

Full Length Research Paper

A new measurement method of separation percentage for human blood plasma based on ultrasound attenuation

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In this research, the separation accuracy of blood-plasma-based multi-decay pulse-power technique (MDPPT) was presented. The MDPPT provides adjustable-path measurements according to the amplitude of transmitted pulses and accurate attenuation measurements of propagation echoes through blood and blood plasma; thus, reduction of time and power consumption during the plasma separation process was achieved using the centrifuge technique. A mathematical model of separation accuracy and attenuation was derived and it was used for concluding the blood plasma concentration based on attenuation. Multi levels of concentration yield 20, 35, 50, 80 and 93% of blood plasma separation percentage; whereas attenuation that is more than 80% of the separation percentage may prove useful for purity inspection and contaminant detection. The platform of separation process was built with multi-level pulse power controlled by the fuzzy logic approach and broadband transducers affixed on the outside of a low-reflection coefficient thin vessel. The signal in the receive transducer permits the measurement of the attenuation and the velocity by measuring the time of flight. The fast Fourier transform (FFT) of the appropriate plasma signal for each echo was obtained and was compared with that of blood to yield the attenuation as a function of separation percentage. The data show the feasibility for measuring a plasma separation of 97%. Therefore, such measurements can be proven useful for detecting contaminants in blood plasma and blood serum. The blood plasma separation accuracy measurements prove advantageous for saving time and power consumption of the separation process required.

Key words: Attenuation measurements, blood plasma percentage, centrifuge device separation accuracy, contaminant detection.

INTRODUCTION

The exponential decay echoes method is a well-known attenuation measurement method that has long been used (Papadakis, 1990). However, a multi-level of echoes power was achieved by a stable and programmable pulser device or using a high-reflection coefficient

container, such as stain steel (Bamberger and Greenwood, 2004).

This article used the direct contact method for blood and blood plasma attenuation measurements. However, for use in low-attenuation liquids or solution, the decay pulses power transmitter technique offers the significant advantage of high accuracy detection required in a vessel filled with blood. For very low concentrations and accurate measurement of plasma separation, the use of MDPPT echoes yield an excepted propagation path

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length for measurements of the percentage weight of blood cells suspended in plasma. This technique will be simulated by using multi-level power decay signals.

In this article, the accuracy of plasma separation measurements using the proposed ultrasound attenuation measurements was presented and a mathematical model was derived based on a primarily experimental setup. The attenuation and the multi-level power of ultrasonic signals and echo amplitude with the concentration of blood plasma was shown. Thus, for new blood sample tests, measuring the echo amplitude signals will estimate the concentration of separated plasma in the sample with high accuracy, contrary to the traditional method of centrifugation, which does not provide accurate and high separation measurements of blood plasma.

This technique can be used for the reduction of time and power consumption during the plasma separation process based on feedback controlling for the centrifuge device. The format of the article is as follows. First, the composition of blood and the various attenuation mechanisms were reviewed. The measurement system and experimental procedures were then described, and the measurement results were presented and discussed. These were compared with the experimental data previously reported in the literature. Summary and discussion are then given.

Blood composition

Human blood consists of blood cells (approximately 45% in volume) suspended in plasma under normal physiological conditions. The blood cells were largely made up of red blood cells (erythrocytes), which are the major contents of blood with small proportions of platelets (thrombocytes) and white blood cells (leukocytes). Published normal ranges for cell counts vary slightly with patient population and method of analysis. Normal ranges in a Caucasian population demonstrate a red cell count of 5.66×10^{12} cells/L in men and 4.99×10^{12} cells/L in women, a leukocyte count of 9.5×10^9 cells/L in men and 11.1×10^9 cells/L in women (Bain, 2006), and a platelet count of 396×10^9 cells/L (Giacomini et al., 2001). The proportion of the total volume of the whole blood that red blood cells occupy (commonly called the *packed cell volume* or *hematocrit*) is usually in the range 39 to 51% for men and 36 to 48% for women (Fairbanks and Tefferi, 2000, 2001). The red blood cells themselves are densely packed with the oxygen-carrying protein hemoglobin, with the normal range for the total hemoglobin concentration in the whole blood being 13.3 to 16.7 g/dl for men and 11.8 to 14.8 g/dl for women (Bain, 2006). If the blood cells are removed from the whole blood by centrifugation, then the remaining fluid (plasma) is made up of 91% water by weight with three major protein types: Albumin (4 g/dl), globulin (2.7 g/dl) and fibrinogen (0.3 g/dl) (Schneck, 2003). The remainder of the plasma consists

