JEAS

JOURNAL OF ENGINEERING AND APPLIED SCIENCES

Journal of Engineering and Applied Sciences, Volume 17, Number 1, June 2020, 123-129

Sessile Drop Approach to Surface Energy Determination of Hepatitis C Virus Infected Blood Cells

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Abstract
This study employed the contact angle approach to measure the angle formed using sessile drop techniques with glycerin as probe liquid on HCV infected and uninfected blood samples. The research also is embodied with the determination of CD4 counts on both the infected and uninfected samples of the blood and the surface free energy of the blood samples which served as a useful determinant in the prediction of the activities of the virus on the blood samples. A total of twenty blood samples were used for the study. Smearing was done on the slides at room temperature and allowed to dry, glycerin was dropped on the surface of the smeared slide while the spreading process is captured. The average contact angle obtained for infected white blood cells was 63.4±3.20 which was observed to be the highest while that for uninfected white blood cells was 48.5±2.75 and are in agreement with literature results. The contact angle data was used for MATLAB computation to obtain the surface free energy. The interfacial surface energy for uninfected blood derived as 44.35±1.90mJ/m^2 was reduced to 33.54±2.31mJ/m^2 for the infected blood due to interaction effects of the virus. The findings of this research have provided a clue for the pharmaceutical industries towards gaining an insight to the interaction mechanism of hepatitis c virus to enable the design of drugs for the treatment of the virus.

Keywords: Blood Samples, Glycerin, Surface Free Energy, Contact Angle, Hepatitis C

1. Introduction

Pathological features of diseases vary in their nature and magnitude. Despite this diversity, the common feature of various disorders underlies the physiochemical and biochemical factors such as surface tension. Changes in the surface tension behavior of human biological fluid are characteristic for some diseases. Studying these interfaces and the changes that occur will provide valuable information relating to various diseases and help to monitor the treatment efficacy. Biological tissues are viscoelastic materials and the cells in a tissue behave very much like molecules in a fluid. This property enables them to change their position and move against each other. The morphology and shape of the organism are driven by the events that occur at the cellular level. The shape of a cell is the result of a balance of intracellular and extrinsic forces exerted on it. This behavior is defined through surface tension which tends to minimize the exposed area of the cell aggregate and maximize the cohesive forces. The intracellular forces on the membrane are a result of the cytoskeleton reorganization. Energy at the cellular level is usually measured through physical properties such as cell adhesion, viscosity, and cortical tension. Several approaches including adhesion experiments, freezing point and, contact angle approaches have been used to measure blood cells and protein surface tension. However, all the approaches used agree with the equation of state approach, thus establishing the impact of surface properties in biological systems. Conclusions arrived at by these previous works of Neumann et al (1983) can be summarily outlined as follows;

- Interfacial tension found practical applications in the closing and opening of vessels in circulation of blood, antigens-antibodies interactions and cells adhesion and protein absorption.
- These biomaterials are hydrophilic and posses relatively a high surface tension.
- Several approaches can be used to achieve their measurements.

Other works have also been done on surface tension relating to quantities like thrombocytes with regards to clotting time and platelets adsorption and the values obtained as conventional surface tension of polymers are in accordance with that obtained from the equation of state.

Several thermodynamic approaches have evolved over the years for determining the values of surface energy components of solids; it is applicable to both polar and non polar systems. (van-Oss, 1994). Though defined as work required to build a unit area, when it comes to its measurement using sessile drop technique, the surface energy is not quite as well defined. The values of contact angle obtained through the sessile drop techniques depends not only on the solid samples in use but equally on the properties of the probe liquids being used as well as the particular theory relating mathematically to one another.

Researchers have overtime developed such numerous theories which differ from each others in terms of derivation and conventions, but most importantly they differ in the number of components which they are equipped to analyze. The simpler methods containing fewer components simplify the system by lumping surface energy into one another while the more complex methods having more components are derived to distinguish between various components of the surface energy. (Oura et al, 2001).

