9.50E-17	39	4400	52	-96.75	9.34E-17
52	220	63	-99.16	9.57E-17	36	4700	55	-97.31	9.39E-17
64	374	65	-99.72	9.62E-17	42	4800	62	-98.9	9.54E-17
63	593	64	-99.43	9.60E-17	40	4300	54	-97.12	9.37E-17
53	372	72	-102.02	9.85E-17	30	6000	57	-97.72	9.43E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-100.31</b>	<b>9.7E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-99.0225</b>	<b>9.56E-17</b>
<b>SD</b>	<b>199.301</b>	<b>5.0304</b>	<b>1.5909</b>	<b>1.6E-18</b>		<b>999.4735</b>	<b>7.409098</b>	<b>1.985415</b>	<b>1.91E-18</b>

**APPENDIX D2**

<b>Table 3.42di. Wu: Separated infected Red Cell with diiodomethane</b>					<b>Table 3.42dii. Wu: Separated Uninfected Red Cell with diiodomethane</b>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	56	-26.45	2.55E-17	36	4500	40	-14.77	1.43E-17
51	278	50	-21.61	2.09E-17	30	6000	50	-21.61	2.09E-17
56	282	56	-26.45	2.55E-17	30	8000	48	-20.12	1.94E-17
64	682	56	-26.45	2.55E-17	35	4900	56	-26.45	2.55E-17
62	606	52	-23.16	2.24E-17	36	5000	62	-31.81	3.07E-17
72	20	66	-35.64	3.44E-17	33	4500	65	-34.66	3.35E-17
56	613	55	-25.6	2.47E-17	39	4000	43	-16.66	1.61E-17
52	468	50	-21.61	2.09E-17	48	6200	52	-23.16	2.24E-17
51	853	50	-21.61	2.09E-17	48	4900	40	-14.77	1.43E-17
50	356	60	-29.97	2.89E-17	40	4800	37	-13.02	1.26E-17
52	268	55	-25.6	2.47E-17	38	4000	38	-13.59	1.31E-17
60	625	56	-26.45	2.55E-17	40	5000	35	-11.94	1.15E-17
47	230	65	-34.66	3.35E-17	41	4000	42	-16.01	1.55E-17
41	246	60	-29.97	2.89E-17	34	4000	45	-17.99	1.74E-17
56	339	55	-25.6	2.47E-17	44	6000	39	-14.17	1.37E-17
50	316	50	-21.61	2.09E-17	39	4400	45	-17.99	1.74E-17
52	220	55	-25.6	2.47E-17	36	4700	34	-11.42	1.10E-17
64	374	60	-29.97	2.89E-17	42	4800	47	-19.39	1.87E-17
63	593	50	-21.61	2.09E-17	40	4300	40	-14.77	1.43E-17
53	372	58	-28.18	2.72E-17	30	6000	38	-13.59	1.31E-17
<b>AVE</b>	<b>408.95</b>	<b>55.75</b>	<b>-26.39</b>	<b>2.55E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-18.3945</b>	<b>1.78E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.766937</b>	<b>4.121793</b>	<b>3.97E-18</b>		<b>999.4735</b>	<b>8.593755</b>	<b>6.399366</b>	<b>6.18E-18</b>

**APPENDIX D3**

<b>Table 3.43di. Wu: Separated Infected Red Cell with Glycerine</b>					<b>Table 3.43dii. Wu: Separated Uninfected Red Cell with Glycerine</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	57	-61.05	5.89E-17	36	4500	42	-54.05	5.22E-17
51	278	55	-59.96	5.79E-17	30	6000	55	-59.96	5.79E-17
56	282	59	-62.21	6.00E-17	30	8000	49	-56.97	5.50E-17
64	682	59	-62.21	6.00E-17	35	4900	43	-54.43	5.25E-17
62	606	60	-62.81	6.06E-17	36	5000	58	-61.63	5.95E-17
72	20	60	-62.81	6.06E-17	33	4500	40	-53.32	5.15E-17
56	613	67	-67.37	6.50E-17	39	4000	47	-56.06	5.41E-17
52	468	53	-58.91	5.69E-17	48	6200	56	-60.5	5.84E-17
51	853	58	-61.63	5.95E-17	48	4900	49	-56.97	5.50E-17
50	356	62	-64.04	6.18E-17	40	4800	44	-54.82	5.29E-17
52	268	55	-59.96	5.79E-17	38	4000	46	-55.65	5.37E-17
60	625	60	-62.81	6.06E-17	40	5000	50	-57.44	5.54E-17
47	230	66	-66.68	6.44E-17	41	4000	55	-59.96	5.79E-17
41	246	61	-63.42	6.12E-17	34	4000	58	-61.63	5.95E-17
56	339	57	-61.06	5.89E-17	44	6000	47	-56.06	5.41E-17
50	316	57	-61.06	5.89E-17	39	4400	50	-57.44	5.54E-17
52	220	60	-62.81	6.06E-17	36	4700	53	-58.91	5.69E-17
64	374	62	-64.04	6.18E-17	42	4800	52	-58.41	5.64E-17
63	593	60	-62.81	6.06E-17	40	4300	42	-54.05	5.22E-17
53	372	68	-68.08	6.57E-17	30	6000	50	-57.44	5.54E-17
<b>AVE</b>	<b>408.95</b>	<b>59.8</b>	<b>-62.7865</b>	<b>6.06E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-57.285</b>	<b>5.53E-17</b>
<b>SD</b>	<b>199.301</b>	<b>3.90142</b>	<b>2.40307</b>	<b>2.3E-18</b>		<b>999.4735</b>	<b>5.458938</b>	<b>2.545747</b>	<b>2.46E-18</b>

**APPENDIX D4**

<b>Table 3.44di.Fowkes: Separated infected Red Cell with water</b>					<b>Table 3.44dii. Fowkes :Separated uninfected Red Cell using water</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	78	-97.48	9.41E-17	36	4500	64	-88.3	8.52E-17
51	278	65	-88.93	8.58E-17	30	6000	65	-88.93	8.58E-17
56	282	69	-91.49	8.83E-17	30	8000	70	-92.14	8.89E-17
64	682	69	-91.49	8.83E-17	35	4900	69	-91.49	8.83E-17
62	606	63	-87.68	8.46E-17	36	5000	63	-87.68	8.46E-17
72	20	76	-96.12	9.28E-17	33	4500	76	-96.12	9.28E-17
56	613	67	-90.2	8.70E-17	39	4000	60	-85.84	8.28E-17
52	468	60	-85.84	8.28E-17	48	6200	60	-85.84	8.28E-17
51	853	71	-92.79	8.96E-17	48	4900	63	-87.68	8.46E-17
50	356	68	-90.84	8.77E-17	40	4800	50	-80.16	7.74E-17
52	268	68	-90.84	8.77E-17	38	4000	73	-94.11	9.08E-17
60	625	60	-85.84	8.28E-17	40	5000	58	-84.65	8.17E-17
47	230	68	-90.84	8.77E-17	41	4000	69	-91.49	8.83E-17
41	246	64	-88.3	8.52E-17	34	4000	60	-85.84	8.28E-17
56	339	62	-87.06	8.40E-17	44	6000	50	-80.16	7.74E-17
50	316	60	-85.84	8.28E-17	39	4400	52	-81.24	7.84E-17
52	220	63	-87.68	8.46E-17	36	4700	55	-82.91	8.00E-17
64	374	65	-88.93	8.58E-17	42	4800	62	-87.06	8.40E-17
63	593	64	-88.3	8.52E-17	40	4300	54	-82.35	7.95E-17
53	372	72	-93.45	9.02E-17	30	6000	57	-84.07	8.11E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-89.997</b>	<b>8.7E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-86.903</b>	<b>8.39E-17</b>
<b>SD</b>	<b>199.301</b>	<b>5.03043</b>	<b>3.23698</b>	<b>3.1E-18</b>		<b>999.4735</b>	<b>7.409098</b>	<b>4.529814</b>	<b>4.37E-18</b>

APPENDIX D5

<i>Table 3.45di.Fowkes :Separated infected Red Cell with diiodomethane.</i>					<i>Table 3.45dii.Fowkes:SeparatedUninfected Red Cell with diiodometehane.</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	56	-24.21	2.34E-17	36	4500	40	-13.94	1.35E-17
51	278	50	-20.08	1.94E-17	30	6000	50	-20.08	1.94E-17
56	282	56	-24.21	2.34E-17	30	8000	48	-18.75	1.81E-17
64	682	56	-24.21	2.34E-17	35	4900	56	-24.21	2.34E-17
62	606	52	-21.4	2.07E-17	36	5000	62	-28.66	2.77E-17
72	20	66	-31.77	3.07E-17	33	4500	65	-30.99	2.99E-17
56	613	55	-23.49	2.27E-17	39	4000	43	-15.66	1.51E-17
52	468	50	-20.08	1.94E-17	48	6200	52	-21.4	2.07E-17
51	853	50	-20.08	1.94E-17	48	4900	40	-13.94	1.35E-17
50	356	60	-27.14	2.62E-17	40	4800	37	-12.32	1.19E-17
52	268	55	-23.49	2.27E-17	38	4000	38	-12.85	1.24E-17
60	625	56	-24.21	2.34E-17	40	5000	35	-11.3	1.09E-17
47	230	65	-30.99	2.99E-17	41	4000	42	-15.08	1.45E-17
41	246	60	-27.14	2.62E-17	34	4000	45	-18.86	1.63E-17
56	339	55	-23.49	2.27E-17	44	6000	39	-13.39	1.29E-17
50	316	50	-20.08	1.94E-17	39	4400	45	-18.86	1.63E-17
52	220	55	-23.49	2.27E-17	36	4700	34	-10.81	1.04E-17
64	374	60	-27.14	2.62E-17	42	4800	47	-18.11	1.75E-17
63	593	50	-20.08	1.94E-17	40	4300	40	-13.94	1.35E-17
53	372	50	-20.08	1.94E-17	30	6000	38	-12.85	1.24E-17
<b>AVE</b>	<b>408.95</b>	<b>55.35</b>	<b>-23.843</b>	<b>2.3E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-17.3</b>	<b>1.65E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.90193</b>	<b>3.54292</b>	<b>3.4E-18</b>		<b>999.4735</b>	<b>8.59375</b>	<b>5.599241</b>	<b>5.39E-18</b>

