# A Hyperglycemic Blood Mimicking Fluid with Good Acoustic and Physical Properties for In-Vitro Ultrasound Flow Studies

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**Research**

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# TITLE: A Hyperglycemic Blood Mimicking Fluid with Good Acoustic and Physical Properties for In-Vitro Ultrasound Flow Studies

**RUNNING TITLE: Hyperglycemic Blood Mimicking Fluid**

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## Abstract

**Background:** A blood mimicking fluid (BMF) is made up of a mixture fluid and ultrasound scattering particles that stay neutrally buoyant in the fluid. For these particles to be able to remain suspended in the fluid, their density must be very close or equal to the density of the mixture fluid and the BMF must also have its acoustic properties (speed of sound, attenuation and backscatter power) and physical properties (density and viscosity) close to the internationally acceptable standard. This paper introduces D(+)-Glucose (DG) as an important component of a mixture fluid consisting of Propylene Glycol (PG) and water for preparing a hyperglycemic blood mimicking fluid (BMF). **Methodology:** The BMF was prepared by first preparing different samples of ternary mixture fluids in which a fixed amount of PG was mixed with various amounts of water and DG to get a particular and suitable percentage combination that yielded a density similar to that of poly (4-methylstyrene) scatter particles. A required amount of the scatter particles was mixed with the suitable mixture fluid to form a BMF with good physical and acoustic properties accepted by the International Electrochemical Commission. **Results:** A very good BMF was produced consisting of 84% distilled water, 5% of PG and 11% of DG as the ternary mixture fluid mixed with 0.8% Poly (4-methylstyrene) scatter particles. It has a density of 1.040 g/cm$^3$, viscosity of 4.30 mpa.s, speed of sound of 1580 m/s and attenuation of 0.017 dB/cm at 5MHz, having a good back scatter property. **Conclusion:** DG is a good substance that forms part of the ternary mixture fluid for the preparation of a hyperglycemic BMF with suitable physical and acoustic properties.


**Key Words:** Propylene Glycol; D(+)-Glucose; Blood Mimicking Fluid; Physical Properties; Acoustic Properties; Ultrasound.

## 1 Introduction

Human blood samples are difficult to handle in the laboratory as fluids for bench work studies because of the complexity of blood and for other health related worries. This has led to several researches to chemically prepare fluids that can closely mimic the acoustic and physical properties of real blood and can be used as a good replacement in preclinical experiments. Earlier blood mimicking fluids (BMF) were made of water mixed with cutting fluid and nylon as scatter particles (Rickey et al., 1995); glycerol, water, dextran, orgasol mixed with synperonic N surfactant as scatters and Sodium azide as a preservative (Ramnarine et al., 1998; Lubbers, 1999; Ramnarine et al., 2001; Browne et al., 2003; Harin & Ince, 2004; Samavat & Evans, 2006; Meagher et al., 2007). Other substances introduced as part of the BMF mixtures towards getting better acoustic and physical properties include sodium iodide salt (Majid et al., 2009), silicon oil and glycol (Tomoji etbal., 2013; Yoshida et al., 2014), Polyamide particles as scatters (Fhrmann et al., 2016). Recently, propylene glycol and polyethylene glycol were found to provide a good combination with water and glycerol as BMF mixture fluid while poly (4-methylstyrene) was used as scatter particles to mimic the red blood cells (Oglat et al., 2017; Oglat et al., 2018). A good BMF must have physical properties (density, viscosity and particle size) and acoustic properties (speed of sound and attenuation) closed to the internationally acceptable standards (Yoshida et al., 2014; Oglat et al., 2018).

