A Review of Suspension-Scattered Particles Used in Blood-Mimicking Fluid for Doppler Ultrasound Imaging

Ammar A. Oglat1, Nursakinah Suardi1, M. Z. Matjafri1*, Mohammad A. Oqlat2, Mostafa A. Abdelrahman3, Ahmad A. Oqlat4

1Department of Medical Physics and Radiation Science, School of Physics, Universiti Sains Malaysia, 11800 Penang, Malaysia, 2Department of Biological Sciences, School of Science, Yarmouk University, Irbid, Jordan, 3Department of Emergency, Faculty of Medicine, JUST, Irbid, Jordan

Abstract

Doppler ultrasound imaging system description and calibration need blood-mimicking fluids (BMFs) for the test target of medical ultrasound diagnostic tools, with known interior features and acoustic and physical properties of this fluid (BMF). Physical and acoustical properties determined in the International Electrotechnical Commission (IEC) standard are specified as constant values, the materials used in the BMF preparation should have values similar to the IEC standard values. However, BMF is ready-made commercially from a field of medical usage, which may not be appropriate in the layout of ultrasound system or for an estimate of novel imaging mechanism. It is often eligible to have the capability to make sound properties and mimic blood arrangement for specific applications. In this review, sufficient BMF materials, liquids, and measures are described which have been generated by utilizing diverse operation mechanism and materials that have sculptured a range of biological systems.

Keywords: Acoustical properties, blood-mimicking fluid, physical properties, suspension scatter particle materials

INTRODUCTION

Blood that precisely mimics real human blood systems in all its features is often referred to as blood-mimicking fluid (BMF). BMF consists of blood-mimicking materials with simple and similar physical properties. Chemical fluid or powder materials are occasionally mixed to mimic both physical and chemical properties.[1,2] Furthermore, BMF was applied for calibration and description of Doppler ultrasound imaging systems since the 1980s. BMF is also used to compare the act of ultrasound systems for the practice of Doppler ultrasound technicians, to allow comparison of backscatter properties of Doppler ultrasound and to evaluate it in a Doppler flow test object or diagnostic techniques. Items used in the preparation of BMF that could be constructed with general acoustic properties, particle features, and physical properties are helpful in studying the ultrasound imaging. To achieve this purpose (preparation of a suitable BMF), the acoustical and physical properties of BMF should be close to the International Electrotechnical Commission (IEC) standard with constant values [Table 1]. BMF is available commercially, imitating the properties of human blood such as density, concentration, scattering, the speed of sound, and viscosity. The range price of the commercial BMF differs depending on the volume of required blood. However, commercial BMF is not customizable because it is made for broad markets and specific implementation. For this reason, customization and preparation of BMF are needed for more precise usage.

This article reviews numerous materials and methods used to prepare BMFs, focusing mainly on those advanced for Doppler ultrasound imaging rather than those developed more specifically for alternative ultrasound mechanisms such as high-intensity focused ultrasound or elasticity imaging (electrography).[3] Many of the physical properties, acoustic properties, and other measurements were done with different methods applied to develop general BMF. Although different types of mixture fluids and scatter particle materials

Address for correspondence: Prof. M. Z. Matjafri, Department of Medical Physics and Radiation Science, School of Physics, University Science Malaysia, 11800 Penang, Malaysia. E-mail: mjafri@usm.my

How to cite this article: Oglat AA, Suardi N, Matjafri MZ, Oqlat MA, Abdelrahman MA, Oqlat AA. A review of suspension-scattered particles used in blood-mimicking fluid for doppler ultrasound imaging. J Med Ultrasound 2018;26:68-76.
used in BMF preparation have been reviewed by Hoskins, the current review aims to provide a better understanding of the pros and cons of various techniques in ultrasound research. Artificial or mimic blood is prepared as a particle suspension in fluids, there are many particles used in the preparation of BMF, where many of these particles have a diameter close to the diameter of human’s red blood cells. The hardened red blood cells studied by Law et al.[14] showed that the backscattered power increased due to the change in the density and compressibility factor of the hardening process. In other words, blood gives the same backscattered power with 4% suspension by volume of hardened red cells in saline, and the viscosity of hardened cells was 1.06 Pa at room temperature which means less than water’s viscosity. These hardened red blood cells were used in both pulse wave and continuous wave (CW) studies. However, the main disadvantage of this process is that it takes 8 h for preparation, which is relatively longer than the time compared to other mimic blood fluid preparation. Also, separating the red cells and suspending them again in saline is a significant way to prevent red cell aggregation. [5] In general, the BMF using in vitro hemodynamic styles of Doppler ultrasound should preferably have identical properties to real human blood and be prepared in a simple method.