The total surface energy of solids and liquids depends on different types of molecular interactions such as dispersion (van der Waals), polar and acid /base interactions and is taken to be the sum of these independent components. Some theories account for more of these phenomena than the others. Surface energy of solids is dependent on the workdone on the surface area of the solid against elastic forces and plastic resistance of the solids. The difficulty of determining surface free energy of solids directly from contact angle leads to the simplification of young's equation by combing the work of adhesion from Dupre equation at the liquid interface with the surface and interfacial tensions of solid-vapour, liquid-vapour and solid-liquid interfaces (Ozoihu, 2014)

The atoms at the surface of the condensed phase materials are in different environment compared to those from its interior, this difference arises from the asymmetric environment. In a bulk material, each atom is surrounded by similar ones and they experience no net force. In addition, environmental influence acts only on the outermost atoms (van-Oss and Giese, 2002). These atoms consequently have a different energy distribution from the inside and are at a higher state at the surface. This excess energy can be measured. The differences in the energy of the atoms or molecules located at the surface and in the bulk of the material manifest themselves as surface energy/surface tension γ. For a non-metallic material, the surface free energy has an apolar component $\gamma^{lw}$and may also have a polar component γ^{ab}. Qualitatively, surface tension acts on any surface trying to minimize the surface area.

1.1 Sessile Drop Techniques

Several techniques are available for the measurement of contact angle such as wilhelmy balance method, capillary rise method, dunoy ring method and sessile drop method but the use of sessile drop method as a means of characterizing surfaces is increasingly on the lead. The advantages of this method are easy handling and rapid data collection, high reproducibility through automatic dosing and positioning of test liquids and user-independent measurement through software controlled contact angle determination. It can be stated here that contact angle are not limited to liquid vapor interface on solids, they are also applicable to liquid-liquid interface on a solid.

The sessile drop technique is a method used for the characterization of surface energies of both solids and liquids. Parameters like contact angle, known surface energy of the liquid can be used to calculate the surface energies of the solid and the liquids used for such experiments are called the probe liquids. The measured angle θ is the contact angle as shown in figure1.

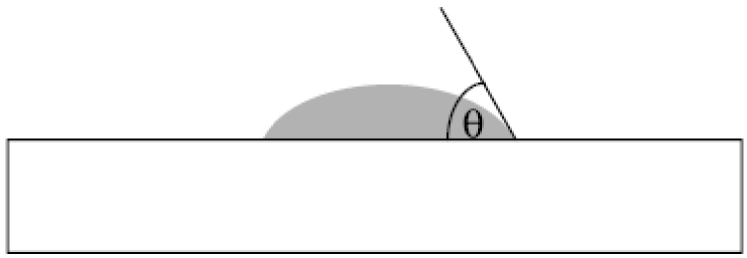

Figure 1: Contact angle diagram

The surface tension of liquids is measured in Newton per meter (Nm^{-1}) and can be obtained through various methods. The interfacial tension can be viewed as being products of different intermolecular forces. The values obtained through sessile drop depend not only on the solid samples in contact but also as a result of the properties of the probe liquids used.

The Zisman plot used for the determination of surface energy of the liquid is limited to single parameter rather than accounting for the fact polar interactions may be encountered which greatly may alter the calculations

1.2 Sessile Drop Measurement of Surface Free Energy of Bacterial Cell Surfaces

Ozoihu (2014) in his work reviewed the experimental determination of contact angle by Hendrik and his team using sessile drop techniques on bacteria layers deposited on cellulose triacetate filters which were completely smeared with bacteria. Water, water-n-propanol mixtures and $\alpha -$ bromonaphthalene were the probe liquids used and calculation of surface energy of the various bacteria was done. Methods of calculation yieldingγ_s^d, γ_s^p together with spreading pressure πe and γ_{sv} separately were employed where d and p represents dispersion and polar component of the fluid. He determined the surface energy of human HIV infected cells reported that the average surface energy of HIV infected white blood cell is 31.81±2.36mJ/$m^2$and that of the uninfected is recorded to be 39.94±2.82mJ/m^2.

1.3 Glycerol as Probe Liquid

Glycerol is completely soluble in water and alcohol. It is slightly soluble in ether, ethyl acetate, and dioxane and insoluble in hydrocarbons. Glycerol has useful solvent properties similar to those of water and simple aliphatic alcohols because of its three-hydroxyl groups. Glycerol is a useful solvent for many solids, both organic and inorganic which is particularly important for the preparation of pharmaceuticals. The solubility of gases in glycerol, like other liquids is temperature and pressure dependent.