APPENDIX D6

<b>Table 3.46di.Fowkes :Separated Infected Red Cell with Glycerine</b>					<b>Table 3.46dii. Fowkes :Separated Uninfected Red Cell with Glycerine</b>				
<i>Netropil</i> (counts/ $mm^3$ of blood)	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ ( $mJ/m^2$ )	$A_{132}$ ( $mJ/m^2$ )	<i>PcV</i> ( $mm^3$ )	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ ( $mJ/m^2$ )	$A_{132}$ ( $mJ/m^2$ )
54	438	57	-65.95	5.40E-17	36	4500	42	-46.69	4.51E-17
51	278	55	-54.6	5.27E-17	30	6000	55	-54.6	5.27E-17
56	282	59	-57.33	5.53E-17	30	8000	49	-50.75	4.90E-17
64	682	59	-57.33	5.53E-17	35	4900	43	-47.24	4.56E-17
62	606	60	-58.03	5.60E-17	36	5000	58	-56.63	5.47E-17
72	20	60	-58.03	5.60E-17	33	4500	40	-45.62	4.40E-17
56	613	67	-63.13	6.09E-17	39	4000	47	-49.54	4.78E-17
52	468	53	-53.28	5.14E-17	48	6200	56	-55.27	5.33E-17
51	853	58	-56.63	5.47E-17	48	4900	49	-50.75	4.90E-17
50	356	62	-59.45	5.74E-17	40	4800	44	-47.8	4.61E-17
52	268	55	-54.6	5.27E-17	38	4000	46	-48.95	4.72E-17
60	625	60	-58.03	5.60E-17	40	5000	50	-51.37	4.96E-17
47	230	66	-62.38	6.02E-17	41	4000	55	-54.6	5.27E-17
41	246	61	-58.74	5.67E-17	34	4000	58	-56.63	5.47E-17
56	339	57	-65.95	5.40E-17	44	6000	47	-49.54	4.78E-17
50	316	57	-65.95	5.40E-17	39	4400	50	-51.37	4.96E-17
52	220	60	-58.03	5.60E-17	36	4700	53	-53.28	5.14E-17
64	374	62	-69.45	5.74E-17	42	4800	52	-52.63	5.08E-17
63	593	60	-58.03	5.60E-17	40	4300	42	-46.69	4.51E-17
53	372	68	-63.88	6.16E-17	30	6000	50	-51.37	4.96E-17
<b>AVE</b>	<b>408.95</b>	<b>59.8</b>	<b>-59.94</b>	<b>5.6E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-51.066</b>	<b>4.93E-17</b>
<b>SD</b>	<b>199.301</b>	<b>3.90142</b>	<b>4.45056</b>	<b>2.7E-18</b>		<b>999.4735</b>	<b>5.458938</b>	<b>3.362256</b>	<b>3.25E-18</b>

APPENDIX D7

<i>Table 3.47di.Owens &amp; WendtI: Separated infected Red Cell with Water.</i>					<i>Table 3.47dii. Owens &amp; Wendt : Separated Uninfected Red Cell with Water</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	78	-57.66	5.57E-17	36	4500	64	-40.89	3.95E-17
51	278	65	-42.03	4.06E-17	30	6000	65	-42.03	4.06E-17
56	282	69	-46.71	4.51E-17	30	8000	70	-47.9	4.62E-17
64	682	69	-46.71	4.51E-17	35	4900	69	-46.71	4.51E-17
62	606	63	-39.75	3.84E-17	36	5000	63	-39.75	3.84E-17
72	20	76	-55.19	5.33E-17	33	4500	76	-55.19	5.33E-17
56	613	67	-44.36	4.28E-17	39	4000	60	-36.4	3.51E-17
52	468	60	-36.4	3.51E-17	48	6200	60	-36.4	3.51E-17
51	853	71	-49.1	4.74E-17	48	4900	63	-39.75	3.84E-17
50	356	68	-45.53	4.39E-17	40	4800	50	-26.01	2.51E-17
52	268	68	-45.53	4.39E-17	38	4000	73	-51.52	4.97E-17
60	625	60	-36.4	3.51E-17	40	5000	58	-34.22	3.30E-17
47	230	68	-45.53	4.39E-17	41	4000	69	-46.71	4.51E-17
41	246	64	-40.89	3.95E-17	34	4000	60	-36.4	3.51E-17
56	339	62	-38.62	3.73E-17	44	6000	50	-26.01	2.51E-17
50	316	60	-36.4	3.51E-17	39	4400	52	-27.98	2.70E-17
52	220	63	-39.75	3.84E-17	36	4700	55	-31.04	3.00E-17
64	374	65	-42.03	4.06E-17	42	4800	62	-38.62	3.73E-17
63	593	64	-40.89	3.95E-17	40	4300	54	-30.01	2.90E-17
53	372	72	50.3	4.85E-17	30	6000	57	-33.15	3.20E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-38.959</b>	<b>4.2E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-38.3345</b>	<b>3.7E-17</b>
<b>SD</b>	<b>199.301</b>	<b>5.03043</b>	<b>21.7756</b>	<b>5.7E-18</b>		<b>999.4735</b>	<b>7.409098</b>	<b>8.279914</b>	<b>7.99E-18</b>

APPENDIX D8

<b>Table 3.48di.Owen&amp;Wendt:Separated infected Red Cell with diiodomethane.</b>					<b>Table 3.48dii.Owen&amp;Wendt: Separated Uninfected Red Cell with diiodomethane.</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	56	-22.39	2.16E-17	36	4500	40	-11.88	1.15E-17
51	278	50	-18.15	1.75E-17	30	6000	50	-18.15	1.75E-17
56	282	56	-22.39	2.16E-17	30	8000	48	-16.81	1.623-17
64	682	56	-22.39	2.16E-17	35	4900	56	-22.39	2.16E-17
62	606	52	-19.52	1.88E-17	36	5000	62	-26.95	2.60E-17
72	20	66	-30.14	2.91E-17	33	4500	65	-29.33	2.83E-17
56	613	55	-21.66	2.09E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	1.75E-17	48	6200	52	-19.52	1.88E-17
51	853	50	-18.15	1.75E-17	48	4900	40	-11.88	1.15E-17
50	356	60	-25.4	2.45E-17	40	4800	37	-10.23	9.87E-18
52	268	55	-21.66	2.09E-17	38	4000	38	-10.77	1.04E-17
60	625	56	-21.66	2.09E-17	40	5000	35	-9.19	8.87E-18
47	230	65	-29.33	2.83E-17	41	4000	42	-13.05	1.26E-17
41	246	60	-25.4	2.45E-17	34	4000	45	-45.88	1.44E-17
56	339	55	-21.66	2.09E-17	44	6000	39	-11.32	1.09E-17
50	316	50	-18.15	1.75E-17	39	4400	45	-45.88	1.44E-17
52	220	55	-21.66	2.09E-17	36	4700	34	-8.68	8.38E-17
64	374	60	-25.4	2.45E-17	42	4800	47	-16.15	1.56E-17
63	593	50	-18.15	1.75E-17	40	4300	40	-11.88	1.15E-17
53	372	58	-23.88	2.30E-17	30	6000	38	-10.77	1.04E-17
<b>AVE</b>	<b>408.95</b>	<b>55.75</b>	<b>-22.265</b>	<b>2.1E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-18.218</b>	<b>1.85E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.76694</b>	<b>3.54</b>	<b>3.4E-18</b>		<b>999.4735</b>	<b>8.593755</b>	<b>11.04622</b>	<b>1.67E-17</b>

APPENDIX D9

<i>Table 3.49di.Owen&amp;Wendt:Separated infected Red Cell with glycerine.</i>					<i>Table 3.49dii.Owen&amp;Wendt:Separated Uninfected Red Cell glycerine.</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	57	-29.14	2.81E-17	36	4500	42	-16.44	1.59E-17
51	278	55	-27.29	2.63E-17	30	6000	55	-27.29	2.63E-17
56	282	59	31.04	3.0-E-17	30	8000	49	-22.01	2.12E-17
64	682	59	31.04	3.0-E-17	35	4900	43	-17.19	1.66E-17
62	606	60	-32	3.09E-17	36	5000	58	-30.09	2.90E-17
72	20	60	-32	3.09E-17	33	4500	40	-14.97	1.45E-17
56	613	67	-38.99	3.76E-17	39	4000	47	-20.35	1.96E-17
52	468	53	-25.48	2.46E-17	48	6200	56	-28.21	2.72E-17
51	853	58	-30.09	2.90E-17	48	4900	49	-22.01	2.12E-17
50	356	62	-33.95	3.28E-17	40	4800	44	-17.96	1.73E-17
52	268	55	-27.29	2.63E-17	38	4000	46	-19.54	1.89E-17
60	625	60	-32	3.09E-17	40	5000	50	-22.86	2.21E-17
47	230	66	-37.97	3.66E-17	41	4000	55	-27.29	2.63E-17
41	246	61	-32.97	3.18E-17	34	4000	58	-30.09	2.90E-17
56	339	57	-29.14	2.81E-17	44	6000	47	-20.35	1.96E-17
50	316	57	-29.14	2.81E-17	39	4400	50	-22.86	2.21E-17
52	220	60	-32	3.09E-17	36	4700	53	-25.48	2.48E-17
64	374	62	-33.95	3.28E-17	42	4800	52	-24.6	2.37E-17
63	593	60	-32	3.09E-17	40	4300	42	-16.44	1.59E-17
53	372	68	-40.03	3.86E-17	30	6000	50	-22.86	2.21E-17
<b>AVE</b>	<b>408.95</b>	<b>59.8</b>	<b>-25.668</b>	<b>3.1E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-22.4445</b>	<b>2.17E-17</b>
<b>SD</b>	<b>199.301</b>	<b>3.90142</b>	<b>19.76</b>	<b>3.9E-18</b>		<b>999.4735</b>	<b>5.458938</b>	<b>4.615584</b>	<b>4.44E-18</b>

APPENDIX D10

<b>Table 3.50di.Zisman: Separated infected Red Cell with water</b>					<b>Table 3.50dii.Zisman Model: Separated Uninfected Red Cell with Water</b>				
<i>Netropil</i> (counts/ $mm^3$ of blood)	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> ( $mm^3$ )	<i>CD4+</i> (counts/ $mm^3$ of blood)	$\theta$ ( $^{\circ}C$ )	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	78	-57.66	5.57E-17	36	4500	64	-40.89	3.95E-17
51	278	65	-42.03	4.06E-17	30	6000	65	-42.03	4.06E-17
56	282	69	-46.71	4.51E-17	30	8000	70	-47.9	4.62E-17
64	682	69	-46.71	4.51E-17	35	4900	69	-46.71	4.51E-17
62	606	63	-39.75	3.84E-17	36	5000	63	-39.75	3.84E-17
72	20	76	-55.19	5.33E-17	33	4500	76	-55.19	5.33E-17
56	613	67	-44.36	4.28E-17	39	4000	60	-36.4	3.51E-17
52	468	60	-36.4	3.51E-17	48	6200	60	-36.4	3.51E-17
51	853	71	-49.1	4.74E-17	48	4900	63	-39.75	3.84E-17
50	356	68	-45.53	4.39E-17	40	4800	50	-26.01	2.51E-17
52	268	68	-45.53	4.39E-17	38	4000	73	-51.52	4.97E-17
60	625	60	-36.4	3.51E-17	40	5000	58	-34.22	3.30E-17
47	230	68	-45.53	4.39E-17	41	4000	69	-46.71	4.51E-17
41	246	64	-40.89	3.95E-17	34	4000	60	-36.4	3.51E-17
56	339	62	-38.62	3.73E-17	44	6000	50	-26.01	2.51E-17
50	316	60	-36.4	3.51E-17	39	4400	52	-27.98	2.70E-17
52	220	63	-39.75	3.84E-17	36	4700	55	-31.04	3.00E-17
64	374	65	-42.03	4.06E-17	42	4800	62	-38.62	3.73E-17
63	593	64	-40.89	3.95E-17	40	4300	54	-30.01	2.90E-17
53	372	72	-50.3	4.85E-17	30	6000	57	-33.15	3.20E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-43.989</b>	<b>4.2E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-38.3345</b>	<b>3.7E-17</b>
<b>SD</b>	<b>199.301</b>	<b>5.030</b>	<b>5.91521</b>	<b>5.7E-18</b>		<b>999.4735</b>	<b>7.40909</b>	<b>8.279914</b>	<b>7.99E-18</b>