In this paper, we have discussed another method for preparing a BMF made up of water, propylene glycol, glucose and poly (4-methylstyrene) as scatter particles. The new item introduced is glucose as part of the ternary mixture fluid with a bulk density of 1.50 g/cm3 higher than the densities of water (0.998 g/cm$^3$) and propylene glycol (1.036 g/cm$^3$). The choice

of glucose is justified because it increases the acoustic and physical properties of the mixture fluid until the right amount produces a BMF fluid with properties accepted internationally. A real human blood contains glucose as part of the components of its plasma and serum, the presence of high level of glucose in the blood plasma ($\geq \mathbf{130mg/dl}$ ) makes it hyperglycemic (diabetic) and a risk factor of cardiovascular disease (Rita *et al.,* 2015; Flora & Nayak, 2019; Hamid et al., 2019; Bochra et al., 2020). Blood sugar (glucose) plays an important role as the energy requirement of the body. Excess of glucose in the blood results to diabetes which can be categorized as type 1 or type 2 depending on its severity on human health. This makes it important to have a BMF with glucose as a component of the mixture fluid to be used for in-vitro ultrasound flow measurements.

## 2 Materials and Methods

### A Materials

The materials used for this research are grouped under chemical items and hard ware apparatus.

### I Chemical Items

The chemical components of the BMF include the following;

a. Distilled water: A distilled water (density of 0.998 $g/cm^3$) was used as the largest proportion of the fluid mixture to mimic the water component of the real blood plasma
b. Sigma Aldrich Propylene glycol (PG): This is a viscous organic compound that is colorless and nearly odorless with a faint sweet taste represented by the chemical formula,

$CH_3CH(OH)CH_2OH$. It is soluble in water and other organic solvents with a molecular mass of 76.09 g/mol, density of 1.036 g/cm$^3$ and viscosity of 0.042 pa.s (Haynes, 2016). PG also known as propan-1,2-diol is used for production of polymers, in various edible items such as coffee-based drinks, liquids sweeteners and ice cream, but for this research, it was used to achieve the physical and acoustic properties of a mixture fluid for BMF preparation.

c. D(+)-Glucose (DG)(Merck, Germany): Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$ as the most abundant monosaccharide (Bastioli, 2014), mainly made by plants and most algae during photosynthesis from water and carbon dioxide using energy from the sunlight. It has a molar mass of 180.156 g/mol and a bulk density of 1.50 g/cm$^3$, soluble in water with applications as food sweeteners, in the industries and in medicine. For this research, glucose was used also to study the physical and acoustic properties of BMF mixture fluid and to find out its effect on BMF flow velocity in the carotid artery phantom.

d. Poly 4-methylstyrene (Sigma Aldrich, Germany): This substance is a mixture of polymer (styrene) and monomer (methyl) used for making polymers and plastics. It is used as a scatter particle in this research because it has a combined influence as a Rayleigh-scattering with a density of 1.040 g/cm$^3$ and particle size of $3 - 8\mu m$ (Oglat et al., 2018). The ability for this substance to remain suspended (neutrally buoyant) in the BMF mixture fluid, its spherical nature which avoids clothing inside a vessel during flow, its closeness to the density of BMF and its commercial availability makes it a good choice as a scatter particle since it is insoluble in liquid substances.

e. Benzalkonium Chloride (Sigma Aldrich, Germany): This is an organic salt of cationic surfactant type classified as a quaternary ammonium compound having a density of 0.98 g/cm$^3$ with variable chemical formula, molar mass and very soluble in water. Its solutions

are fast-acting biocidal agents with a moderately long duration of action used as fungicides, medical surgical device disinfectants and anti-bacterial agent.

### II Hard ware apparatus

The apparatus used in this research are the chemical balance to measure the mass of substances, electric density meter to measure fluid density, electric rotational viscometer (ERV) to measure viscosity and A-scan GAMPT (German Society for Applied Medical Physics and Technology) to measure acoustic properties. Other apparatus are the magnetic stirrer hot plate and bar for heating and stirring, vacuum pump for degassing, beakers, measuring cylinders, thermometer and a dropper.

## B Methods

### I Blood Mimicking Fluid (BMF) Preparation

The BMF was prepared in the medical and solid state physics laboratories of Universiti Sains Malaysia as outline through the following steps:

a. First, the glass beaker with size about twice the volume of the BMF was washed with distilled water and wiped clean with a tissue paper. The choice of this size of beaker was to avoid the overflowing of the fluid while stirring. This was followed by placing a magnetic stirrer (with size commensurate with the size of the beaker) inside the beaker.
b. 90 weight percentage (wt% ) of distilled water was measured using a measuring cylinder and 5 wt% of PG (Oglat et al., 2017) was weight as well as 5 wt% of DG in a fume cupboard using a chemical balance. The distilled water was first poured into the beaker, followed by the DG with thorough stirring to ensure that the DG dissolves completely.