2.0 Materials and Methods

Thermodynamically, surface tension γ is interpreted as the increase in Gibbs free energy of a system when the area of the interface under consideration is increased reversibly by an infinitesimal amount at a constant temperature (t), pressure (p) and composition (n).

$$\gamma = \left(\frac{\delta G}{\delta A}\right)_{t,p,n} \quad (1)$$

The total surface energy of solids and liquids depends on different types of molecular interactions such as dispersion (van-der Waals), polar and acid /base interactions and is taken to be the sum of these independent components. Good and Girifalco (1960) expressed the work of adhesion as a geometric mean of the surface tension of pure components;

$$w_{sl} = 2\phi\,(\gamma_{sv,}\gamma_{lv})^{1/2} \quad (2)$$

ϕ is a correction factor for intermolecular interactions, which depends on the chemical properties of the solid and liquid.

ϕ = 1 for similar forces, ϕ < 1 for dissimilar forces of adhesion and cohesion.

Combining the Young-Dupre and Good and Girifalco equations yields;

$$\gamma_{lv}\ (1 + \cos\theta) = (\gamma_{sv}, \gamma_{lv})^{1/2} \quad (3)$$

γ_{sv} can be found once contact angle and the surface tension of the liquid γ_{lv} is known from;

$$\mathrm{Cos}\theta = \frac{(0.015\gamma_{sv} - 2.00)(\gamma_{sv}\gamma_{lv})^{1/2} + \gamma_{lv}}{\gamma_{lv}0.0015\,(\gamma_{sv}\gamma_{lv})^{1/2} - 1} \quad (4)$$

Having obtained γ_{sv} from equation (1.4), the corresponding value of γ_{sl} can be calculated from;

$$\gamma_{sv} - \gamma_{sl} = \gamma_{lv}\mathrm{Cos}\theta \quad (5)$$

2.1 Methods

The materials used for this study are as follows; hepatitis C positive and negative blood samples, glass funnels, gloves, 150mm spreader, slide racks, disposable syringes and needles, microlitre pipette, HCV test kits, HIV test kits, glycerin solution, test tubes, test tube racks, prepared slides and 5.0μl microlitre syringe.

Standard and universal precautions followed in this research were in accordance with the blood-borne pathogen standards enacted by the Centre for Disease Control (CDC) and also the NCCLS standards were equally observed in the collection, transportation, preparation, and safe storage of the blood specimens used for the experiment. Personal protective equipment (PPE) was equally used in the course of the experiment.

The blood used for this study includes ten samples of hepatitis C infected blood and ten samples of uninfected blood. The infected samples were collected from Mount Horeb Clinic and Dialysis Centre, Warri and Anambra State University Teaching Hospital, Amaku. The blood component separation, inoculation and smearing of the slides were carried out at the laboratory unit of the Anambra State University Teaching Hospital, Amaku.

2.2 Determination of CD4 Cell Count

The CD4 cells count on the whole blood samples was obtained using a Partec Flow Cytometry instrument. This helped to determine the level and progression of the HCV depletion of the immune system of the patients. The machine displays automatically the number of CD4+T cells.

2.3 Smearing of Slides with Blood Samples

The ten uninfected blood samples kept below room temperature in an EDTA container were separated into their components using a centrifuge machine. This separated the blood samples in the order of serum, white blood cell and red blood cell at the bottom. A pipette was used to draw 1000μl of each of the components from their boundary layer to smear the slides respectively using a spreader bent at 45^0 to achieve an even distribution on the slides. The blood samples in the container were placed in a blood roll mixer to unify the components and another 1000μl whole blood withdrawn to smear the slide. A total of four slides were obtained from each sample and the slides were allowed to dry naturally at room temperature because exposing the slides to the sun is likely to cause oxidation and the surface energy might be increased unconditionally. In a similar manner 100μl each of infected blood serum, white blood cell and red blood cells were smeared on their respective slides using the method of smearing explained earlier. The separated blood samples were then placed in the blood roll mixer and all the separated components unified so as to get the whole blood also smeared.