APPENDIX D11

<b>Table 3.51di.Zisman: Separated infected Red Cell with diiodomethane</b>					<b>Table 3.51dii.Zisman: Separated Uninfected Red Cell with diiodomethane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	56	-22.39	8.87E-18	36	4500	40	-11.88	1.15E-17
51	278	50	-18.15	1.15E-17	30	6000	50	-18.15	1.75E-17
56	282	56	-22.39	1.75E-17	30	8000	48	-16.81	1.623-17
64	682	56	-22.39	7.00E-18	35	4900	56	-22.39	2.16E-17
62	606	52	-19.52	7.45E-18	36	5000	62	-26.95	2.60E-17
72	20	66	-30.14	1.04E-17	33	4500	65	-29.33	2.83E-17
56	613	55	-21.66	1.26E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	7.91E-18	48	6200	52	-19.52	1.88E-17
51	853	50	-18.15	1.44E-17	48	4900	40	-11.88	1.15E-17
50	356	60	-25.4	4.59E-18	40	4800	37	-10.23	9.87E-18
52	268	55	-21.66	6.57E-18	38	4000	38	-10.77	1.04E-17
60	625	56	-21.66	9.87E-18	40	5000	35	-9.19	8.87E-18
47	230	65	-29.33	1.20E-17	41	4000	42	-13.05	1.26E-17
41	246	60	-25.4	9.87E-17	34	4000	45	-45.88	1.44E-17
56	339	55	-21.66	8.38E-18	44	6000	39	-11.32	1.09E-17
50	316	50	-18.15	7.45E-18	39	4400	45	-45.88	1.44E-17
52	220	55	-21.66	6.15E-18	36	4700	34	-8.68	8.38E-17
64	374	60	-25.4	1.75E-17	42	4800	47	-16.15	1.56E-17
63	593	50	-18.15	1.50E-17	40	4300	40	-11.88	1.15E-17
53	372	58	-23.88	1.32E-17	30	6000	38	-10.77	1.04E-17
<b>AVE</b>	<b>408.95</b>	<b>55.75</b>	<b>-22.265</b>	<b>1.5E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-18.218</b>	<b>1.85E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>4.766937</b>	<b>3.540004</b>	<b>2.01E-17</b>		<b>999.4735</b>	<b>8.593755</b>	<b>11.04622</b>	<b>1.67E-17</b>

APPENDIX D12

<b>Table 3.42di. Zisman: Separated infected Red Cell with glycerine</b>					<b>Table 3.42dii. Zisman Separated Uninfected Red Cell with glycerine</b>					
Netropil (counts/ mm <sup>3</sup> of blood)	Netropil (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	PcV (mm <sup>3</sup> )	CD4+ (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	
54	54	57	-29.14	2.81E-17	36	4500	42	-16.44	1.59E-17	
51	51	55	-27.29	2.63E-17	30	6000	55	-27.29	2.63E-17	
56	56	59	31.04	3.0-E-17	30	8000	49	-22.01	2.12E-17	
64	64	59	31.04	3.0-E-17	35	4900	43	-17.19	1.66E-17	
62	62	60	-32	3.09E-17	36	5000	58	-30.09	2.90E-17	
72	72	60	-32	3.09E-17	33	4500	40	-14.97	1.45E-17	
56	56	67	-38.99	3.76E-17	39	4000	47	-20.35	1.96E-17	
52	52	53	-25.48	2.46E-17	48	6200	56	-28.21	2.72E-17	
51	51	58	-30.09	2.90E-17	48	4900	49	-22.01	2.12E-17	
50	50	62	-33.95	3.28E-17	40	4800	44	-17.96	1.73E-17	
52	52	55	-27.29	2.63E-17	38	4000	46	-19.54	1.89E-17	
60	60	60	-32	3.09E-17	40	5000	50	-22.86	2.21E-17	
47	47	66	-37.97	3.66E-17	41	4000	55	-27.29	2.63E-17	
41	41	61	-32.97	3.18E-17	34	4000	58	-30.09	2.90E-17	
56	56	57	-29.14	2.81E-17	44	6000	47	-20.35	1.96E-17	
50	50	57	-29.14	2.81E-17	39	4400	50	-22.86	2.21E-17	
52	52	60	-32	3.09E-17	36	4700	53	-25.48	2.48E-17	
64	64	62	-33.95	3.28E-17	42	4800	52	-24.6	2.37E-17	
63	63	60	-32	3.09E-17	40	4300	42	-16.44	1.59E-17	
53	53	68	-40.03	3.86E-17	30	6000	50	-22.86	2.21E-17	
<b>AVE</b>	<b>AVE</b>	<b>59.8</b>	<b>-25.668</b>	<b>3.1E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-22.4445</b>	<b>2.17E-17</b>	
<b>SD</b>	<b>SD</b>	<b>3.90142</b>	<b>19.76</b>	<b>3.9E-18</b>		<b>999.4735</b>	<b>5.458938</b>	<b>4.615584</b>	<b>4.44E-18</b>	

APPENDIX D13

<b>Table 3.53di.Neuman: Separated infected Red Cell with water</b>					<b>Table 3.53dii.Neuman Model: Separated Uninfected RedCell with Water</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ (°C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	78	-36.32	3.51E-17	36	4500	64	-23.98	2.31E-17
51	278	65	-24.93	2.41E-17	30	6000	65	-24.93	2.41E-17
56	282	69	-28.62	2.76E-17	30	8000	70	-29.52	2.85E-17
64	682	69	-28.62	2.76E-17	35	4900	69	-28.62	2.76E-17
62	606	63	-23.02	2.22E-17	36	5000	63	-23.02	2.22E-17
72	20	76	-34.68	3.35E-17	33	4500	76	-34.68	3.35E-17
56	613	67	-26.8	2.59E-17	39	4000	60	-20.07	1.94E-17
52	468	60	-20.07	1.94E-17	48	6200	60	-20.07	1.94E-17
51	853	71	-30.4	2.93E-17	48	4900	63	-23.02	2.22E-17
50	356	68	-27.72	2.67E-17	40	4800	50	-8.89	8.58E-17
52	268	68	-27.72	2.67E-17	38	4000	73	-32.15	3.10E-17
60	625	60	-20.07	1.94E-17	40	5000	58	-18.02	1.74E-17
47	230	68	-27.72	2.67E-17	41	4000	69	-28.62	2.76E-17
41	246	64	-23.98	2.31E-17	34	4000	60	-20.07	1.94E-17
56	339	62	-22.05	2.13E-17	44	6000	50	-8.89	8.58E-17
50	316	60	-20.07	1.94E-17	39	4400	52	-11.38	1.10E-17
52	220	63	-23.02	2.22E-17	36	4700	55	-14.82	1.43E-17
64	374	65	-24.93	2.41E-17	42	4800	62	-22.05	2.13E-17
63	593	64	-23.98	2.31E-17	40	4300	54	-13.7	1.32E-17
53	372	72	-31.28	3.02E-17	30	6000	57	-16.97	1.64E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-26.3</b>	<b>2.5E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-21.1735</b>	<b>2.82E-17</b>
<b>SD</b>	<b>199.301</b>	<b>5.03043</b>	<b>4.58737</b>	<b>4.4E-18</b>		<b>999.4735</b>	<b>7.409098</b>	<b>7.392093</b>	<b>2.06E-17</b>

APPENDIX D14

<b>Table 3.54di.Neuman: Separated infected Red Cellwith diiodomethane</b>					<b>Table 3.54dii.Neuman: Separated Uninfected Red Cell with diiodomethane</b>				
<i>Netropil</i> (counts/ mm <sup>3</sup> of blood)	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )	<i>PcV</i> (mm <sup>3</sup> )	<i>CD4+</i> (counts/ mm <sup>3</sup> of blood)	$\theta$ ( $^{\circ}$ C)	$F^{adh}$ (mJ/m <sup>2</sup> )	$A_{132}$ (mJ/m <sup>2</sup> )
54	438	56	-16.08	8.34E-18	36	4500	40	-8.64	8.34E-18
51	278	50	-13.12	1.27E-17	30	6000	50	-13.12	1.27E-17
56	282	56	-16.08	1.18E-17	30	8000	48	12.18	1.18E-17
64	682	56	-16.06	1.55E-17	35	4900	56	-16.06	1.55E-17
62	606	52	-14.09	1.36E-17	36	5000	62	-19.08	1.84E-17
72	20	66	-21.11	2.04E-17	33	4500	65	-20.6	1.99E-17
56	613	55	-15.56	1.50E-17	39	4000	43	-9.92	9.57E-18
52	468	50	-13.12	1.27E-17	48	6200	52	-14.09	1.36E-17
51	853	50	-13.12	1.27E-17	48	4900	40	-8.64	8.34E-18
50	356	60	-18.07	1.74E-17	40	4800	37	-7.43	7.17E-18
52	268	55	-15.56	1.50E-17	38	4000	38	-7.83	7.56E-18
60	625	56	-16.06	1.18E-17	40	5000	35	-6.67	6.44E-18
47	230	65	-20.6	1.99E-17	41	4000	42	-9.48	9.15E-18
41	246	60	-18.07	1.74E-17	34	4000	45	-10.8	1.04E-17
56	339	55	-15.56	1.50E-17	44	6000	39	-8.23	7.94E-18
50	316	50	-13.12	1.27E-17	39	4400	45	-10.8	1.04E-17
52	220	55	-15.56	1.50E-17	36	4700	34	-6.3	6.08E-18
64	374	60	-18.07	1.74E-17	42	4800	47	-11.71	1.13E-17
63	593	50	-13.12	1.27E-17	40	4300	40	-8.64	8.34E-18
53	372	58	-17.06	1.65E-17	30	6000	38	-7.83	7.56E-18
<b>AVE</b>	<b>408.95</b>	<b>55.75</b>	<b>-15.96</b>	<b>1.5E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-9.6845</b>	<b>1.05E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.76694</b>	<b>2.37456</b>	<b>2.9E-18</b>		<b>999.4735</b>	<b>8.593755</b>	<b>6.496632</b>	<b>3.84E-18</b>