of other minerals, trace elements and lipids.

Ultrasound absorption mechanisms in blood

Historically, the attenuation coefficient in blood is commonly cited as varying with $0.14f^{1.21}$ (these parameters appear in several reference texts). However, analogous to earlier discussion, such equations only have a limited range of validity due to the varying frequency dependence of the different attenuation mechanisms (Bradley et al., 2011). Several mechanism factors contribute toward generating acoustic attenuation in whole blood. These are present on molecular as well as cellular levels within the intracellular and extracellular fluids themselves. The absorption mechanisms of the molecular level include viscosity and thermal conduction along with additional structural and thermal relaxation processes (Bamber, 2004). Absorption mechanisms of the cellular level include relative motion of viscous and thermal conduction due to the inhomogeneous regions of acoustic and thermal properties, in addition to scattering.

EXPERIMENTAL SETUP

The experimental set-up tools as schematically shown in Figure 1 consist of a Plexiglas container; programmable pulser, receiver and PC interface. Two transducers having center frequencies of 1, 2, 3 and 4 MHz are affixed to the outside of the container on opposite sides. In the attenuation measurements reported here, data were obtained only for the 2 MHz transducers, because the 2 MHz gives detection depth and sufficient clear of separation process of blood plasma in our experience. The walls of the vessel have a thickness (w) of 3.2 mm and the inside walls are separated by a distance D of 4 cm. This vessel has been used to measure density of a liquid, velocity of sound and the attenuation.

The programmable pulser-receiver has a maximum unipolar square wave voltage of 300 V and a maximum gain of 80 dB. For the 2 MHz transducers, the square wave signal to the send transducer has a width of 53 ns. A computer controlled pulser through exponential decay power degree, number of transmitted pulses and operation mode selection (pulse-echo mode or the pitch-catch mode). Besides the dependence of ultrasound attenuation on the concentration of red blood cells and blood proteins, the experimental results have also shown dependencies on the measurement temperature and the level of haemoglobin oxygenation. For example, the attenuation coefficient at 10 MHz in solutions of human haemoglobin at 25 and 15°C when compared with 35°C increased by 7 and 18%, respectively. Similarly, a 4% increase in the attenuation coefficient at 10 MHz in deoxygenated haemoglobin solutions when compared with oxygenated solutions (Bradley et al., 2011), therefore the temperatures measured by two thermocouples on the pipeline sensor, are recorded. When the vessel was filled with blood, then 11.1% of the ultrasound reflected at the Plexiglas-blood interface, and the rest is propagated through the blood. At the opposite wall, 88.8% was transmitted into Plexiglas. The use of the Plexiglas container produces advantages that decreases the echo reflected from the container wall and in our procedure, these reflected echoes were considered noisy to the received interest echo. Since the interest echo has high power, there is no need to use high amplification gain in the receiver device.