Why the red blood cells (erythrocytes) are responsible for the blood scattering in ultrasound, although that the blood consists of erythrocytes, platelets (thrombocytes), and white blood cells (leukocytes)?

The spectrum of Doppler signal depends on the red blood cells of whole blood components. Blood consists of suspension materials, namely, red blood cells (erythrocytes), platelets (thrombocytes), and white blood cells (leukocytes). Due to the presence of a comparatively low number of platelets and leukocytes, it is typically supposed that the red blood cells (erythrocytes) are responsible for blood scattering in ultrasound imaging. The average diameter of the erythrocyte is 7 µ, which is much less than the ultrasound wavelength that is around 0.2 mm–0.5 mm. Thus, the single erythrocyte works as a point scatter, the combined influence is referred to as Rayleigh scattering. The size of the pulse-echo (PE) from blood is tiny compared to that resulted via specular reflection with tissue interfaces. One result relating to the Rayleigh scatter process is that the power of the scattered signal wave rises with the fourth power of frequency \((1-f^4)\).[6,67]

### Table 1: Specifications of the BMF defined as the IEC standard. \(f\) is the acoustic frequency (Hz)

| Acoustical and physical properties of BMF | Values |
|-----------------------------------------|--------|
| Viscosity \((\times 10^{-3} \text{ Pas})\) | 4.0±0.4 |
| Attenuation \((\text{dB/cm/MHz})\) | \(<0.1\times10^{-6} f\) |
| Acoustic speed \((\text{m/s})\) | 1570±30 |
| Density \((\times 10^3 \text{ kg/m}^3)\) | 1.050±0.040 |

\(F\) is the acoustic frequency (Hz)[67]

### PHYSICAL PROPERTIES OF BLOOD-MIMICKING FLUID

#### Density

The particle materials used in BMF preparation should be able to remain suspended (not float or ascent) inside a liquid; in other words, it is important to remain neutrally buoyant even at minimum speeds.[1,7-10] The density of particles should approximate as much as possible to the human blood density which is between 1.01 and 1.09 g/cm³. Some particles were used but were inappropriate in this regard, like Sephadex, which has an announced density of 1.7 g/cm³. However, some particles are suitable as suspension particles in the mixture fluid such as nylon and polystyrene with a density of 1.03 and 1.05 g/cm³, respectively.[11] The density of particles should be appropriate to allow them to suspend in the fluid, particularly when these particles flow along the tube or vessel. During steady flow, with a zero-speed zone at the bottom of the tube, the velocity profile should be parabolic. The main problems related to the densities of particles will happen if the density of particle is lower or higher than the fluid density.[1]

#### Particle size and concentration

Erythrocytes are concave on both sides, their diameter, thickness, and mean volume are 7–8, 2–3, and 87 µm, respectively.[12] Hematocrit (which is the volume concentration of erythrocyte) ranges from 40% to 50% in normal people.[13-15] Particles that should be used in BMF are mostly spherical or shaped like a disk, for example, polystyrene microspheres with particle diameters 10–30 µm,[8,10,16] 3–20 µm for nylon,[10,17-20] and 20–70 µm for Sephadex.[21] Low scattering particle with a minimum volume concentration is mostly used because backscatter from erythrocyte is lower than from the particles.

#### Viscosity

Viscosity has an immediate effect on the velocity in the including vessel.[22] For instance, in the so-called inlet zone to tube, the expansion of the tube after the velocity is steady is inversely proportional to the fluid viscosity. The velocity will increase with a higher viscosity fluid, and this velocity occurs before the beginning of turbulence, and this is an eligible advantage of a flow test object. High velocity occurs due to high viscosity fluid, and this happens before the start of turbulence, which is the primary disadvantage of a flow test object.[23] The measurements of dynamic viscosity for overall human blood with high shear rate are reported as 3.5–4.5 mPas[24,25] and with a different shear rate at 2.25–4.5 mPas.[26-28] Pedley stated that we could consider blood as Newtonian in large vessels (>0.5 mm) with viscosity measured under situations of high shear rate, that is, 4 mPas.[29]

Viscosity coefficient is constant for a Newtonian fluid, and this viscosity is not based on the shear rate at a specific temperature. When the viscosity depends on PCV and shear rate, we can consider that blood is non-Newtonian.[30,31] The viscosity that depends on the shear rate is displayed at most tiny vessels of arterial measure. Blood acts as a Newtonian fluid when treating with the major vessels of the body, and in this case, there is benefit for Doppler ultrasound with major vessels.
with a viscosity of 0.004 kg/ms.\textsuperscript{[29]} When the red blood cells flow, the signal of Doppler ultrasound appears. Also, several theoretical models have progressed to a better understanding of viscosity depending on the shear rate method.\textsuperscript{[32,42]} The strength of the Doppler signal does not linearly rise with PCV\textsuperscript{[33,44]} Furthermore, the main disadvantage of Doppler signal is that it produces noise, which is the product of the vast difference between the numeric and geometric order of the sample volume within the scattering elements, the occurrence of this noise causes many problems. For example, the exhibited spectrum of Doppler ultrasound has a speckle, the casual difference in the frequency being of similar size to the spectrum itself.