Finally, the PG was added and the whole component placed on a magnetic stirrer hot plate as shown in figure 1a.

c. The stirring plate was set to operate at 700 revolutions per minute (rpm) for 20 minutes at a temperature of $37^0$C after which the vacuum pump is used to degas the fluid for about 30 minutes.

d. The procedures above were repeated four more times but with different wt% of distilled water and DG while the amount of PG remained fixed at 5 wt%. The five (5) samples (figure 1b) together with 100 wt% distilled water were then tested for their densities, viscosities, speed of sound and attenuation properties

e. A linear relationship was established using the statistical package for social sciences (SPSS) soft ware between the density and the amount of DG in the fluids resulting to the equation:

$$\text{Density} = 0.00426(\text{amount of DG}) + 0.993 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \; 1$$

With equation 1, 11 wt% of DG by weight was required to mix with 5 wt% of PG and 84 wt% of distilled water to produce exactly a mixture fluid with a density of 1.040 g/cm$^3$, a value required to match with the density of poly (4-methylstyrene) scatter. This allows the scatter particles to remain neutrally buoyant in the fluid mixture.

f. Finally, the hyperglycemic BMF was produced by mixing the required amount of scatter particles, poly 4-methylstyrene (0.8 wt%) with the mixture fluid (5 wt% of PG, 11 wt% of DG and 84 wt% of water). The BMF was then placed on the stirrer plate and two drops of 50 wt% anti-fungal agent (Benzalkonium Chloride) was added, it was then stirred for about 30 minutes and degassed for about an hour to remove any air bubbles.

**II Measurements of Density and Viscosity**

The densities of the BMF fluids were measured using a portable Density Meter (DMA 35). The strip attached to the meter was pressed down and dipped in the fluid, and then it was released to draw up the fluid inside the strip by suction pressure, while the density reading was recorded automatically by the meter in just few seconds. The density increased with decrease in temperature, therefore the liquid was heated up to about $40^0$C before dipping the meter strip. The readings were measured when the temperature was exactly at $37^0$C, the normal human body temperature.

About 700 $cm^3$ of each BMF fluid was required for the viscosity measurements. The spindle L1 was selected for viscous liquid, it was attached to the ERV and then lowered into the fluid (figure 1c) while the ERV was switched on. The spindle rotates inside the liquid for few minutes until a steady value of the viscosity was recorded due to the viscous resistance from the fluid (Kim et al., 2015; Ihm et al., 2020)

**III Measurements of speed of sound, Attenuation and Backscatter power**

The speed of sound was measured by pulse echo (PE) method using the A-scan Gampt machine. The arrangement for this measurement is shown in (figure 2) where the time of flight (TOF) between the highest two peaks of the transmitted and reflected waves was measured. For good accuracy, the protective layer thickness of the probe was first calculated and added to the distance between the walls of the vessel containing the liquid. This was done by using two acrylic plates of thicknesses 40 mm and 80 mm with the same speed of sound of 2700 m/s. The Pulse echo method using A-scan GAMPT technique was able to provide time of flight for the

two plates which was used to calculate the thickness of the protective layer of the probe using the equation:

$$dp_1 = \frac{\left(\frac{t_1}{t_2}da_2\right) - da_1}{2\left(1 - \frac{t_1}{t_2}\right)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where $dp_1$is the thickness of the transducer protective layer, $da_1$is the thickness of the acrylic plate (40 mm), $da_2$is the thickness of the acrylic plate (80 mm), $t_1$ is the time of flight through the plate (40 mm) and $t_2$is the time of flight through the plate (80 mm). The values for the speed of sound for the liquids were calculated using the equation:

$$Speed\ of\ sound = \frac{2(x + dp_1)}{t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where $x$ is the distance between the walls of the vessel containing the mixture liquid and $t$ is the time of flight

The amplitudes of the highest two similar peaks were also measured and the attenuation coefficients ($\alpha$) were also calculated using the attenuation equation:

$$\alpha = \frac{2 \times 0.868}{x} \ln \frac{A_2}{A_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Such that $A_1$ and $A_2$ are the amplitudes of the two highest but similar wave peaks respectively and $x$ is the distance.