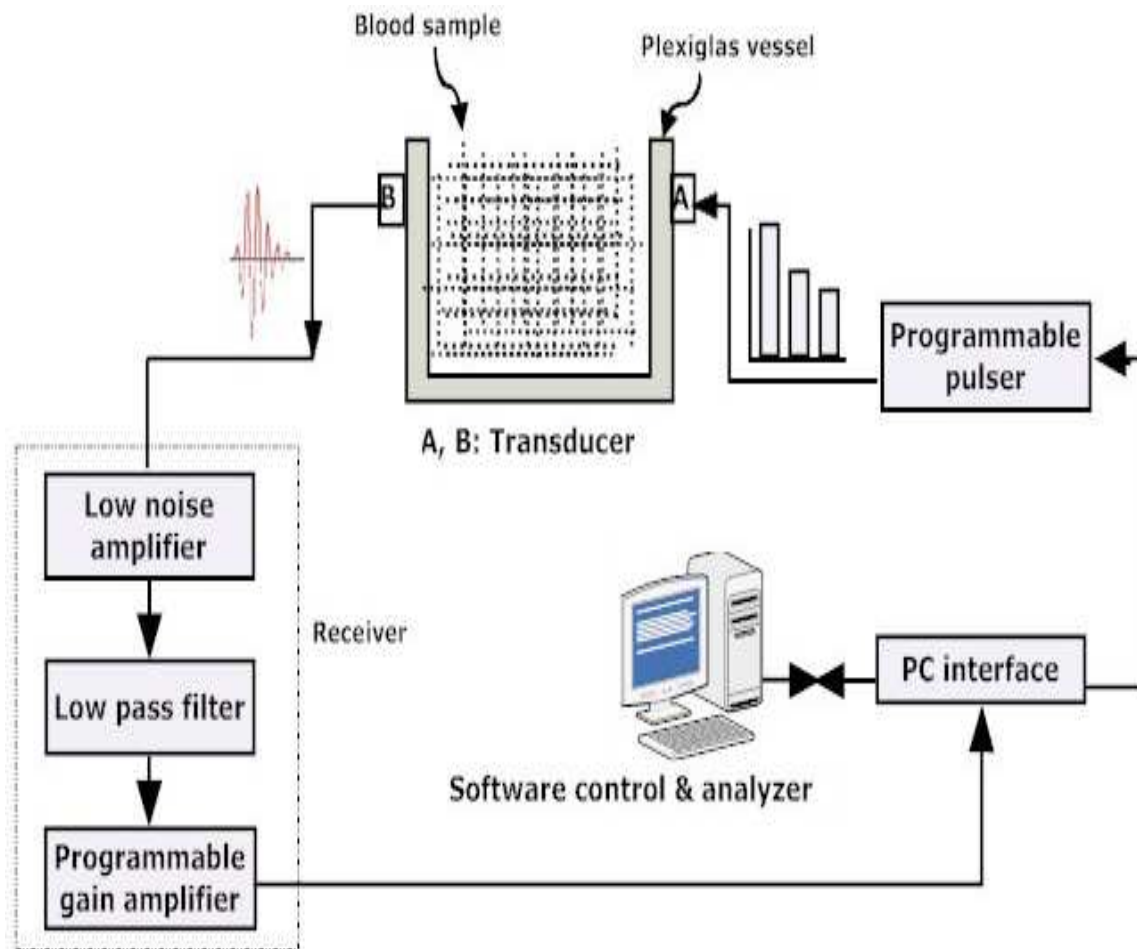


Figure 1. Schematic diagram of experimental setup.

When operating in the pitch-catch mode, the receive transducer records only the first echo signal for each six decay amplitude pulse transmitted. The first echo signal, signal of interest, occurs at the shortest time, and the path length in the pitch-catch mode is $2w + D$. Fast Fourier transform (FFT) of the peak of interest was used to obtain the attenuation measurements as a function of frequency, the first for each echo was obtained for blood and for the plasma. A deep minimum in the time signal can be used to make time-of-flight measurements through the plasma, thus leading to an accurate method for measuring the velocity of sound and finding out the relation between plasma separation accuracy measurements and plasma velocity of sound.