The correlation between shear rate and viscosity is inversely proportional because the blood is a non-Newtonian fluid. Thus, the viscosity increases with the decreased shear rate. A shear rate of 23/s measured at 37°C with natural range is 0.84–0.56 Pas (mean value = 0.7 Pas) and 0.51–0.38 Pas (mean value = 0.44 Pas) at a shear rate of 230/s.\textsuperscript{[23]} The main cause that makes the blood behave like a non-Newtonian is an aggregation of red blood cells created in fixed blood and at low shear rates (<50/s) [Figure 1].\textsuperscript{[35]} It is affected by the existence of fibrinogen in the blood (a soluble protein in blood plasma).\textsuperscript{[36]} The aggregation will separate when the shear rate rises, which gives a change in viscosity. This separation of aggregates also minimizes the size of the scattering centers so that it will result in a decrease in the backscattered power.\textsuperscript{[32,33,35]} In small vessels (<0.6 mm diameter), the non-Newtonian differences in viscosity become significant.\textsuperscript{[37,38]} By applying high frequency and color flow mapping, we can see the small vessels on scanner.\textsuperscript{[39]} The model of a non-Newtonian fluid should work with small vessels accurately. However, in the large anatomical structures such as heart chambers or vessel bifurcations, the effect of flow will increase by a non-Newtonian flow.\textsuperscript{[40–42]} The large anatomical structure will be clear in any vessels with the pulsatile flow.\textsuperscript{[38,43]} The flattening of the velocity profile occurs in vessels with the laminar flow when the shear rate over the streamlined center is zero.\textsuperscript{[36]}

Hence, the best fluid to use is the blood itself.\textsuperscript{[34]} However, there are some obstacles related to using blood and its ingredients. There is a possibility of biohazard and attention must be taken to reduce this hazard. Red blood cells can easily damage in vitro because the expiration date of erythrocytes is limited, and this prevents the use of blood as a normal fluid in long-range studies of measurement and quality monitor. Blood properties such as physical and acoustical properties differ according to temperature, humidity, and atmospheric pressure. Furthermore, the complexity of flow models occurs at higher temperature. However, non-Newtonian model fluid should work with small vessels accurately. But, in the large anatomical structures like heart chambers or vessel bifurcations, the effect of flow will be non-Newtonian flow.\textsuperscript{[40–42]} The large anatomical structure will be apparent in any vessel with the pulsatile flow.\textsuperscript{[38,43]}

**Acoustical Properties of a Blood-mimicking Fluid**

**Ultrasound backscattering of blood**

One of the essential features of a possible suspension in the BMF is constancy and the ultrasound backscatter.\textsuperscript{[44,45]} The theoretical and experimental studies of scattering ultrasound from blood have been done extensively. Theoretical models depend on both of discrete Rayleigh scattering\textsuperscript{[46]} and the continuum approach.\textsuperscript{[32,47,48]} Some studies have used an integrated approach, they have presented that the backscatter coefficient is proportional to both the product of backscattering cross-section and packing factor.\textsuperscript{[31]} The physical properties (compressibility, size, and density) and the packing factor of the individual scatter determine the distribution and interaction of scatters. However, the mixture fluid is determined by the backscattering cross section.\textsuperscript{[49]}

In general, two main factors affect the scattering properties of blood: first, physical properties of the erythrocyte (distribution of size, compressibility, acoustical properties, and density) and second, distribution properties (flow disturbance, hematocrit, and plasma proteins that encourage red cell grosening). However, when the proportion of particle diameter for an erythrocyte to ultrasound wavelength is in the field (0.009–0.036) and with an ideal frequency of 2–8 MHz at minimal scatter concentrations, the Rayleigh scattering with a frequency reliance of 1–f\(^4\) will occur.\textsuperscript{[50,51]} The rate based on the scattering overtake 1-f\(^4\) when the scatter concentration is more than 50%.\textsuperscript{[52]} The difference of backscatter measurement with hematocrit is linear up to nearly 8%, arrives a top that is hugely based on flow conditions around 12%–26%, and then reduces with the rising hematocrit. Due to the backscatter based on flow conditions, the measurements should be carried out under regular flow and at physiologically closely connected shear rates. Backscatter and the suspended red cells have been used in many studies. In 1977, the IEC had commented that a standard BMF should have similar backscatter to that of real human red cell suspensions under regular flow (draft IEC 1685 standard), Shung et al. presented a brief review about that.\textsuperscript{[53]}