The backscatter power of the BMF was measured at different radio frequency signals by calculating the average power spectrum through applying the Fast Fourier Transform (FFT) generated by the A-scan GAMPT software at 5 MHz. This was done at different concentrations of the scatter particles poly (4-methylstyrene) and cholesterol to find out if the BMF simulates

the real human blood. The backscatter coefficients were directly read on the screen of the A-mode PE technique during the ultrasound scanning.

**IV Ultrasound measurements of Flow velocity and Indices**

The BMF was tested by running it through an 8.0 mm lumen diameter common carotid artery wall-less phantom prepared with a konjac-carrageenan and gelatin based tissue mimicking material ((Ammar et al., 2018). The BMF was pumped with the aid of a gear pump (multi-flow GAMPT) at a steady flow rate of 1500 ml/min to measure the flow properties using a digital Hitachi ultrasound scanner connected with a linear array transducer (EUP-L74M).

## 3 Results and Discussion

The result of changing the amount of DG with a fixed amount of PG shows that the density, viscosity, speed of sound and attenuation increased linearly (Tomoji et al., 2013; Yoshida et al., 2014; Oglat et al., 2017; Oglat et al., 2018) with increase in the percentage weight of the DG as seen in table 1 and figure 3. This was made possible because the density of DG (1.50 g/cm$^3$) is higher than those of water (0.998 g/cm$^3$) and PG (1.036 g/cm$^3$). We had to put the amount of PG fixed at 5 wt% because its density is less than the density of the scatter particle (poly 4-methystyrene) whose density is 1.040 g/cm$^3$. By implication, it means that increasing the amount of DG increased the densities of water and PG until a suitable amount of DG gave the required density of the mixture fluid that enabled the scatter particle to stay neutrally buoyant in the fluid because they have the same densities. By equation 1, 11 wt% of DG (91.52g) was required to mix with 84 wt% of water (700 cm$^3$) and 5 wt% of PG (40 cm$^3$) to obtain a suitable mixture fluid which in turn was used to mix with 0.8wt% of poly 4-methly styrene scatter (6.656g) to produce 800cm$^3$ of the BMF. This result means that 91.52g of DG was mixed with

740cm$^3$ of fluid to give a good mixture fluid for the hyperglycemic BMF. It also implies that the amount of glucose in the BMF is 12,369 mg/dl which is far much higher than the normal range of glucose level of less than 125 mg/dl (Poretsky, 2017) in the healthy human blood.

For Doppler flow test object, it is relevant that the BMF backscatter power be well established, reconfirmed and stable. The ultrasound backscatter from the BMF should be close to that of flowing human blood. This will make measurements of penetration depth and sensitivity very reliable (Evan & James, 1988). In this research, we used the PE method which involves using a single plane transducer as emitter and receiver to measure the backscatter power at different concentrations of the Poly (4-methystyrene) scatter particles in the BMF. The mean power spectrum was calculated by performing FFT on the radio frequency (RF) signals while the backscatter power in decibel (dB) was determined by the ratio of the mean power at 5 MHz from the flowing BMF. Results showed a linear dependence of backscatter power on increasing the particle concentrations of Poly (4-methystyrene) (Oglat et al., 2018) as shown in figure 4.