The data were obtained in five steps; according to discrete spinning time duration of the centrifuge device, each step received the sixth echo using appropriate decay pulser voltages and receiver gain. More illustration of experimental steps are as follows:

1. The programmable pulser device will generate six decay pulses;
2. The receiver transducer will receive only the first echo for each transmitted pulse;
3. Before centrifugation process for blood sample, the blood will obey the six decay pulses and the receiver will evaluate the data for received signal intensity (Table 1, column one). These collected data will be as referenced investigation data;

4. The blood sample then spinning for five minutes duration using centrifuge device. The time duration was divided into five periods, each period has one minute;

5. For each spinning per minute for blood sample, the sample will obey the six transmitted pulses, hence echo intensity measurement after one minute spinning is achieved as shown in Table 1 column two (the first spinning per minute).

Overlapping echoes were used to determine the effect of changing the pulser voltage, and the data were normalized to a decay pulser voltage of 100, 80, 65, 50, 35 and 25 V. Indeed, the data were corrected for the receiver gain. The result of each echo interest for the blood is as shown in Figure 2.

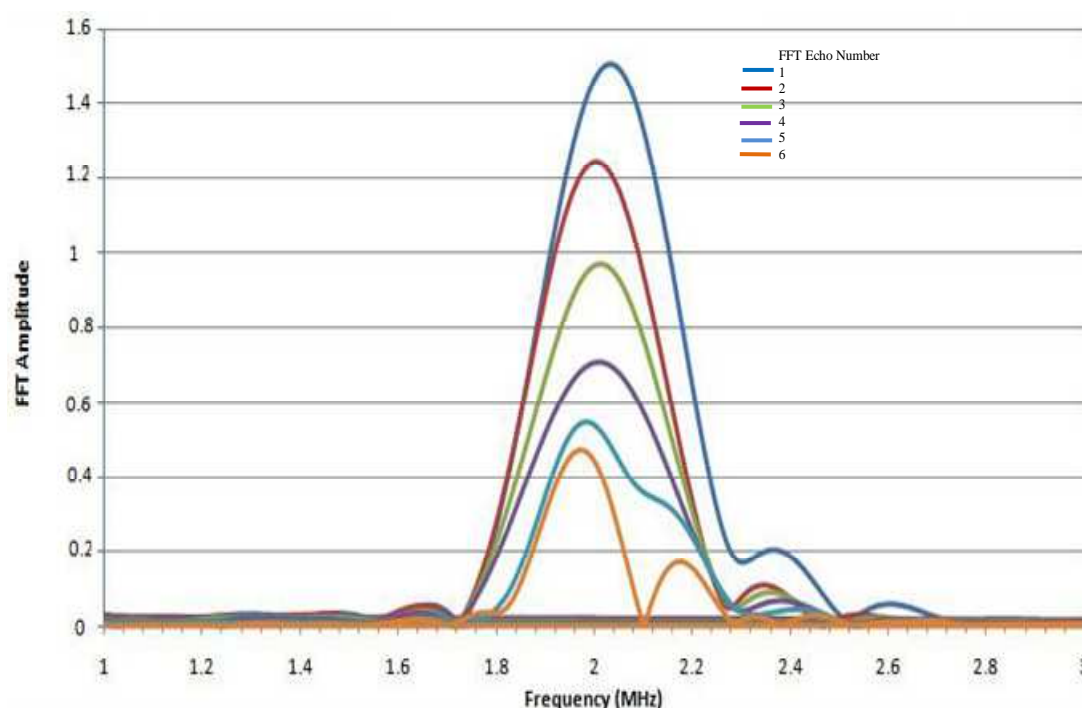
In the case of increasing the number of echoes for each step, the attenuation effect for blood will be seen as shifting the peak amplitude corresponding to a smaller frequency (Greenwood, 2004).

DATA ANALYSIS

The additional data obtained for blood based on the multi-level power transmitted are as shown in Table 1.

Table 1. Comparison of FFT amplitude for blood and blood plasma after one minute spinning by centrifuge device.