![Figure 1: (a) Erythrocytes showing – a^ : One cell and b^ : a gross cell. (b) Orgasol™ or Nylon particles showing – a^ : solo particle and b^ : an aggregate of particles. Small divisions equal 2.2 μm.](image)
The backscatter measurements depend on exchange method. Backscatter is defined as a reflected power of sample surface. The backscatter power measurement is affected by two main system parameters: first, the magnitude of the useful scattering (beam zone, spatial sensitivity response of the detector, and time section forms) and second, the capacity of the system (sensitivity, gain and receiver area, signal-to-noise ratio, and incident intensity). For any real value of backscatter coefficient, an adjustment process is required to remove system-particular parameters. Measurements of the backscatter are usually carried out using a single plane transducer works as a sender and receiver. Furthermore, focused transducers have been used, but they demand additional complex normalization procedures. Despite different backscatter values of measurements, there is no final backscatter scale test object. This is necessary to evaluate mistakes and find the precision of real backscatter measurements between several laboratories. In this case, because of the hardness of actual backscatter measurements, relative measures are necessary, so the measurements of backscatter of the BMF should be compared with human blood flow measurements by applying the same measurement system.

When using the backscatter power of BMF in a Doppler flow test, the object should be steady, reproducible, and entirely described. The measurements of penetration deepness and sensitivity are necessary because they should be similar to backscatter from the BMF and flow human blood. Sometimes, the BMF has an inappropriate characterization of scattering properties and appears to be frequently higher than human blood scattering. For instance, fresh pig blood may have similar scatter to that of real human blood, but for experimental researches and high-range firmness, a proper synthetic blood mimic is more desirable. The backscatter should be recognized by the draft IEC 1685, with relative mistakes not more than a factor of 2 ± 3 dB. In some experiments, for example, this will participate to slip lower than ± 7% in the measurement of penetration deepness using a standard flow Doppler test object.

The Doppler signal should be shaped like a Gaussian method when the value of scatters in the sample volume is very high, which is the situation of human red blood cells with the count of 5 × 10⁷/mm³. However, the scattering cannot be Gaussian, when the number of red blood cells is incomplete or deficient. For comparing the statistical properties of BMF and human blood, the small data is required. Two methods are used to scan the statistical kind of the Doppler signal: first, calculation of the first and second demand statistics after spectral analysis from the acoustic Doppler signal and second, testing the radiofrequency (RF) spectrum of the received Doppler signal. Using this process, Hoskins et al. explained that there is no variation in the statistical properties of human blood and particles like a Sephadex for concentrations as minimal as 1% by volume. Furthermore, the vessel itself will impact on the scaling of Doppler frequencies within the Doppler spectrum because of the difference in backscattered power over a vessel such as ultrasound beam shape. The scaling of Doppler signals will also be affected by the attenuation from different parts of the vessel lumen. A small value of attenuation in real red blood cells is 0.9 dB/cm at 3.5 MHz.

Backscattered power rises when flow becomes turbulent because of the difference in density between the erythrocytes and plasma. During the turbulence, the flow will be highly accelerating because the erythrocyte and plasma have to be "drawn apart" to form larger scattering position. When the hematocrit is >0.10, this effect will be visible. Thus, it is essential to apply a fluid with a good physiological hematocrit. The speed of sound, attenuation, and backscattered power are the acoustic properties of interest. The important point is having a correct speed of sound in applications like measurements of volume flow wherever the vessel’s cross-sectional area is being measured by ultrasonic means. The shear rate, turbulence, and hematocrit can affect the backscattered power.

**Speed of sound and attenuation**

The acoustic speed in the BMF should be identical to the tubes and tissue-mimicking material (TMM) to prevent refraction artifacts. The speed of sound in BMF and TMM is usually 540 m/s. The refraction artifacts can be noticed when using tubes with a high velocity of sound. The speed of BMF in the draft IEC 1685 standard is 1570 ± 30 m/s. This vast range permits the speed to correspond vessel wall, blood, and the TMM of a flow test object. The rate of speed of sound and attenuation has been studied for human blood. The attenuation of the BMF must be <0.1 dB/cm MHz, as recommended by the draft of IEC 1685 standard. Hence, to reduce inhomogeneity of the sound scope into the tube, the attenuation of the BMF must be minimal. The acoustic speed of the BMF was measured by PE signal technique while the attenuation was measured by proceeding a Fast Fourier Transform on the RF signal from the reflector.