The BMF prepared has a density of 1.040 g/cm$^3$, viscosity of 4.30 mpa.s, speed of sound of 1580 m/s and an attenuation of 0.017 dB/cm at 5 MHz, which are within the acceptable ranges for the physical and acoustic properties of a BMF by the International Electrochemical Commission (IEC) for Doppler ultrasound flow phantoms (Ramnarine et al., 1998; Browne et al., 2003; Samavat & Evans, 2006; Yoshida et al., 2012; Yoshida et al., 2014). Results of flow velocity measurements (figure 5) show that the peak systolic flow velocity (74.1 cm/s), end diastolic velocity (23.2 cm/s), resistivity index (0.69) and pulsatility index (1.06) were within accepted limits (Oglat et al., 2018). This observation suggest that even though the BMF is hyperglycemic, its hemodynamic indices are within the normal range for a healthy blood flow

implying that high blood sugar does not affect the flow of blood in the human carotid artery since this type of flow is non-Newtonian in-vivo but Newtonian in-vitro (Ozlem et al., 2015).

## 4 Conclusion

The BMF produced in this research work makes it clear that glucose is a better replacement for polyethylene glycol as a component of a mixture fluid because it gives better results for the physical and acoustic properties of the fluid. It was prepared by mixing 84.0 wt% of distilled water, 5.0 wt% of Propylene glycol and 11.0 wt% of D(+)-Glucose with 0.8 wt% of poly (4-methylstyrene) scatter particles for proper suspension in the fluid. The scatter particles fit in well in the mixture fluid as it provides good backscatter strength making the BMF very suitable for ultrasound in-vitro studies. The preparation took just few minutes and the cost of producing it is very low compared to commercially available BMF phantoms. The BMF lasted for about 3 months due to the addition of a preservative or anti-fungal agent to the fluid.

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

Prof. MZ initiated the research, provided support (under the grant number 1001/PFIZIK/822173) and supervisory role. Dr NS gave technical advice, support (under the grant number 1001/PFIZIK/822173) and editing of the manuscript. Mr DK performed the experiment and contributed in drafting of the manuscript. Dr SA and Dr AA contributed with essential materials needed for the manuscript drafting and also participated in prove reading the manuscript. All authors read and approved the final manuscript

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**Table 1:** Physical and acoustic properties of mixture fluid for the preparation of a hyperglycemic blood mimicking fluid at a temperature of $37^0$C

| S/No. | Distilled water (wt%) | Propylene Glycol (wt%) | D(+)-Glucose (wt%) | Density (g/cm$^3$) | Viscosity (mpa.s) | Speed of Sound (m/s) | Attenuation (dB/cm) |
|---|---|---|---|---|---|---|---|
| 1 | 100 | 0 | 0 | 0.993 | 2.8 | 1513.4 | 0.0046 |
| 2 | 90 | 5 | 5 | 1.016 | 3.4 | 1549.3 | 0.0065 |
| 3 | 85 | 5 | 10 | 1.035 | 3.8 | 1566..4 | 0.0105 |
| 4 | 80 | 5 | 15 | 1.057 | 4.4 | 1588.4 | 0.0190 |
| 5 | 75 | 5 | 20 | 1.079 | 5.2 | 1614.1 | 0.0194 |
| 6 | 70 | 5 | 25 | 1.100 | 5.8 | 1634.3 | 0.0198 |

## Figure Legends

**Figure 1:** (a) Magnetic stirring mixing the components properly and to become homogeneous

(b)Density meter (DMA 35) to measure the density of the aqueous solutions

(c) Rotational Viscosity Meter measuring the viscosity of the mixture fluids

**Figure 2:** Measurement of the acoustic properties of solutions using the A-scan Gampt technique

**Figure 3:** Linear graphical representation of relationship between Density and D(+)-Glucose concentration (DGCon)

**Figure 4:** Relative backscatter from BMF under uniform flow plotted against particle concentration for Poly (4-methylstyrene) particles of size 3-8 $\mu$m.