APPENDIX D15

<i>Table 3.55di.Neuman: Separated infected Red Cell with glycerine</i>					<i>Table 3.55dii.Neuman: Separated Uninfected Red Cell with glycerine</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	57	-18.78	1.81E-17	36	4500	42	-9.09	8.77E-18
51	278	55	-17.41	1.68E-17	30	6000	55	-17.41	1.68E-17
56	282	59	-20.16	1.95E-17	30	8000	49	-13.41	1.29E-17
64	682	59	-20.16	1.95E-17	35	4900	43	-9.68	9.34E-18
62	606	60	-20.86	2.01E-17	36	5000	58	-19.47	1.88E-17
72	20	60	-20.86	2.01E-17	33	4500	40	-7.95	7.68E-18
56	613	67	-25.72	2.48E-17	39	4000	47	-12.13	1.17E-17
52	468	53	-16.06	1.55E-17	48	6200	56	-18.09	1.75E-17
51	853	58	-19.47	1.88E-17	48	4900	49	-13.41	1.29E-17
50	356	62	-22.25	2.15E-17	40	4800	44	10.28	9.92E-18
52	268	55	-17.41	1.68E-17	38	4000	46	-11.5	1.11E-17
60	625	60	-20.86	2.01E-17	40	5000	50	-14.06	1.36E-17
47	230	66	-25.03	2.42E-17	41	4000	55	-17.41	1.68E-17
41	246	61	-21.55	2.08E-17	34	4000	58	-19.47	1.88E-17
56	339	57	-18.78	1.81E-17	44	6000	47	-12.13	1.17E-17
50	316	57	-18.78	1.81E-17	39	4400	50	-14.06	1.36E-17
52	220	60	-20.86	2.01E-17	36	4700	53	-16.06	1.55E-17
64	374	62	-22.25	2.15E-17	42	4800	52	-15.39	1.48E-17
63	593	60	-20.86	2.01E-17	40	4300	42	-9.09	8.77E-18
53	372	68	-26.41	2.55E-17	30	6000	50	-14.06	1.36E-17
<b>AVE</b>	<b>408.95</b>	<b>59.8</b>	<b>-20.726</b>	<b>2E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-12.6795</b>	<b>1.32E-17</b>
<b>SD</b>	<b>199.301</b>	<b>3.90142</b>	<b>2.70018</b>	<b>2.6E-18</b>		<b>999.4735</b>	<b>5.458938</b>	<b>6.399333</b>	<b>3.4E-18</b>

APPENDIX D16

<i>Table 3.56di. Girifalco &amp; Good Separated infected Red cell with Water</i>					<i>Table 3.56dii. Girifalco &amp; Good Separated infected Red cell with Water</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	78	-57.66	5.57E-17	36	4500	64	-40.89	3.95E-17
51	278	65	-42.03	4.06E-17	30	6000	65	-42.03	4.06E-17
56	282	69	-46.71	4.51E-17	30	8000	70	-47.9	4.62E-17
64	682	69	-46.71	4.51E-17	35	4900	69	-46.71	4.51E-17
62	606	63	-39.75	3.84E-17	36	5000	63	-39.75	3.84E-17
72	20	76	-55.19	5.33E-17	33	4500	76	-55.19	5.33E-17
56	613	67	-44.36	4.28E-17	39	4000	60	-36.4	3.51E-17
52	468	60	-36.4	3.51E-17	48	6200	60	-36.4	3.51E-17
51	853	71	-49.1	4.74E-17	48	4900	63	-39.75	3.84E-17
50	356	68	-45.53	4.39E-17	40	4800	50	-26.01	2.51E-17
52	268	68	-45.53	4.39E-17	38	4000	73	-51.52	4.97E-17
60	625	60	-36.4	3.51E-17	40	5000	58	-34.22	3.30E-17
47	230	68	-45.53	4.39E-17	41	4000	69	-46.71	4.51E-17
41	246	64	-40.89	3.95E-17	34	4000	60	-36.4	3.51E-17
56	339	62	-38.62	3.73E-17	44	6000	50	-26.01	2.51E-17
50	316	60	-36.4	3.51E-17	39	4400	52	-27.98	2.70E-17
52	220	63	-39.75	3.84E-17	36	4700	55	-31.04	3.00E-17
64	374	65	-42.03	4.06E-17	42	4800	62	-38.62	3.73E-17
63	593	64	-40.89	3.95E-17	40	4300	54	-30.01	2.90E-17
53	372	72	-50.3	4.85E-17	30	6000	57	-33.15	3.20E-17
<b>AVE</b>	<b>408.95</b>	<b>66.6</b>	<b>-43.989</b>	<b>4.25E-17</b>		<b>5000</b>	<b>61.5</b>	<b>-38.3345</b>	<b>3.7E-17</b>
<b>SD</b>	<b>199.3013</b>	<b>5.030434</b>	<b>5.915213</b>	<b>5.71E-18</b>		<b>999.4735</b>	<b>7.409098</b>	<b>8.279914</b>	<b>7.99E-18</b>

APPENDIX D17

<i>Table 3.57di.Girifalco&amp;Good: Separated infected Red Cell with diiodomethane</i>					<i>Table 3.57dii.Girifalco &amp;Good: Separated Uninfected Red Cell with diiodomethane</i>				
<i>Netropil (counts/mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	56	-22.39	2.16E-17	36	4500	40	-11.88	1.15E-17
51	278	50	-18.15	1.75E-17	30	6000	50	-18.15	1.75E-17
56	282	56	-22.39	2.16E-17	30	8000	48	-16.81	1.623-17
64	682	56	-22.39	2.16E-17	35	4900	56	-22.39	2.16E-17
62	606	52	-19.52	1.88E-17	36	5000	62	-26.95	2.60E-17
72	20	66	-30.14	2.91E-17	33	4500	65	-29.33	2.83E-17
56	613	55	-21.66	2.09E-17	39	4000	43	-13.65	1.32E-17
52	468	50	-18.15	1.75E-17	48	6200	52	-19.52	1.88E-17
51	853	50	-18.15	1.75E-17	48	4900	40	-11.88	1.15E-17
50	356	60	-25.4	2.45E-17	40	4800	37	-10.23	9.87E-18
52	268	55	-21.66	2.09E-17	38	4000	38	-10.77	1.04E-17
60	625	56	-21.66	2.09E-17	40	5000	35	-9.19	8.87E-18
47	230	65	-29.33	2.83E-17	41	4000	42	-13.05	1.26E-17
41	246	60	-25.4	2.45E-17	34	4000	45	-45.88	1.44E-17
56	339	55	-21.66	2.09E-17	44	6000	39	-11.32	1.09E-17
50	316	50	-18.15	1.75E-17	39	4400	45	-45.88	1.44E-17
52	220	55	-21.66	2.09E-17	36	4700	34	-8.68	8.38E-17
64	374	60	-25.4	2.45E-17	42	4800	47	-16.15	1.56E-17
63	593	50	-18.15	1.75E-17	40	4300	40	-11.88	1.15E-17
53	372	58	-23.88	2.30E-17	30	6000	38	-10.77	1.04E-17
<b>AVE</b>	<b>408.95</b>	<b>55.75</b>	<b>-22.265</b>	<b>2.1E-17</b>		<b>5000</b>	<b>44.8</b>	<b>-18.218</b>	<b>1.85E-17</b>
<b>SD</b>	<b>199.301</b>	<b>4.76694</b>	<b>3.54</b>	<b>3.4E-18</b>		<b>999.4735</b>	<b>8.59375</b>	<b>11.04622</b>	<b>1.67E-17</b>

APPENDIX D18

<i>Table 3.58di: Girifalco &amp; Good: Separated Uninfected White with glycerine</i>					<i>Table 3.58dii: Girifalco &amp; Good: Separated Uninfected White with glycerine</i>				
<i>Netropil (counts/ mm<sup>3</sup> of blood)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>	<i>PcV (mm<sup>3</sup>)</i>	<i>CD4+ (counts/ mm<sup>3</sup> of blood)</i>	<i>θ (°C)</i>	<i>F<sup>adh</sup>(mJ/m<sup>2</sup>)</i>	<i>A<sub>132</sub>(mJ/m<sup>2</sup>)</i>
54	438	57	-29.14	2.81E-17	36	4500	42	-16.44	1.59E-17
51	278	55	-27.29	2.63E-17	30	6000	55	-27.29	2.63E-17
56	282	59	31.04	3.0-E-17	30	8000	49	-22.01	2.12E-17
64	682	59	31.04	3.0-E-17	35	4900	43	-17.19	1.66E-17
62	606	60	-32	3.09E-17	36	5000	58	-30.09	2.90E-17
72	20	60	-32	3.09E-17	33	4500	40	-14.97	1.45E-17
56	613	67	-38.99	3.76E-17	39	4000	47	-20.35	1.96E-17
52	468	53	-25.48	2.46E-17	48	6200	56	-28.21	2.72E-17
51	853	58	-30.09	2.90E-17	48	4900	49	-22.01	2.12E-17
50	356	62	-33.95	3.28E-17	40	4800	44	-17.96	1.73E-17
52	268	55	-27.29	2.63E-17	38	4000	46	-19.54	1.89E-17
60	625	60	-3200	3.09E-17	40	5000	50	-22.86	2.21E-17
47	230	66	-37.97	3.66E-17	41	4000	55	-27.29	2.63E-17
41	246	61	-32.97	3.18E-17	34	4000	58	-30.09	2.90E-17
56	339	57	-29.14	2.81E-17	44	6000	47	-20.35	1.96E-17
50	316	57	-29.14	2.81E-17	39	4400	50	-22.86	2.21E-17
52	220	60	-32	3.09E-17	36	4700	53	-25.48	2.48E-17
64	374	62	-33.95	3.28E-17	42	4800	52	-24.6	2.37E-17
63	593	60	-32	3.09E-17	40	4300	42	-16.44	1.59E-17
53	372	68	-40.03	3.86E-17	30	6000	50	-22.86	2.21E-17
<b>AVE</b>	<b>408.95</b>	<b>59.8</b>	<b>-184.07</b>	<b>3.1E-17</b>		<b>5000</b>	<b>49.3</b>	<b>-22.4445</b>	<b>2.17E-17</b>
<b>SD</b>	<b>199.301</b>	<b>3.90142</b>	<b>710.15</b>	<b>3.9E-18</b>		<b>999.4735</b>	<b>5.4589</b>	<b>4.615584</b>	<b>4.44E-18</b>