**Effect on velocity profile and distribution of particles**

Typically, the red blood cells distribute in large vessels. The particle moves toward the center of the vessel due to its force which results from the presence of a shear gradient. Therefore, it is not suitable to use large particles in small vessels due to the particles’ inability to take over a small proportion of the diameter, and this can have effects on both particle distribution and the velocity profile. Also, this effect can be an unnoticeable effect. It is essential to utilize particles that are tiny and similar to red blood cells for many reasons.

One, to make sure that the concentration of particle is rising even for the small sample volume in the most narrowing focused Doppler beam. Two, to supply a BMF that may probably be helpful at higher frequencies (non-Rayleigh scattering increase due to the high proportion of diameter to wavelength for massive particles). Finally, to provide a BMF that may probably be helpful in tiny or small vessels. However, one of the main cons of using large particles in small vessels is the aggregation or clotting of these particles inside the flow track and then causing flow obstructions and obstacles.
The rheological or physical parameters of BMF are the viscosity, the density, and the particle concentration or hematocrit. Blood behaves like a Newtonian liquid at high shear rates; in other words, its viscosity is not based on velocity gradient when the direct and equivalent flow is determined. However, the velocity of improving turbulent flow is based on hematocrit because the presence of red blood cell rises the constancy of the flow.[64]

**TYPES OF SUSPENSION PARTICLES USED FOR BLOOD-MIMICKING FLUID PREPARATION**

**Blood-mimicking fluid using Orgasol™ (nylon) particles**

BMF based on the use of the smooth powder of Orgasol™ (nylon) suspended in a mixture of water and glycerol is used as an alternative to blood.[20] However, there is a disadvantage of applying nylon particles despite having densities and diameters close to red blood cell that these particles aggregate at low shear rates to give non-Newtonian manner which are the physical features of real blood.[65] This method does not only affect the flow in small diameter vessels, but also in large structures [Figure 1].[38]

Some studies have used different diameters of nylon particles such as 5, 10, and 20 µm with 1.03 g/cm³ as density. The reliance of attenuation, speed or velocity of sound, and backscatter power with nylon particle diameter, at a constant particle concentration by weight 1.82%, is tabulated in Tables 2 and 3.[42] Small effect on the speed of sound and attenuation from nylon diameter can be observed when the scattered concentrations are deficient. This was set fundamentally by the glycerol-to-water ratio. The outcome of backscatter measurements on BMF with particle scattering of 5, 10, and 20 mm diameter is explained in Figure 2 as a mission of scattering particle concentration.[11, 42]

A suitable BMF for utilizing with Doppler ultrasound was applied with (5-µm-diameter nylon particles) as a scatter particle. This BMF has the velocity of sound of 1547 m/s; attenuation of 0.26 dB/cm at 5MHz, density of 1037 kg/m³, and viscosity of 4.1 ± 0.1 mPa/s. In addition, a new type of surfactant called household surfactant is used to reduce air bubbles.[17] For testing the influence of “red blood cell density,” the basic structure of the BMF was preserved. The BMF was established on a suspension of nylon or Orgasol™ particles. The norm solution was made by mixing pure water, surfactant, dextran, and glycerol at concentrations of 83.86%, 0.9%, 3.36%, and 10.06%, respectively, with 1.82% 5-µm Orgasol™ particles by weight.[18]

For inspecting the influence of red blood cell density, the basic structure of the BMF was preserved. The BMF was established on a suspension of nylon or Orgasol™ particles for examining the blood flow in flow phantom. The norm solution was made by mixing pure water, surfactant, dextran 185,000 D, and glycerol at concentrations of 83.86%, 0.9%, 3.36%, and 10.06%, respectively, with 1.82% 5-µm Orgasol™ particles by weight. Magnetic stirrer device was used for mixing the items for preparation BMF and then filtered it to eliminate any residual mass clumps by vacuum pump; this filtering was done through a 32-µm sieve. Ultimately, before measuring the acoustical and physical properties of BMF, it was mixed and degassed by vacuum pump technique until removal of the air bubbles.[18]