Echo number	Blood FFT amplitude at 2 MHz	Blood after one minute spinning process, FFT amplitude at 2 MHz	Separation percentage
1	1.58	1.612	20% plasma 80% blood (RBC, WBC and platelets)
2	1.2268	1.2769	
3	0.9526	1.0115	
4	0.7397	0.8013	
5	0.5743	0.6347	
6	0.4459	0.5028	

**Figure 2.** FFT amplitudes of echoes 1 through 6 for blood.

The decay pulse power provides an adjustable path for echo propagation through a liquid. Echoes (1 to 6) achieved echo number based on the multi-level power transmission technique of 100, 80, 65, 50, 35 and 25 V, and for high accuracy of measurements, each transmitted power level was taken for ten trails. The traditional centrifuge device works with 3500 rpm for a 5 min time period during the blood separation process. In this research, the experimental setup works through two scenarios.

Scenario 1

Using the centrifuge device and a one-minute period of blood spinning yields a concentration of blood cells suspended in plasma.

Scenario 2

The multi-level power technique was applied to the blood sample of scenario one and this process results in the second column of Table 1; whereas the first column of Table 1 was obtained with pure blood sample, which obeys the same multi-level power.

By tuning the spinning time of the centrifuge device for 2, 3, 4 and 5 min, which simulate the change of blood concentration and looping through scenario 1 and 2, the data result from this process is as shown in Figure 3, which accompanied the echo number and natural logarithm of FFT plasma and blood ratio of echo for each concentration. Table 1 second column shows the echo power received for the first step of the one-minute spinning experiment.

In addition, Table 1 shows the separation percentage of

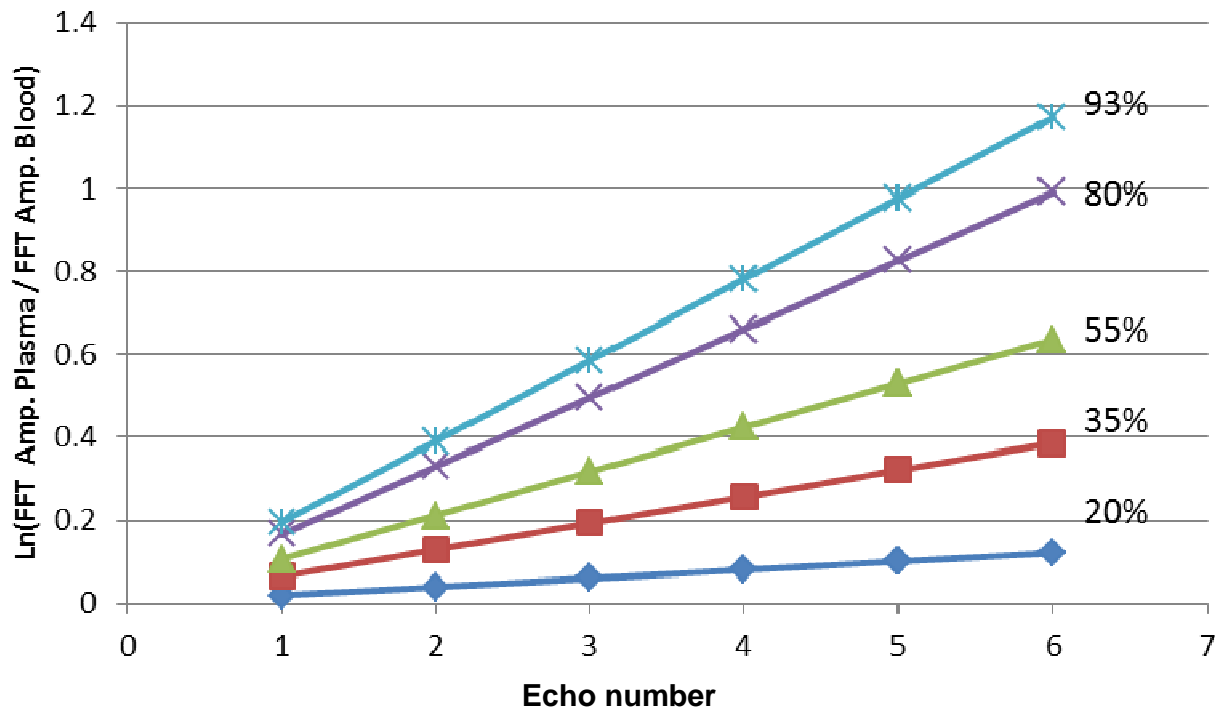


Figure 3. FFT amplitude natural logarithm for the blood plasma concentration of blood versus the echo number at 2 MHz frequency.