**APPENDIX E: SCILAB CALCULATIONS (VALIDATION OF APPENDIX A-D) USING NEUMANN MODEL**

**APPENDIX E1(Infected WBC-water)**

$\theta_{water}=64:1:85; st=72.8$

$B=\cos(\%pi*\theta_{water}/180);x=0.25*st*(1+B)^2;$

$Gamasv=0.25*st*(1+B)^2$

$F=(Gamasv.^{0.5}-st.^{0.5})./(1-0.015*(Gamasv*st).^{0.5})$

$S=Gamasv-st-F$

$N=-12*\%pi*(1.6*10^{(-10)})^2*S$

$\theta_{water}=64:1:85; st=72.8$

--> $B=\cos(\%pi*\theta_{water}/180);x=0.25*st*(1+B)^2;$

-> $Gamasv=0.25*st*(1+B)^2$

37.65419 36.833937 36.016125 35.201222 34.389688 33.581975 32.778529 31.979783 31.186164 30.398088 29.615962  
28.840182 28.071134 27.309193 26.554722 25.808074 25.069591 24.3396 23.618419 22.906353 22.203693 21.510718

--> $F=(Gamasv.^{0.5}-st.^{0.5})./(1-0.015*(Gamasv*st).^{0.5})$

- 11.162347 - 11.033326 - 10.912937 - 10.800387 - 10.694974 - 10.596076 - 10.503141 - 10.415678 - 10.333245 - 10.255448 - 10.181932  
- 10.112375 - 10.046489 - 9.9840102 - 9.9247006 - 9.8683429 - 9.8147389 - 9.7637076 - 9.7150832 - 9.6687137 - 9.6244592 - 9.5821911

$$\rightarrow S = \text{Gamav} - st - F$$

- 23.983463 - 24.932737 - 25.870937 - 26.798391 - 27.715339 - 28.621949 - 29.51833 - 30.404539 - 31.280591 - 32.146464 - 33.002106  
 - 33.847443 - 34.682377 - 35.506797 - 36.320578 - 37.123583 - 37.91567 - 38.696692 - 39.466497 - 40.224933 - 40.971848 - 41.70709

$$\rightarrow N = -12 * \pi * (1.6 * 10^{-10})^2 * S$$

$$10^{-16} *$$

0.2314637 0.2406252 0.2496797 0.2586305 0.2674800 0.2762296 0.2848806 0.2934334 0.3018881 0.3102446 0.3185024  
 0.3266607 0.3347187 0.3426751 0.3505289 0.3582787 0.3659231 0.3734607 0.3808901 0.3882097 0.3954182 0.4025140

### APPENDIX E2(Uninfected WBC-water)

$$\text{Theta}_{\text{water}} = 50:1:78; \text{st} = 72.8$$

$$B = \cos(\pi * \text{Theta}_{\text{water}} / 180); x = 0.25 * \text{st} * (1 + B)^2;$$

$$\text{Gamav} = 0.25 * \text{st} * (1 + B)^2$$

$$F = (\text{Gamav}^{0.5} - \text{st}^{0.5}) / (1 - 0.015 * (\text{Gamav} * \text{st})^{0.5})$$

$$S = \text{Gamav} - \text{st} - F$$

$$N = -12 * \pi * (1.6 * 10^{-10})^2 * S$$

$$\text{Theta}_{\text{water}} = 50:1:78; \text{st} = 72.8.$$

--> $B = \cos(\%pi * \text{Theta\_water} / 180); x = 0.25 * st * (1 + B)^2;$

--> $\text{Gamav} = 0.25 * st * (1 + B)^2$

49.117271 48.315266 47.508588 46.697767 45.883329 45.065799 44.245702 43.423557 42.599884 41.775195 40.950000  
40.124805 39.300109 38.476408 37.65419 36.833937 36.016125 35.201222 34.389688 33.581975 32.778529 31.979783  
31.186164 30.398088 29.615962 28.840182 28.071134 27.309193 26.554722

--> $F = (\text{Gamav}^{0.5} - st^{0.5}) / (1 - 0.015 * (\text{Gamav} * st)^{0.5})$

- 14.78989 - 14.325192 - 13.913113 - 13.545401 - 13.215436 - 12.917847 - 12.64823 - 12.402939 - 12.178934 - 11.973654 - 11.784933  
- 11.610922 - 11.450036 - 11.300906 - 11.162347 - 11.033326 - 10.912937 - 10.800387 - 10.694974 - 10.596076 - 10.503141 - 10.415678  
- 10.333245 - 10.255448 - 10.181932 - 10.112375 - 10.046489 - 9.9840102 - 9.9247006

--> $S = \text{Gamav} - st - F$

- 8.8928397 - 10.159542 - 11.378298 - 12.556832 - 13.701235 - 14.816354 - 15.906068 - 16.973503 - 18.021182 - 19.051151 - 20.065067  
- 21.064273 - 22.049855 - 23.022686 - 23.983463 - 24.932737 - 25.870937 - 26.798391 - 27.715339 - 28.621949 - 29.51833 - 30.404539  
- 31.280591 - 32.146464 - 33.002106 - 33.847443 - 34.682377 - 35.506797 - 36.320578

--> $N = -12 * \%pi * (1.6 * 10^{(-10)})^2 * S$

$10^{(-16)}$  \*

0.0858246 0.0980495 0.1098116 0.1211856 0.1322302 0.1429922 0.1535090 0.1638108 0.1739219 0.1838621 0.1936474  
0.2032907 0.2128025 0.2221913 0.2314637 0.2406252 0.2496797 0.2586305 0.2674800 0.2762296 0.2848806 0.2934334  
0.3018881 0.3102446 0.3185024 0.3266607 0.3347187 0.3426751 0.3505289

**APPENDIX E3(Infected WBC-Diodomethane)**

$$\text{Theta\_dio}=41:1:65; \text{st}=50.8$$

$$B=\cos(\%pi*\text{Theta\_dio}/180);x=0.25*\text{st}*(1+B)^2;$$

$$\text{Gamasv}=0.25*\text{st}*(1+B)^2$$

$$F=(\text{Gamasv}.^0.5-\text{st}.^0.5)./(1-0.015*(\text{Gamasv}*\text{st}).^0.5)$$

$$S=\text{Gamasv}-\text{st}-F$$

$$N=-12*\%pi*(1.6*10^(-10))^2*S$$

$$\text{Theta\_dio}=41:1:65; \text{st}=50.8$$

$$\text{-->}B=\cos(\%pi*\text{Theta\_dio}/180);x=0.25*\text{st}*(1+B)^2;$$

$$\text{-->}Gamasv=0.25*\text{st}*(1+B)^2$$

Gamasv =

39.103373 38.589634 38.069338 37.542843 37.010512 36.472711 35.929805 35.382162 34.83015 34.274139 33.714499  
33.151597 32.585804 32.017487 31.447014 30.874748 30.301054 29.726293 29.150823 28.575 27.999177 27.423703  
26.848922 26.275177 25.702803

$$\text{-->}F=(\text{Gamasv}.^0.5-\text{st}.^0.5)./(1-0.015*(\text{Gamasv}*\text{st}).^0.5)$$

- 2.6372847 - 2.7253955 - 2.8128823 - 2.8996781 - 2.9857215 - 3.0709563 - 3.155331 - 3.2387991 - 3.3213187 - 3.4028522 - 3.4833659  
- 3.5628304 - 3.6412197 - 3.7185114 - 3.7946866 - 3.8697291 - 3.9436259 - 4.0163665 - 4.087943 - 4.15835 - 4.227584 - 4.2956436 -  
4.3625293 - 4.4282434 - 4.4927895

--> $S = \text{Gamav} - st - F$

- 9.0593427 - 9.4849702 - 9.9177801 - 10.357479 - 10.803766 - 11.256333 - 11.714864 - 12.179039 - 12.648531 - 13.123008 - 13.602135  
- 14.085572 - 14.572976 - 15.064001 - 15.5583 - 16.055523 - 16.55532 - 17.057341 - 17.561234 - 18.06665 - 18.573239 - 19.080654 -  
19.588548 - 20.09658 - 20.604408

--> $N = -12 * \pi * (1.6 * 10^{(-10)})^2 * S$

$10^{(-16)}$  \*

0.0874315 0.0915392 0.0957162 0.0999597 0.1042669 0.1086346 0.1130598 0.1175396 0.1220706 0.1266498 0.1312738  
0.1359395 0.1406434 0.1453823 0.1501527 0.1549514 0.1597749 0.1646199 0.1694830 0.1743607 0.1792498 0.1841469  
0.1890485 0.1939515 0.1988526

#### **APPENDIX E4 (Uninfected WBC-diodomethane)**

$\text{Theta\_dio} = 32:1:65; st = 50.8$

$B = \cos(\pi * \text{Theta\_dio} / 180); x = 0.25 * st * (1 + B)^2;$

$\text{Gamav} = 0.25 * st * (1 + B)^2$

$F = (\text{Gamav}^{0.5} - st^{0.5}) / (1 - 0.015 * (\text{Gamav} * st)^{0.5})$

$S = \text{Gamav} - st - F$

$N = -12 * \pi * (1.6 * 10^{(-10)})^2 * S$

$\text{Theta\_dio} = 32:1:65; st = 50.8$

-> $B = \cos(\pi * \text{Theta\_dio} / 180); x = 0.25 * st * (1 + B)^2;$

$$\rightarrow \text{Gamasy} = 0.25 * st * (1+B)^2$$

Gamasy =

43.374078 42.93501 42.486306 42.02829 41.56129 41.085639 40.601677 40.109747 39.610195 39.103373 38.589634  
 38.069338 37.542843 37.010512 36.472711 35.929805 35.382162 34.83015 34.274139 33.714499 33.151597 32.585804  
 32.017487 31.447014 30.874748 30.301054 29.726293 29.150823 28.575 27.999177 27.423703 26.848922 26.275177  
 25.702803

$$\rightarrow F = (\text{Gamasy}^{0.5} - st^{0.5}) / (1 - 0.015 * (\text{Gamasy} * st)^{0.5})$$

- 1.8300895 - 1.9198496 - 2.0098518 - 2.0999755 - 2.1901061 - 2.2801351 - 2.3699605 - 2.4594862 - 2.5486222 - 2.6372847 - 2.7253955  
 - 2.8128823 - 2.8996781 - 2.9857215 - 3.0709563 - 3.155331 - 3.2387991 - 3.3213187 - 3.4028522 - 3.4833659 - 3.5628304 - 3.6412197  
 - 3.7185114 - 3.7946866 - 3.8697291 - 3.9436259 - 4.0163665 - 4.087943 - 4.15835 - 4.227584 - 4.2956436 - 4.3625293 - 4.4282434 -  
 4.4927895

$$\rightarrow S = \text{Gamasy} - st - F$$

- 5.5958321 - 5.9451403 - 6.303842 - 6.6717347 - 7.0486044 - 7.4342257 - 7.8283623 - 8.2307672 - 8.641183 - 9.0593427 - 9.4849702 -  
 9.9177801 - 10.357479 - 10.803766 - 11.256333 - 11.714864 - 12.179039 - 12.648531 - 13.123008 - 13.602135 - 14.085572 - 14.572976  
 - 15.064001 - 15.5583 - 16.055523 - 16.55532 - 17.057341 - 17.561234 - 18.06665 - 18.573239 - 19.080654 - 19.588548 - 20.09658  
 - 20.604408

$$\rightarrow N = -12 * \pi * (1.6 * 10^{(-10)})^2 * S$$

$10^{(-16)}$  \*

0.0540052 0.0573764 0.0608382 0.0643887 0.0680259 0.0717475 0.0755513 0.0794349 0.0833958 0.0874315 0.0915392  
 0.0957162 0.0999597 0.1042669 0.1086346 0.1130598 0.1175396 0.1220706 0.1266498 0.1312738 0.1359395 0.1406434  
 0.1453823 0.1501527 0.1549514 0.1597749 0.1646199 0.1694830 0.1743607 0.1792498 0.1841469 0.1890485 0.1939515  
 0.1988526