BMF is necessary for the vessel flow phantom, for reflected ultrasonic sound waves, and for assessing the speed of flow in the same method as in arteries. Utilizing 5-µm-diameter Orgasol™ particles (same size to red cell) is more prevalent in the literature and also cited in IEC 61685.[19] Although this fluid (BMF) is a Newtonian, it has extremely similar features to human blood, with a sound speed of 1548 m/s, the viscosity of 4.1 ± 0.1 MPa/s, density of 1037 ± 2 kg/m³, and attenuation coefficient of 0.05 ± 0.01 dB.cm/MHz.[19] However, the BMF was prepared in a similar method to the previous methods used to prepare BMF. First, a plastic beaker was used with a size of two times the fluid needed to prepare BMF to avoid overflow of the components during stirring, components of the fluid which are required for the sample are weighed in a fume hood and poured into the plastic beaker, and the stirrer was turned on for 2 h. Second, a vacuum pump device was used to degas the fluid mixture for 2 h. Finally, both the acoustical

---

**Table 2: Dependence of backscatter, attenuation and speed of sound for the plasma base, BMFs with Orgasol™ concentration (1.82% and diameter 5, 10 and 20 mm) and human red blood cells resuspended in saline[43]**

| Backscatter power at 5 MHz (dB) | Attenuation at 5 MHz (dB/cm) | Speed of sound (m/s) |
|---------------------------------|-----------------------------|---------------------|
| 5 µm Orgasol™                   | 0                           | 1547                |
| 10 µm Orgasol™                  | 7.9                         | 1547                |
| 20 µm Orgasol™                  | 13                          | 1548                |
| Human blood                      | 0                           | 1580                |

---

**Figure 2: Regular flow from blood-mimicking fluid with relative backscatter plotted versus nylon particle ratio with different particle diameters of 5, 10, and 20 µm[18]**


| Properties                      | IEC 1685 draft specifications | Human blood (37°C) | Recommended BMF (22°C) |
|---------------------------------|-------------------------------|--------------------|------------------------|
| Scatterer size (µm)             |                               |                    |                        |
| Hematocrit (percentage volume)  | 7                             | 5                  |                        |
| Density (kg m⁻³)                | 1050±40                       | 1053               | 1037±2                 |
| Viscosity (mPas)                | 4±0.4                         | 3                  | 4.1±0.1                |
| Velocity (m/s)                  | 1570±30                       | 1583               | 1548±5                 |
| Attenuation (dB/cm MHz)         | <0.1                          | 0.15               | 0.05±0.01              |
| Backscatter (F⁻⁴/m/sr)          | cf human blood                | 4×10⁻⁴            | cf human blood         |
| Fluid properties                | Newtonian                     | Non-Newtonian      | Newtonian              |

BMF: Blood-mimicking fluid, IEC: International Electrotechnical Commission

For studying the thermal and acoustic properties of high intensity-focused ultrasound, the BMF has been advanced. The BMF relies on a degassed water solution scattered with polyethylene microspheres, Gellan gum, glycerol, and Orgasol™ particles. A wide range of physical properties are inclusive of thermal conductivity, viscosity, attenuation coefficient, the velocity of sound, and diffusivity. Moreover, the BMF utilized for the tortuous vascular wall with fewer flow phantoms was a standardized model with acoustic and viscosity properties agreeing to those of human blood. The added materials to prepare BMF substances are a mixture of Dextran 185000D, Tergitol™ surfactants, Orgasol™, glycerol, distilled water, and potassium sorbate with specific weight amounts of 3.3%, 0.9%, 1.8%, 10.0%, 83.7%, and 0.3%, respectively. With adding 5-µm-diameter of Orgasol™ as a scattering material, the physical and acoustical properties matched the real human blood cells.

Several research studies used the Orgasol™ material as suspension particles for preparing BMF. For example, BMF was utilized for the flow test object phantoms, and it was a standardized model with acoustic and viscosity properties consistent with those of human blood. The materials added for the preparation of BMF substance mixture were dextran of average molecular weight 185,000 D, Synermonic N surfactants, Orgasol™, pure glycerol, and distilled water, the mixture’s ratios of weights were 3.363%, 0.9%, 1.82%, 10.06%, 83.86%, and 0.3%, respectively. With adding 5-µm-diameter of Orgasol™ as a scattering material, the physical and acoustical properties matched the real human blood cells.