2.4 Contact Angle Experiment and Measurement

The glycerin used as probe liquid for the study was dropped on the surface of the prepared slides using a microliter syringe. Contact was not made between the syringe and the test surface and the droplet volume was small enough to avoid impact effect on the surface and gravity effect negligible. The process of spreading was captured with a high definition Nikkon digital camera and the images were eventually printed on paper. The contact angles were measured carefully at the solid-vapour, solid-liquid and liquid-vapour interface.

3.0 Results and Discussion

Table1 depicts the CD4+ results obtained from the PartecCytoflow Counter Machine for both the infected and uninfected samples. In general, infected samples have low CD4 counts while the uninfected usually have high CD4 cell counts.

Table1: CD4 Counts of Infected and Uninfected Blood Samples

Blood samples (B)	Infected (counts/mm^3)	Uninfected(counts/mm^3)
B1	428	660
B2	600	872
B3	625	1780
B4	312	1450
B5	464	1500
B6	247	930
B7	852	1360
B8	115	1520
B9	704	1580
B10	798	1020
Average	**514.5**	**1267.2**
SD	**243.1059**	**368.2731**

CD4+ cell counts indicate the severity of the hepatitis c virus infection. The highest CD4 count was seen on the uninfected samples while the lowest count was noticed on the infected samples. On the average, infected samples have lower CD4 count which signifies the impact of the hepatitis C virus in the depletion of the immune system on the infected patients.The regression trends analysis cannot be used here because the infected samples do not fit into the linear, exponential and the polynomial trends because its value cannot be approximated to 1. This is as a result of the depleted immune system of the infected patients offering different levels of resistance to the virus and also a difference in the HCV genotypes. It could also be seen that a scenario arises where an uninfected sample may have low value of CD4 count this means that CD4 counts can also be lowered by other forms of infection besides HCV.

The results of contact angle measured can be seen in table 2. The infected and uninfected samples of the blood were separated according to their respective blood components and their results tabulated.

Table 2: Contact Angles of Blood Components

	Infected Samples(Ө)				**Uninfected Samples(Ө)**			
Blood Samples	**Whole Blood**	**WBC**	**RBC**	**Serum**	**Whole Blood**	**WBC**	**RBC**	**Serum**
1	60	65	59	60	50	47	48	58
2	55	61	64	62	51	46	57	55
3	58	63	58	63	40	48	45	45
4	60	64	57	61	50	52	50	56
5	60	67	56	59	45	50	52	53
6	56	66	62	64	56	51	51	57
7	55	58	63	58	55	45	49	54
8	58	68	61	65	50	49	54	52
9	54	62	60	66	43	44	47	50
10	55	60	64	57	55	51	53	51
Average	**57.1**	**63.4**	**60.4**	**61.5**	**49.5**	**48.5**	**50.6**	**53.1**
SD	**2.3781**	**3.2045**	**2.8752**	**3.0277**	**5.3593**	**2.7508**	**3.5653**	**3.8427**

Table 2 summarizes the impact of the hepatitis c virus infection and the various drug treatments on the blood components using the average contact angle and standard deviation. It can be observed on critical examination of table 2 that infected samples have higher contact angles measured on the different components of the blood when compared to the angles measured on the uninfected and the treated samples. From the foregoing, it can be deduced that the hepatitis c virus has the ability of increasing the contact angle of infected surfaces. Hence, infected surfaces are poorly wetted, leading to an increase in contact angle of such surfaces. Among the infected blood components, the white blood cell has the highest contact angle as evident in Fig 3.1 with a large gap existing between the infected and the uninfected surfaces.This leads to a logical conclusion that the white blood cells are the principal target of the virus with sole action of depleting the lymphocytes.