**APPENDIX E5 (Infected WBC-gly)**

$$\text{Theta\_gly}=58:1:73; \text{st}=64$$

$$B=\cos(\%pi*\text{Theta\_gly}/180);x=0.25*\text{st}*(1+B)^2;$$

$$\text{Gamasv}=0.25*\text{st}*(1+B)^2$$

$$F=(\text{Gamasv}.^{0.5}-\text{st}.^{0.5})./(1-0.015*(\text{Gamasv}*\text{st}).^{0.5})$$

$$S=\text{Gamasv}-\text{st}-F$$

$$N=-12*\%pi*(1.6*10^{(-10)})^2*S$$

$$\text{Theta\_gly}=58:1:73; \text{st}=64$$

$$\text{-->}B=\cos(\%pi*\text{Theta\_gly}/180);x=0.25*\text{st}*(1+B)^2;$$

$$\text{-->}Gamasv=0.25*\text{st}*(1+B)^2$$

37.450447 36.725446 36. 35.274554 34.549547 33.825414 33.102585 32.381483 31.662528 30.946129 30.232693  
29.522616 28.816289 28.114095 27.416408 26.723594

$$\text{-->}F=(\text{Gamasv}.^{0.5}-\text{st}.^{0.5})./(1-0.015*(\text{Gamasv}*\text{st}).^{0.5})$$

- 7.0784963 - 7.1113551 - 7.1428571 - 7.1730711 - 7.2020616 - 7.2298895 - 7.2566122 - 7.2822835 - 7.3069546 - 7.3306735 - 7.3534854  
- 7.3754332 - 7.3965573 - 7.4168958 - 7.4364848 - 7.4553584

$$\text{-->}S=\text{Gamasv}-\text{st}-F$$

- 19.471056 - 20.163199 - 20.857143 - 21.552375 - 22.248392 - 22.944696 - 23.640803 - 24.336233 - 25.030518 - 25.723197 - 26.413822  
- 27.101951 - 27.787154 - 28.469009 - 29.147107 - 29.821048

$$\rightarrow N = -12 * \pi * (1.6 * 10^{-10})^2 * S$$

$$10^{-16} *$$

0.1879146 0.1945945 0.2012917 0.2080014 0.2147186 0.2214386 0.2281567 0.2348683 0.2415688 0.2482539 0.2549191  
0.2615602 0.2681731 0.2747536 0.2812979 0.2878021

### APPENDIX E6(Uninfected WBC-gly).

$$\text{Theta\_gly} = 45:1:61; \text{st} = 64$$

$$\rightarrow B = \cos(\pi * \text{Theta\_gly} / 180); x = 0.25 * \text{st} * (1 + B)^2;$$

$$\rightarrow \text{Gamasv} = 0.25 * \text{st} * (1 + B)^2$$

$$\text{Gamasv} =$$

46.627417 45.949872 45.265896 44.575952 43.880504 43.180018 42.474959 41.765792 41.052982 40.336992 39.618285  
38.89732 38.174556 37.450447 36.725446 36. 35.274554

$$\rightarrow F = (\text{Gamasv}^{0.5} - \text{st}^{0.5}) / (1 - 0.015 * (\text{Gamasv} * \text{st})^{0.5})$$

- 6.4875188 - 6.5466362 - 6.6029971 - 6.6567523 - 6.7080438 - 6.7570056 - 6.8037636 - 6.8484365 - 6.891136 - 6.9319669 - 6.9710282 -  
7.0084127 - 7.044208 - 7.0784963 - 7.1113551 - 7.1428571 - 7.1730711

$$\rightarrow S = \text{Gamasv} - \text{st} - F$$

- 10.885064 - 11.503492 - 12.131107 - 12.767296 - 13.411452 - 14.062976 - 14.721277 - 15.385771 - 16.055882 - 16.731041 - 17.410687  
- 18.094267 - 18.781236 - 19.471056 - 20.163199 - 20.857143 - 21.552375

$$\rightarrow N = -12 * \pi * (1.6 * 10^{-10})^2 * S$$

$$10^{-16} *$$

0.1050515 0.1110199 0.1170770 0.1232168 0.1294336 0.1357214 0.1420746 0.1484877 0.1549549 0.1614708 0.1680301  
0.1746273 0.1812572 0.1879146 0.1945945 0.2012917 0.2080014

## APPENDIX F

Test of reliability on WBC using Cronbach's alpha on HIV infected and uninfected blood using the probe liquids.

Samples:	Infected()	Uninfected( $\theta$ )	Average, $\theta$	Individual variance	Total variance
1	67.66	48.66	58.16	90.25	180.50
2	67.33	64.66	65.99	1.78	3.56
3	64.00	43.00	53.50	110.25	220.50
4	64.66	49.33	56.99	58.74	117.49
5	67.00	54.66	60.83	38.06	76.00
6	68.00	54.66	61.33	44.48	88.97
7	66.33	50.33	58.33	64.00	128.00
8	66.33	54.00	60.16	38.00	76.00
9	65.66	55.66	60.66	25.00	50.00
10	66.66	52.33	59.49	51.33	102.66
11	59.00	54.00	56.50	6.25	12.50
12	58.66	53.66	56.16	6.25	12.50
13	58.00	49.33	53.66	18.78	37.57
14	62.33	55.33	58.83	12.25	24.50
15	66.33	50.33	58.83	64.25	128.50
16	59.66	53.66	56.66	9.00	18.00
17	59.66	52.33	57.83	66.74	26.85
18	61.66	47.66	54.00	49.00	98.00
19	59.00	51.00	55.00	16.00	32.00
20	66.00	49.66			133.48
Total				783.838	1567.08

## APPENDIX G1

Table 4.2. Interfacial energies and T4 lymphocytes for infected blood.

CD4	Interfacial free energies of Infected Blood ( $\gamma_{sv}$ ) mJ/m <sup>2</sup>											
	Whole Blood			WBC			SERUM			RBC		
	Wat	Gly	Diiod	Wat	Gly	Diiod	Wat	Gly	Diiod	Wat	Gly	Diiod
438	28.84	34.55	42.03	25.07	37.45	25.70	33.58	36.73	32.02	26.55	38.17	30.87
278	36.02	36.00	39.61	26.55	36.73	25.70	33.58	37.45	28.58	36.83	39.62	34.27
282	30.40	37.45	34.27	28.07	31.66	34.27	28.84	39.62	27.42	33.58	36.73	30.87
682	25.51	36.73	43.80	37.65	30.95	26.85	31.19	32.38	28.58	33.58	29.20	30.87
606	29.62	36.00	43.37	32.78	30.23	26.85	32.78	34.55	32.02	38.48	36.00	33.15
20	25.07	40.60	40.60	21.52	36.73	28.58	31.19	36.00	35.38	28.07	36.00	25.13
613	35.20	33.83	38.59	31.98	30.23	28.58	40.95	28.82	35.93	35.20	30.95	31.45
468	28.07	31.66	42.94	28.07	26.72	34.27	40.95	38.90	34.83	40.95	41.05	34.27
853	36.83	33.83	37.01	29.62	29.52	32.02	41.78	40.34	34.27	31.98	37.45	34.27
356	25.07	33.83	48.15	28.84	32.38	28.58	32.78	37.45	25.70	34.39	34.55	28.58
268	34.39	39.62	44.22	37.65	35.27	33.15	36.83	43.18	34.27	34.39	39.62	31.45
625	32.78	41.05	41.09	28.84	36.00	39.10	33.58	33.83	30.30	40.95	36.00	30.87
230	31.19	40.34	39.10	33.58	33.83	38.59	32.78	33.83	26.70	34.39	31.66	25.70
246	32.78	39.62	41.09	32.78	36.00	30.30	43.42	43.18	30.87	37.30	35.27	28.58
339	35.20	41.77	42.49	31.98	32.38	26.85	36.02	37.45	34.27	39.30	38.17	31.45
316	25.55	33.10	43.37	36.02	33.83	34.27	40.95	36.73	29.73	40.95	38.17	34.27
220	30.40	36.00	44.63	30.40	36.00	36.47	36.83	40.34	39.10	38.48	36.00	31.45
374	34.39	34.55	34.27	33.58	31.66	34.27	49.12	43.88	35.38	36.83	34.55	28.58
593	23.62	36.73	36.47	36.83	36.00	33.15	40.95	37.45	38.59	37.65	36.00	34.27
372	28.84	38.17	38.07	26.55	32.38	31.45	32.78	33.83	37.54	31.19	30.23	29.73

**APPENDIX G2**

**Table 4.2. Interfacial energies and T4 lymphocytes for uninfected blood.**

*Interfacial free energies of uninfected Blood ( $\gamma_{SV}$ ) mJ/m<sup>2</sup>*

CD4	Whole Blood			WBC			SERUM			RBC		
	Wat	Gly	Diiod	Wat	Gly	Diiod	Wat	Gly	Diiod	Wat	Gly	Diiod
4500	47.51	45.27	44.22	41.78	46.63	38.59	45.88	46.63	34.27	37.85	48.62	39.61
6000	45.07	55.15	42.49	26.55	42.47	25.70	40.95	41.05	34.27	36.83	39.62	34.27
8000	40.95	45.27	42.94	49.12	45.27	43.37	49.12	49.90	37.01	32.78	43.88	35.38
4900	36.83	43.88	43.80	43.42	44.58	38.07	42.60	39.62	35.38	33.58	47.96	30.87
5000	41.78	39.62	43.37	38.48	44.58	32.59	42.60	43.18	40.60	38.48	37.45	27.42
4500	43.42	40.34	40.60	37.65	43.18	34.27	40.12	43.88	35.93	28.07	49.70	25.70
4000	42.60	45.95	42.03	40.95	44.58	38.07	49.12	43.18	37.01	40.95	45.27	38.07
6200	41.78	39.62	40.60	38.48	42.47	35.38	42.60	41.05	37.54	40.95	38.90	33.15
4900	49.12	49.90	40.11	42.60	39.62	32.02	38.48	43.18	33.15	38.48	43.88	39.61
4800	49.12	46.63	39.61	40.95	43.18	35.93	42.60	41.77	34.27	49.12	47.30	41.09
5000	45.07	44.58	41.56	37.65	43.18	35.38	42.60	42.47	38.59	30.40	45.95	40.60
4000	46.70	48.62	43.37	40.95	35.27	39.61	41.78	37.45	30.30	42.60	43.18	42.03
4000	48.32	49.90	41.56	38.48	43.18	42.03	43.42	41.05	34.27	33.58	39.62	38.59
4000	42.60	43.18	41.56	37.65	41.05	34.83	39.30	41.77	34.27	40.95	37.45	37.01
6000	40.95	43.18	39.61	45.07	44.58	35.38	42.60	44.58	33.71	49.12	45.27	40.11
4400	45.07	43.18	37.01	36.83	42.47	37.01	39.30	42.47	35.93	47.51	43.18	37.01
4700	46.70	49.90	40.60	42.60	41.77	35.93	45.88	40.34	39.61	45.07	41.05	42.49
4800	44.25	45.27	38.07	41.78	45.95	40.60	49.12	43.88	38.07	39.30	41.77	35.93
4300	45.07	43.18	36.47	42.60	43.18	37.01	49.12	44.58	38.59	45.88	48.62	39.61
6000	47.51	42.47	37.01	40.95	43.88	39.61	49.12	41.05	37.54	43.42	43.18	40.60

APPENDIX G3

Table 4.2: Average interfacial energies and T 4 lymphocytes.