Another example was that a BMF was prepared with polyethylene microspheres, Gellan gum, glycerol, and Orgasol™ particles. A wide range of physical properties are inclusive of thermal conductivity, viscosity, attenuation coefficient, the velocity of sound, and diffusivity. Moreover, the BMF utilized for the tortuous vascular wall with fewer flow phantoms was a standardized model with acoustic and viscosity properties agreeing to those of human blood. The added materials to prepare BMF substances are a mixture of Dextran 185000D, Tergitol™ surfactants, Orgasol™, glycerol, distilled water, and potassium sorbate with specific weight amounts of 3.3%, 0.9%, 1.8%, 10.0%, 83.7%, and 0.3%, respectively. With adding 5-µm-diameter of Orgasol™ as a scattering material, the physical and acoustical properties matched the real human blood cells. They have used different types of Orgasol™ (nylon) powder with different densities and with the same diameters (5–15 mm), Orgasol™ 2001, Orgasol™ 3501, and Orgasol™ 1009. The densities of three different types are 1.02, 1.06, and 1.13 g/ml, respectively. Finally, the Orgasol™ (nylon) powder 2001 was used for preparing BMF and it was mixed with mixture fluid (water and glycerol). Acoustical and physical properties of BMF were suitable and agreed the values at IEC standard.

Blood-mimicking fluid using Sephadex particle

Law et al. briefly studied and used nonbiological blood. The used particles in BMF preparation should regularly be distributed in the fluid. When the density of particles is more than that of the surrounding liquid, the settling may happen, this can surely be visible at low speeds for particles such as Sephadex. The Sephadex particle has been studied by many researchers. Sometimes, the BMF is prepared with the powder material Sephadex, which is a scattered particle and suspended in water. Sephadex particles have a diameter and density almost similar to the human blood. However, according to past studies, although the Sephadex’s density and size are much greater than the real human density and size, the backscattered measurements and other measurements of Sephadex are not too much different from that of human blood such as 20–50 µm at a concentration of 1.5 g/L. Moreover, they aggregate or clot at low shear rates to give non-Newtonian manner which is the physical feature of blood.

The backscattered power of the scatter suspension is based on several factors such as the size of scattering, concentration volume of scattering, and variation in size modulus and density between the suspending fluid and the scatter. When the erythrocyte concentration increases, the backscattered power will increase, but the backscattered power reduces with plateaus at PCV percentage with 15%–30%. The backscattered power of Sephadex particle suspension in fluids follows an identical style of human blood when the particle concentration increases, and this is possible to be an event for other particles. The backscattered power of any size and strength will be nearly relative to the third power of particle diameter. Furthermore, the backscattered power will increase with the variance between the size elastic modulus and the density of liquid suspension and the scatter. The particles’ diameter and density distribution of Sephadex usually are 20–70 µm and 1.7 g/cm³, respectively. Some researchers discovered that when using Sephadex particles with identical volume concentration to blood, the backscattered power will be massive. For example, dialysis tubing phantom used a Sephadex...
Blood-mimicking fluid using polystyrene particle

As we said before, when the density of particles is more than the surrounding fluid, the settling might happen. Thus, when preparing the BMF, we should take into consideration that the fluids and scatter particles should be suitable and simulate the acoustical and physical properties of real human blood. Numerous studies used polystyrene as a scattering material for BMF preparation since the polystyrene density and particle size are close to that of the red blood cells. A BMF with polystyrene has been studied and used by Kimme-Smith, he prepared BMF and used it with Doppler phantom. BMF was prepared with the total amount of 500 mL, composed of glycerol (96 mL), polystyrene divinylbenzene microspheres (11 mL), and degassed distilled water (393 mL). The diameter of microsphere was 29.4 µm, and also the viscosity, density, and scattering properties of blood were suitable to be used in Doppler flow.[10] The liquid is a mixture of glycerol and degassed water in a proper ratio to give a gravity density of 1.043 g/cm³. This specific density was chosen to minimize deposition of the third element of the BMF. Polystyrene microspheres with 30 µm as a particle size were used. The polystyrene microspheres supply scattering from the liquid; their concentration and size distribution were chosen to provide an equal level of backscatter and to the actual blood. The velocity of sound of this liquid was 1546 m/s and the attenuation coefficient was 0.1 dB/cm.[58]

Since polystyrene microspheres are suitable to scatter particles in BMF, many of the liquids are prepared with proper density and viscosity which is close to polystyrene density of 1.05 g/cm³. For example, a preparation of BMF with a density of 1.05 g/ml that was complies with the density mentioned in the IEC standard, however, this BMF prepared by a suitable mixture fluid that made of glycerin aqueous solution and water-soluble and dispersed with polystyrene microsphere particles.[10]

The BMF was utilized for the flow test object phantoms, and it was a standardized model with acoustic and viscosity properties agreeing to those of human blood. The materials added for the preparation of BMF substance mixture were, silicone oil, poly (ethylene glycol) 400, and distilled water, with the specific amount of wt% at 25.0%, 18%, and 57%, respectively. With 5-µm-diameter polystyrene as a scattering material, the physical and acoustical properties were matched to those of the real human blood cells.[31]

A specific instrument measured the physical properties (the viscosity and the density) and the acoustical properties (the speed of sound and the attenuation) of BMF. For example, the electronic rotational and U-tube viscometers were used for the viscosity measurement, pycnometer and densitometer were used for density measurement, and signal PE by A-scan GAMPT ultrasonic device was used for the acoustical measurements (attenuation and speed of sound). However, several types of particle scatters are used in BMF preparation and in several methods.[74–77] The most common BMF materials and preparation methods are shown in Table 4.