blood plasma value of 20% at 2 MHz. The blood FFT amplitude for first echo is 1.58 and 1.612 after a one-minute spinning process; thus, there is a slight increase in the amplitude value after one-minute spinning due to the decrease of concentration of red blood cells.

It can be seen that the FFT of blood amplitude decreases with an increase of echo numbers, based on the programmable transmitted echo power.

The result of 20, 35%, 55, 80 and 93% plasma concentration based on blood data was plotted, and the slope was derived as depicted in Figure 3.

When the echo number increases, the natural logarithm increases for FFT ratio of plasma and blood. The measurements of plasma separation that were obtained were 20, 35, 55, 80 and 93% by weight during the full experimental setup.

BLOOD ULTRASOUND ATTENUATION MODEL

Subsequently, the relationship between slope and attenuation was discovered due to the attenuation of ultrasound waves propagated through the blood plasma; afterward, the attenuation, slope and plasma separation percentage have been concluded as in Equations 9 and 10.

Also, we would discuss the pitch-catch mode of two transducers that are seen operating. When the container was filled with blood plasma, then the FFT amplitude at a

specified frequency, 2 MHz, for echo N equal to m , was defined as V_{Pm} and when filled with blood by V_{Bm} . D is the distance between the walls of the vessel, and the path length through the blood or plasma is $2w + D$. The value V_{Pm} was influenced by the attenuation through the blood plasma, the transmission coefficients at the Plexiglas-blood interfaces, the reflection coefficients at the walls and the spreading of the beam due to diffraction and similarly for blood. In traveling a distance D through the plasma, the amplitude was reduced by a factor f_p due to the particles (red blood cells, white blood cells and platelets) in the plasma and the attenuation through the liquid in which the particles are suspended. Equation 1 shows the amplitude (V_m) for transmitted pulse m :

$$V_m = V_o e^{(1-m)/2} \quad (1)$$

where V_o is the initial amplitude and m is the number of transmitted pulse. The attenuated amplitude obtained is as follows:

$$V_{Pm} = K_P f_P^m V_m \quad (2)$$

Table 2. Illustration of attenuation and separation percentage of plasma calculation.

Time of spinning (Min)	Slope on logarithmic graph	Attenuation factor (f)	Attenuation (dB/cm)	Separation percentage	
				Plasma (%)	Blood (%)
1	0.02	1.0101	-0.0232	20	80
2	0.0643	1.0327	-0.0745	35	65
3	0.106	1.0544	-0.1228	55	45
4	0.1652	1.0861	-0.1913	80	20
5	0.1954	1.1026	-0.2263	93	7

and

$$V_{Bm} = K_B f_B^m V_m \quad (3)$$

The constants K_p and K_B include the transmission and reflection coefficients at the interfaces and the beam diffraction. For the blood plasma discussed here, these effects closely resemble those for blood (the velocity of sound and density of blood and plasma are 1540 m/s, 1060 Kg/m³ and 1489 m/s, 1025 Kg/m³, respectively), and so K_p is equal to K_B . When the transmission and reflection coefficients are not equal (Bamberger, 2004), therefore,

$$\frac{V_{Pm}}{V_{Bm}} = f^m \quad (4)$$

where f is equal to $\frac{f_p}{f_B}$ and f is the factor relative to blood. A similar equation can be written for echo N equal to n :

$$\frac{V_{Pn}}{V_{Bn}} = f^n \quad (5)$$

A relationship between the slope and the attenuation factor f relative to blood can be obtained by taking the natural logarithm and subtracting Equations 4 and 5:

$$S = \frac{(\ln(FFT(f^n)) - \ln(FFT(f^m)))}{(n - m)} \quad (6)$$

$$f = e^{S/2} \quad (7)$$

where S refers to the slope of the natural logarithm of $\frac{V_p}{V_B}$ versus the echo number N . Echoes m and n

are used to determine the slope. Thus, the attenuation is modeled as:

$$\alpha(\text{dB/cm}) = (-20/D) \log_{10}(f) \quad (8)$$

The attenuation (α) as a function of 2 MHz frequency was obtained by using (Equations 7 and 8) for each plasma separation percentage.

An example of complete-test calculation of the plasma separation process is as shown in Table 2. The slopes of the straight lines in Figure 3 are used to calculate the attenuation factor f from Equation 7, as shown in the third column. The attenuation in the fourth column was calculated using Equation 8. To improve the accuracy of the measurements, many echoes should be used.

The computer code calculated the slopes as a function of echoes for each percentage plasma separation at 2 MHz (Figure 3). The attenuation as a function percentage of plasma separation is as shown in Figure 4. Data were obtained with a blood test, and then, plasma was checked for every one-minute spinning of a five-minute total time. For a given frequency, a plot of the attenuation (α) versus the separation percentage X was modeled with Equation 9. For example, when the values of the attenuation at 2 MHz are extracted from Figure 4, then such a plot shows the following linear relationship:

$$\alpha = -0.0027x + 0.027 \quad (9)$$

Therefore, a measurement of the attenuation at a given frequency will yield the weight percentage of the plasma. Relationship between the slopes versus separation percentages yields direct measurements that decrease the computer calculation time and, hence, that of the plasma percentage measurement time, from Figure 5, where it is seen that it is a linear relationship:

$$s = 0.236x - 0.0234 \quad (10)$$

From Equation 10 and for a slope value of 0.05, in Figure 5, a plasma separation percentage of 31.1% was produced. The conclusion is that a wide range with very high accuracy of plasma separation can be detected and

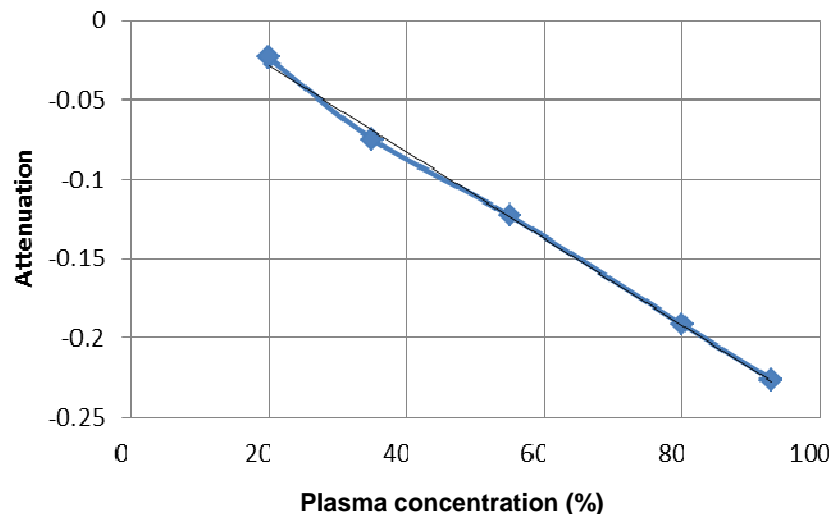


Figure 4. Results of attenuation measurements for plasma concentrations having 20, 35, 50, 80 and 93% by weight in blood.