CD4	$\gamma_{sv}$ (mJ/m <sup>2</sup> ) for infected blood				CD4	$\gamma_{sv}$ (mJ/m <sup>2</sup> ) for uninfected blood			
	Whole	WBC	SERUM	RBC		Whole	WBC	SERUM	RBC
438	35.14	29.40	34.11	31.86	4500	45.66	42.33	42.26	42.02
278	37.21	29.66	33.20	36.90	6000	47.57	31.57	38.75	36.90
282	34.04	29.86	31.96	33.72	8000	43.05	45.92	45.34	37.34
682	35.34	31.81	30.71	31.21	4900	41.50	42.02	39.20	37.47
606	36.33	29.95	33.11	35.87	5000	41.59	38.55	42.12	34.45
20	35.42	28.94	34.19	29.73	4500	41.45	38.36	39.97	34.49
613	35.87	30.26	35.23	32.53	4000	43.52	41.20	43.10	41.43
468	34.22	29.68	38.22	38.75	6200	40.66	38.77	40.39	37.66
853	35.89	30.38	38.79	34.56	4900	46.37	38.08	38.27	40.65
356	35.68	29.93	31.97	32.50	4800	45.12	40.02	39.54	45.83
268	39.41	35.35	38.09	35.15	5000	43.73	38.73	41.22	38.98
625	38.30	34.64	32.57	35.94	4000	46.23	38.61	36.51	42.60
230	36.87	35.33	31.10	30.58	4000	46.59	41.23	39.58	37.26
246	37.83	33.02	39.15	33.71	4000	42.44	37.84	38.44	38.47
339	39.82	30.40	35.91	36.30	6000	41.24	41.67	40.29	44.83
316	34.00	34.70	35.80	37.79	4400	41.75	38.77	39.23	42.56
220	37.01	34.29	38.75	35.31	4700	45.73	40.10	41.94	42.87
374	34.40	33.17	42.79	33.32	4800	42.53	42.77	43.69	39.00
593	32.27	35.32	38.99	35.97	4300	41.57	40.93	44.09	44.70
372	35.02	30.12	34.71	30.38	6000	42.33	41.48	42.57	42.40
AVE	36.00	31.81	35.47	34.10		43.53	39.94	40.82	40.09
SD	1.89	2.36	3.29	2.56		2.17	2.82	2.26	3.36

## APPENDIX H

Table 4.3: Variations of interfacial energies of adhesion with CD+ cell count.

CD4	$F^{adh}$ (mJ/m <sup>2</sup> ) for infected WBC				$F^{adh}$ (mJ/m <sup>2</sup> ) for uninfected WBC				
	WU	FOWK	NEU	G&G	CD4	WU	FOWK	NEU	G&G
438	-67.22	-62.15	-25.99	-39.86	4500	-56.47	-49.56	-13.16	-22.38
278	-67.11	-61.93	-25.69	-39.34	6000	-65.68	-60.16	-23.86	-36.90
282	-63.95	-59.52	-24.27	-37.10	8000	-54.30	-46.52	-8.86	-18.02
682	-66.51	-60.28	-23.09	-35.87	4900	-56.96	-49.96	-13.24	-22.69
606	-67.37	-61.81	-25.17	-38.55	5000	-59.88	-53.30	-16.80	-27.06
20	-66.69	-62.25	-26.64	-40.96	4500	-59.49	-53.26	-17.06	-27.32
613	-66.56	-43.17	-24.96	-38.17	4000	-57.19	-50.54	-14.27	-23.76
468	-65.67	-61.29	-25.87	-39.54	6200	-59.07	52.83	-16.63	-26.77
853	-65.45	-60.73	-25.05	-38.24	4900	-44.37	54.03	-16.83	-27.46
356	-66.38	-61.41	-25.42	-38.77	4800	-58.41	-51.76	-15.30	-25.15
268	-62.00	-56.14	-19.87	-31.12	5000	-58.99	-41.30	-16.76	-26.83
625	-60.45	-55.99	-21.25	-32.81	4000	-58.86	-52.81	-16.78	-27.09
230	-60.55	-55.58	-20.34	-31.56	4000	-56.18	-50.13	-14.59	-23.95
246	-63.80	-58.36	-22.31	-34.34	4000	-59.74	-53.66	-17.60	-27.95
339	-44.96	-61.28	-24.77	-37.59	6000	-57.98	-50.60	-17.60	-22.92
316	-62.10	-56.59	-20.64	-32.09	4400	-58.54	-52.60	-13.26	-26.87
220	-61.29	-56.54	-21.42	-33.01	4700	-58.58	-51.81	-16.80	-21.97
374	-63.08	-57.98	-22.25	-34.27	4800	-55.80	-49.01	-15.00	-21.41
593	-61.89	-56.12	-19.96	-31.18	4300	-57.79	-50.93	-12.81	-22.87
372	-65.35	-60.87	-25.40	-36.16	6000	-56.71	-50.15	-14.30	-23.32

## APPENDIX I

Table 4.4 Values of  $A_{132}$  for CD4 infected-CD4 uninfected-Serum interaction

CD4	$A_{132}$ (mJ/m <sup>2</sup> ) for infected WBC				$A_{132}$ (mJ/m <sup>2</sup> ) for uninfected WBC				
	WU	FOWK	NEU	G&G	CD4	WU	FOWK	NEU	G&G
438	6.50E-17	6.00E-17	2.51E-17	3.86E-17	4500	5.45E-17	4.78E-17	1.27E-17	1.98E-17
278	6.48E-17	5.98E-17	2.48E-17	3.80E-17	6000	6.35E-17	5.81E-17	2.31E-17	3.74E-17
282	6.14E-17	5.75E-17	2.35E-17	3.58E-17	8000	5.24E-17	4.49E-17	3.43E-17	1.33E-17
682	6.59E-17	5.82E-17	2.23E-17	3.46E-17	4900	5.50E-17	4.82E-17	1.28E-17	1.95E-17
606	6.55E-17	5.96E-17	2.43E-17	3.72E-17	5000	5.78E-17	5.14E-17	1.62E-17	2.58E-17
20	6.66E-17	6.01E-17	2.57E-17	3.95E-17	4500	5.74E-17	5.14E-17	1.65E-17	2.48E-17
613	6.42E-17	5.91E-17	2.41E-17	3.68E-17	4000	5.52E-17	4.88E-17	4.25E-17	2.05E-17
468	6.34E-17	5.92E-17	2.50E-17	3.82E-17	6200	5.70E-17	5.10E-17	1.61E-17	2.36E-17
853	6.32E-17	5.86E-17	2.42E-17	3.69E-17	4900	5.88E-17	5.21E-17	1.62E-17	2.45E-17
356	6.41E-17	5.93E-17	2.45E-17	3.74E-17	4800	5.64E-17	5.00E-17	1.48E-17	2.21E-17
268	6.15E-17	5.42E-17	1.92E-17	3.00E-17	5000	5.69E-17	5.10E-17	1.62E-17	2.40E-17
625	5.77E-17	5.40E-17	2.05E-17	3.17E-17	4000	5.68E-17	5.10E-17	1.62E-17	1.94E-17
230	5.88E-17	5.36E-17	1.96E-17	3.05E-17	4000	5.42E-17	4.84E-17	1.41E-17	1.88E-17
246	6.16E-17	5.63E-17	2.15E-17	3.31E-17	4000	5.77E-17	5.30E-17	1.69E-17	2.44E-17
339	6.45E-17	5.92E-17	2.39E-17	3.66E-17	6000	5.59E-17	5.20E-17	1.28E-17	2.08E-17
316	5.98E-17	5.46E-17	1.99E-17	3.10E-17	4400	5.65E-17	5.10E-17	1.62E-17	2.31E-17
220	5.91E-17	5.46E-17	2.07E-17	3.19E-17	4700	5.65E-17	5.30E-17	1.45E-17	2.14E-17
374	6.09E-17	5.60E-17	2.15E-17	3.31E-17	4800	5.38E-17	4.93E-17	1.24E-17	1.83E-17
593	5.97E-17	5.42E-17	1.93E-17	3.01E-17	4300	5.58E-17	5.06E-17	1.38E-17	2.06E-17
372	6.27E-17	5.88E-17	2.45E-17	3.74E-17	6000	5.48E-17	5.22E-17	1.35E-17	1.94E-17
AVE	6.24E-17	5.73E-17	2.27E-17	3.49E-17		5.63E-17	5.08E-17	1.76E-17	2.21E-17
SD	2.43E-18	2.37E-18	2.22E-18	3.19E-18		2.28E-18	3.63E-18	7.61E-18	4.65E-18

## APPENDIX J: SCILAB Program for 3-D plot for infected WBC

### APPENDIX J1

```
Theta_AVE=65:1:85; st=62.53
B=cos(%pi*Theta_AVE./180);x=0.25*st*(1+B)^2;
Gamasv=0.25*st*(1+B)^2;
F=(Gamasv.^0.5-st.^0.5)/(1-0.015*(Gamasv*st).^0.5);
S=Gamasv-st-F;
N=-12*%pi*(1.6*10^(-10))^2*S
[x,y]=meshgrid(x,Theta_AVE);
B2=cos(%pi*y./180);
//Gamasv3=0.25*st*(1+B2).^2;
F2=(x.^0.5-st.^0.5)/(1-0.015*(x.*st).^0.5);
z=x-st-F2;
mesh(x,y,z)
scf;
surf(x,y,z);
xtitle("Inf WBC_AVE", "Surface Energy(mJ/m^2)", "Contact Angle(degrees)", "Change in Free
Energy of Adh(mJ/m^2)")
```