**Figure 5:** BMF flow velocity and indices through 8.0 mm common carotid artery phantom

# Figures

(a)

(b)

(c)

**Figure 1**

(a) Magnetic stirring mixing the components properly and to become homogeneous (b) Density meter (DMA 35) to measure the density of the aqueous solutions, (c) Rotational Viscosity Meter measuring the viscosity of the mixture fluids

**Figure 2**

Measurement of the acoustic properties of solutions using the A-scan Gampt technique

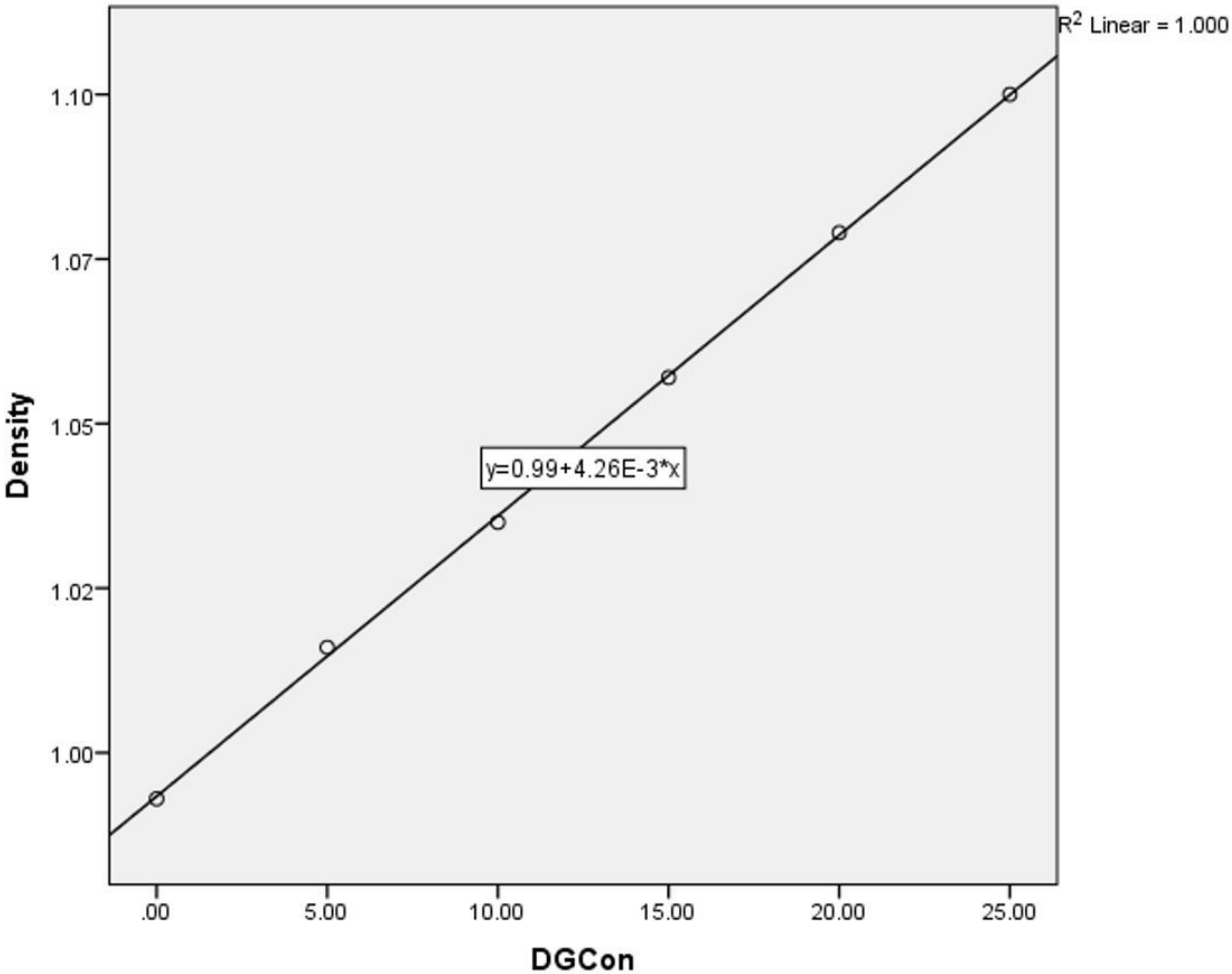


**Figure 3**

Linear graphical representation of relationship between Density and D(+)-Glucose concentration (DGCon)