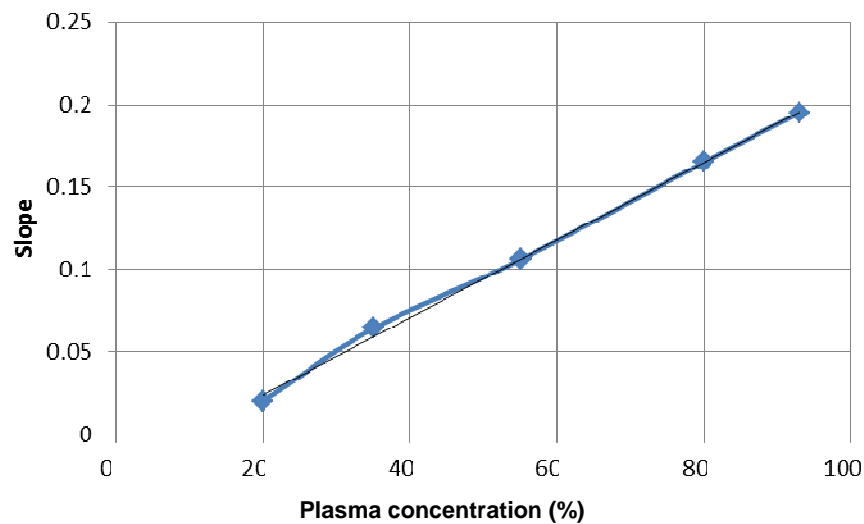


Figure 5. Direct relationship between plasma and concentration slope.

measured. Such a measurement can indeed be used for the detection of contaminants, and for controlling measurement devices.

CALIBRATING FEATURES

The method just described is a standard method, which means that the measurement is dependent on the changes in the pulser voltage. Another method for Calibration was called the self-calibrating method (Greenwood et al., 2006); in this method, the receiver transducer

receives multi echoes reflected from a container made of high-reflection coefficient material.

This method cannot be used in several real-time applications for measuring attenuation. In this research, we use containers made from very low-reflection coefficients, as these containers have advantages that decrease the noisy echo power and pass the maximum transmitted power to the receiver transducer. However, in the laboratory experiments, the results of the two methods are in very good agreement.

The standard method assumes that the pulser voltage and system electronics are stable and that the amplitudes

for blood remain unchanged since the blood calibration files were obtained. This is especially important for the measurement of very small attenuations, such as the detection of less than 1% concentration; however, in our experiments, we have greater than this detection value. Thus, frequent blood calibration data may be needed to ensure accuracy. However, the calibrating method used, and described by Equations 7 and 8), has been pre-calibrating; this calibration was done by using the fuzzy controller for the blood at time ($t = 0$); and this calibration is very important not only to check the stability of the pulser device, but also to check the healthy blood sample, because in this experiment, we select the samples of healthy donor blood, which, for example, is different from the blood sample of a donor infected with diabetes.

Conclusions

Mathematical modeling of the relationship of attenuation, amplitude and percentage of plasma separation was derived; based on these modeling equations, a complete experimental setup was obtained and compared.

Using this model, we were able to know the concentration of blood plasma derived through the centrifugation process, therefore, measuring the intensity of the echo signal received and using the formula for calculating attenuation enables the determination of the concentration of blood cells in plasma. This technique can be developed for controlling the centrifuge device or can be adaptive as a new mythological separation method that offers fast and accurate separation measurement of human blood.

The results of using multiple decay power pulses through the blood, leading to an adjustable long path, show the effectiveness of measuring the attenuation of a low blood contents concentration, thus, also providing a very accurate measurement of the plasma separation percentage. The results show the feasibility for measuring the attenuation of a plasma with a concentration as low as 10% of weight. As discussed in earlier, Figure 4 shows the effect of the contents of the blood percentages on the value of attenuation. The absolute attenuation of blood was calculated by including effects of reflection and transmission at the Plexiglas-blood and blood-Plexiglas interfaces, and also diffraction effects. The plasma separation percentage measurements can be extracted from Figures 4 and 5, of which these plots show a linear relationship between the percentage of plasma separation and attenuation and slope, respectively. The relationship between plasma separation percentage and the slope has advantages: it decreases the time calculation required for blood plasma percentage.

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