### APPENDIX J2

```
Theta_AVE=65:1:85; st=62.53
B=cos(%pi*Theta_AVE./180);x=0.25*st*(1+B)^2;
Gamasv=0.25*st*(1+B)^2;
F=(Gamasv.^0.5-st.^0.5)/(1-0.015*(Gamasv*st).^0.5);
S=Gamasv-st-F;
N=-12*%pi*(1.6*10^(-10))^2*S
[x,y]=meshgrid(x,Theta_AVE);
B2=cos(%pi*y./180);
//Gamasv3=0.25*st*(1+B2).^2;
```

```

F2=(x.^0.5-st^0.5)./(1-0.015*(x.*st).^0.5);
z=-12*%pi*(1.6*10^(-10))^2*x
mesh(x,y,z)
scf;
surf(x,y,z);
xtitle("Inf WBC_AVE", "Surface Energy(mJ/m^2)", "Contact Angle(degrees)", "Hamaker
coefficient(mJ/m^2)")

```

#### APPENDIX J3

```

Theta_AVE=65:1:85; st=62.53
B=cos(%pi*Theta_AVE./180);x=0.25*st*(1+B)^2;
Gamav=0.25*st*(1+B)^2;
F=(Gamav.^0.5-st.^0.5)./(1-0.015*(Gamav*st).^0.5);
S=Gamav-st-F;
N=-12*%pi*(1.6*10^(-10))^2*S
[x,y]=meshgrid(x,S);
B2=cos(%pi*y./180);
//Gamav3=0.25*st*(1+B2).^2;
F2=(x.^0.5-st.^0.5)./(1-0.015*(x.*st).^0.5);
z=-12*%pi*(1.6*10^(-10))^2*x
mesh(x,y,z)
scf;
surf(x,y,z);
xtitle("Inf WBC_AVE", "Surface Energy(mJ/m^2)", "Change in free energy of
Adhesion(mJ/m^2)", "Hamaker coefficient(mJ/m^2)")

```

#### APPENDIX J4: SCILAB Program for 3-D plot for uninfected WBC

```

Theta_AVE=43:1:65; st=62.53
B=cos(%pi*Theta_AVE./180);x=0.25*st*(1+B)^2;
Gamav=0.25*st*(1+B)^2;
F=(Gamav.^0.5-st.^0.5)./(1-0.015*(Gamav*st).^0.5);

```

```

S=Gamasv-st-F;
N=-12*%pi*(1.6*10^(-10))^2*S
[x,y]=meshgrid(x,Theta_AVE);
B2=cos(%pi*y./180);
//Gamasv3=0.25*st*(1+B2).^2;
F2=(x.^0.5-st^0.5)./(1-0.015*(x.*st).^0.5);
z=x-st-F2;
mesh(x,y,z)
scf;
surf(x,y,z);
xtitle("Uninf WBC_AVE","Surface Energy(mJ/m^2)","Contact Angle(degrees)","Change in free
Energy of adhesion(mJ/m^2)")

```

#### APPENDIX J5

```

Theta_AVE=43:1:65; st=62.53
B=cos(%pi*Theta_AVE./180);x=0.25*st*(1+B)^2;
Gamasv=0.25*st*(1+B)^2;
F=(Gamasv.^0.5-st.^0.5)./(1-0.015*(Gamasv*st).^0.5);
S=Gamasv-st-F;
N=-12*%pi*(1.6*10^(-10))^2*S
[x,y]=meshgrid(x,S);
B2=cos(%pi*y./180);
//Gamasv3=0.25*st*(1+B2).^2;
F2=(x.^0.5-st^0.5)./(1-0.015*(x.*st).^0.5);
z=-12*%pi*(1.6*10^(-10))^2*x
mesh(x,y,z)
scf;
surf(x,y,z);
xtitle("UNinf WBC_AVE","Surface Energy(mJ/m^2)","Contact Angle(degrees)","Hamaker
coefficient(mJ/m^2)")

```

APPENDIX J6

$\theta_{AVE}=43:1:65$ ;  $st=62.53$

$B=\cos(\pi*\theta_{AVE}/180)$ ;  $x=0.25*st*(1+B)^2$ ;

$G_{masv}=0.25*st*(1+B)^2$ ;

$F=(G_{masv}^{0.5}-st^{0.5})/(1-0.015*(G_{masv}*st)^{0.5})$ ;

$S=G_{masv}-st-F$ ;

$N=-12*\pi*(1.6*10^{-10})^2*S$

$[x,y]=\text{meshgrid}(x,S)$ ;

$B2=\cos(\pi*y/180)$ ;

$G_{masv3}=0.25*st*(1+B2)^2$ ;

$F2=(x^{0.5}-st^{0.5})/(1-0.015*(x.*st)^{0.5})$ ;

$z=-12*\pi*(1.6*10^{-10})^2*x$

$\text{mesh}(x,y,z)$

$\text{scf}$ ;

$\text{surf}(x,y,z)$ ;

$\text{xtitle}(\text{"UNinf WBC\_AVE"}, \text{"Surface Energy(mJ/m}^2\text{)"}, \text{"Change in free energy of Adhesion(mJ/m}^2\text{)"}, \text{"Hamaker coefficient(mJ/m}^2\text{)"})$

**APPENDIX K: SCILAB Program for the estimation of intermolecular parameter.**

**APPENDIX K1: Model using water.**

Theta AVE wt=58:1:80; st=72.8; se=28:1:50;

-->B=cos(%pi\*Theta\_AVEwt./180);

-->C=(st\*(1+B))./((st\*se)^0.5\*2)

C=

1.0850423 1.0744883 1.0638231 1.0530498 1.0421719 1.0311925 1.020115 1.0089428 0.9976793 0.9863279  
0.9748922 0.9633755 0.9517814 0.9401133 0.9283750 0.9165699 0.9047017 0.8927739 0.8807901 0.8687542  
0.8566696 0.8445401 0.8323694

**APPENDIX K2: Model using glycerine.**

-->Theta\_AVEgly=58:1:80; st=64; se=28:1:50;

-->B=cos(%pi\*Theta\_AVEgly./180);

-->C=(st\*(1+B))./((st\*se)^0.5\*2)

C =

1.1565103 1.125342 1.0954451 1.0667187 1.0390733 1.012429 0.9867145 0.9618655 0.9378244 0.9145392  
0.8919625 0.870051 0.8487681 0.8280759 0.8079428 0.7883393 0.7692383 0.7506147 0.7324456 0.7147099  
0.6973881 0.6804623 0.6639157

**APPENDIX K3: Model using diodomethane**

-->Theta\_AVEdio=58:1:80; st=50.8;se=28:1:50;

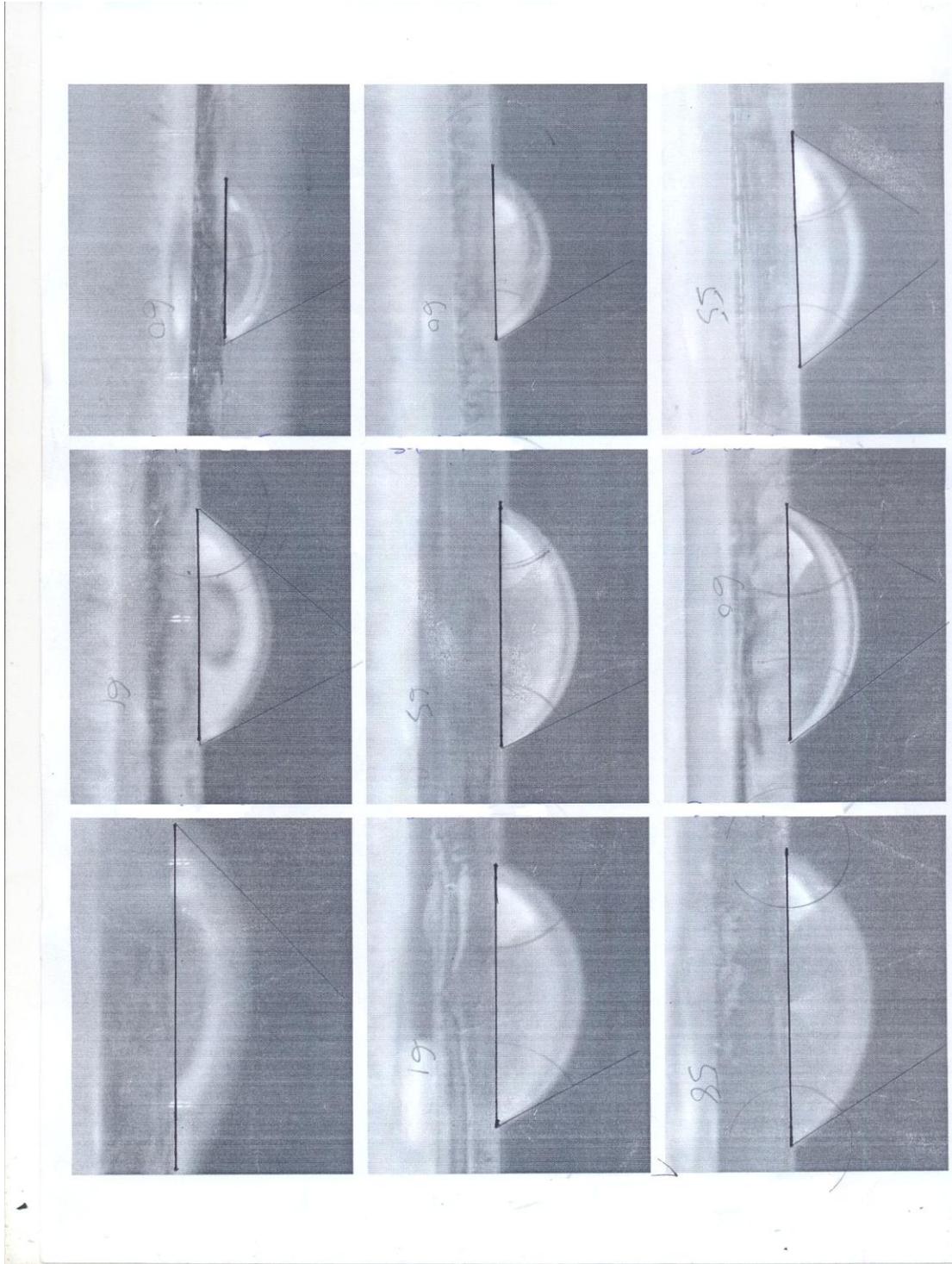
-->B=cos(%pi\*Theta\_AVEdio./180);

**-->C=(st\*(1+B))./((st\*se)^0.5\*2)**

C =

1.0303656	1.002597	0.9759611	0.9503680	0.9257379	0.9019998	0.8790901	0.8569514	0.8355326	0.8147872
0.7946730	0.7751522	0.7561899	0.7377547	0.7198177	0.7023524	0.6853347	0.6687425	0.6525552	0.6367540
0.6213216	0.6062419	0.5915001							

*Contact Angle measurement on infected blood using glycerine*



*Contact Angle measurement on infected blood using diiodomethane*