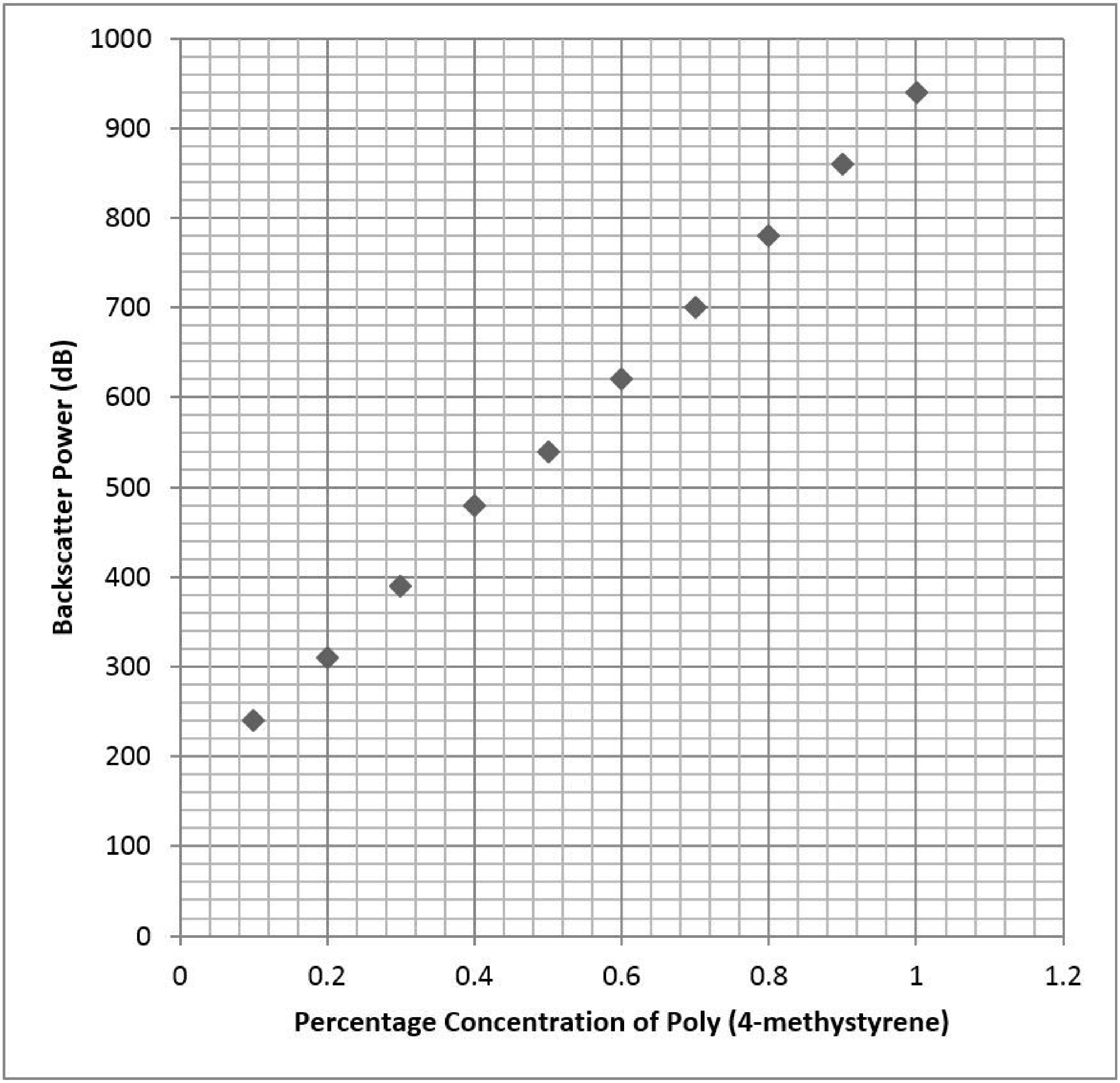


Figure 4

Relative backscatter from BMF under uniform flow plotted against particle concentration for Poly (4-methylstyrene) particles of size 3-8 μm.