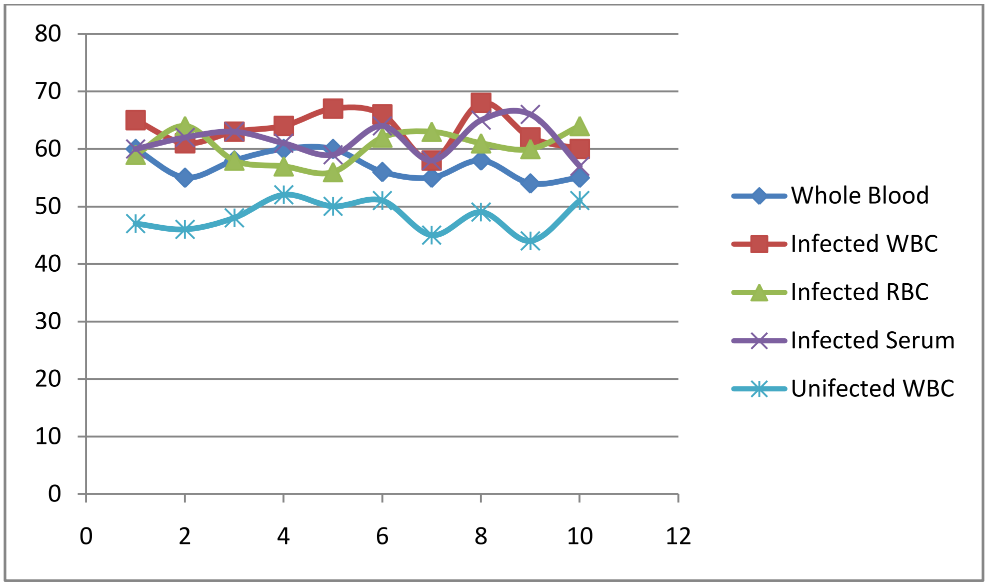

Figure2: Plot of Contact Angle versus Blood Components

The measured contact angle data from experimental set up was used to compute for the surface free energy (γ_{sv}) using Matlab computational tool. Neumman model for equation of state was used in the computation for the determination of the surface free energy.

Table 3: Computed Surface Energies of Blood Cells

	Infected Samples(γsv)in mJ/m^2				Uninfected Samples(γsv)in mJ/m^2			
Blood Samples	**Whole Blood(γsv)**	**WBC (γsv)**	**RBC (γsv)**	**Serum (γsv)**	**Whole Blood(γsv)**	**WBC (γsv)**	**RBC (γsv)**	**Serum (γsv)**
1	36.00	32.38	36.73	36.00	43.18	45.27	44.58	37.45
2	39.62	35.27	33.10	34.55	42.48	45.95	38.17	39.62
3	37.45	33.83	37.45	33.83	49.90	44.58	46.63	46.63
4	36.00	33.10	38.17	35.27	43.18	41.77	43.18	38.90
5	36.00	30.95	38.90	36.73	46.63	43.18	41.77	41.05
6	38.90	31.66	34.55	33.10	38.90	42.48	42.48	38.17
7	39.62	37.45	33.83	37.45	39.62	46.63	43.88	39.62
8	37.45	30.23	35.27	32.38	43.18	43.88	40.34	41.77
9	40.34	34.55	36.00	31.66	47.96	47.30	45.27	43.18
10	39.62	36.00	33.10	38.17	39.62	42.48	41.05	38.90
Average	**38.10**	**33.54**	**35.71**	**34.92**	**43.47**	**44.35**	**42.735**	**40.53**
SD	**1.721233**	**2.31359**	**2.0837**	**2.1920**	**3.70064**	**1.9032**	**2.5148**	**2.7500**

Table3 shows that the surface free energy of the uninfected blood component is higher than that of the infected component which is an indicative of the fact that the presence of hepatitis c virus infection on the samples has a reduction effect on interfacial surface energy. The virus can be concluded to have the capability of reducing the energy at the surface of the blood hence infected blood components have low surface free energy and as such can be described as a polar surfaces characterized by poor wetting abilities.

The reduction of the interfacial energy in whole blood is 12.35%, white blood cell is 24.37%, red blood cell is 16% and serum is reduced by 14%. From the percentage analysis above, it can be deduced that the onus of the infection is on the white blood cell component since it has the highest percentage reduction (24.37%) with its surface energy degraded from 44.35mJ/m^2 to 33.54mJ/m^2. HCV also attack other blood components hence a reduction in their respective surface energy as a result of the viral particles interfering with red blood cells and serum.

4.0. Conclusion

The findings of this study have in no doubt unveiled the result of hepatitis c virus interaction with blood cells. The presence of the virus in the blood component causes the surfaces to be poorly wetted and hence leads to an increase in the contact angle of the surfaces and a reduction in the surface energy. Any drug administered to patients which has the potency of increasing the surface energy of the infected blood components is likely to increase the CD4 counts and boost the immune system and as such virological clearance may be achieved.

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