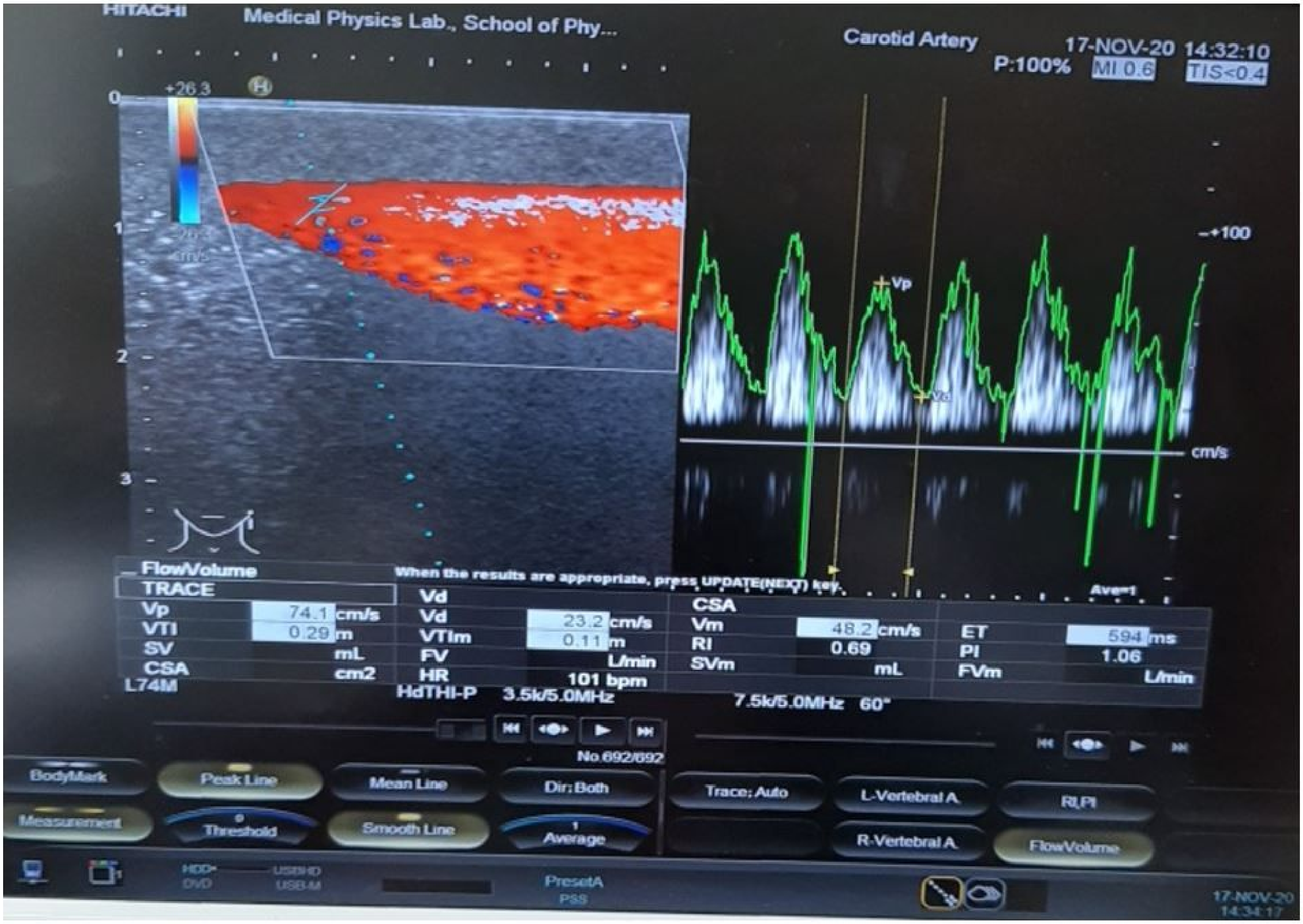


**Figure 5**

BMF flow velocity and indices through 8.0 mm common carotid artery phantom