**Table 4: Most common blood-mimicking fluid items and their preparation methods**

| Material number | Material | Method | Application | References |
|-----------------|----------|--------|-------------|------------|
| 1.0             | Orgasol™ (nylon) | Nylon particles of three different sizes (5, 10, and 20 µm) were mixed with four materials of fluids (dextran, surfactant, pure water, and glycerol) based on weight by the magnetic stirrer and then the mixture was filtered by sieve and degassed by a vacuum pump | Speculating the achievement of Doppler ultrasound tool in the test object by using artificial blood | [1,3,5,11,12,17,40,49] |
| 2.0             | Polystyrene microspheres | BMF was prepared with the acoustic speed and density with polystyrene particles as a scattered material suspension in the fluids and it was mixed with water-soluble silicone oil | Using Doppler ultrasound for measurement of blood flow | [2,8,9,13,15] |

BMF: Blood-mimicking fluid
used as scatters and suspension in fluid should be with a diameter close to the erythrocyte diameter, at least between 5 and 10 µm and with a density of 1.05 ± 0.04 g/ml to give the same physical and acoustical properties, these properties must be close to the values which were determined by the IEC standard.

The density measurement of BMF in most of the research studies was done either by using the pycnometer method or by the densitometer to produce accurate measurements of the liquid mixture in unit g/ml or g/cm³. Furthermore, several previous studies measured the viscosity of BMF using U-tube viscometer and electronic rotational viscometer. Finally, the speed of sound of mixture fluid and BMF was measured in all experimental studies using PE technique.¹,²,³,⁴,⁵,⁶,⁷,⁸,⁹,¹₀,¹¹,¹₂,¹³,¹⁴,¹⁵,¹⁶,¹⁷,¹⁸

Acknowledgment

This research study was supported by Prof. Dr. Mohammad Zubir Mat Jafri. We thank our colleagues from Medical Physics and Radiation Science department, Universiti Sains Malaysia, who provided insight and expertise that greatly assisted the research, although they may not agree with all the interpretations of this article. Moreover, we thank Dr. Ahmed Nouri, Pharm.D., who contributed in the revision of this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rammarine KV, Nassiri DK, Hoskins PR, Lubbers J. Validation of a new blood-mimicking fluid for use in Doppler flow test objects. Ultrasound Med Biol 1998;24:451-9.
2. Lubbers J. Application of a new blood-mimicking fluid in a flow Doppler test object. Eur J Ultrasound 1999;9:267-76.
3. Liu Y, Maruvada S, King RL, Herman BA, Wear KA. Development and characterization of a blood mimicking fluid for high intensity focused ultrasound. J Acoust Soc Am 2008;124:1803-10.
4. Law YF, Johnston KW, Routh HF, Cobbold RS. On the design and evaluation of a steady flow model for Doppler ultrasound studies. Ultrasound Med Biol 1989;15:505-16.
5. Chien S, Usami S, Dellenback RJ, Gregersen MI, Nanninga LB, Guest MM, et al. Blood viscosity: Influence of erythrocyte aggregation. Science 1967;157:829-31.
6. Burns PN. The physical principles of Doppler and spectral analysis. J Clin Ultrasound 1987;15:567-90.
7. Samavat H, Evans JA. An ideal blood mimicking fluid for Doppler ultrasound phantoms. J Med Phys 2006;31:275-8.
8. Yoshida T, Tanaka K, Sato K, Kondo T, Yasukawa K, Miyamoto N, et al. Blood-mimicking fluid for the Doppler Tissue Flow Test of Medical Diagnostic Instruments. In: Ultrasonomics Symposium (IUS), 2012 IEEE International. IEEE, 2012.
9. Tanaka K, Yoshida T, Sato K, Kondo T, Yasukawa K, Miyamoto N, et al. Blood-mimicking fluid for testing ultrasonic diagnostic instrument. Jpn J Appl Phys 2012;51:07GF18.
10. Yoshida T, Sato K, Kondo T, Blood-mimicking fluid using glycols aqueous solution and their physical properties. Jpn J Appl Phys 2014;53:07FK01.
11. Koza JR, Jones LW, Keane MA, Streeter MJ, Al-Sakran SH, et al. Toward automated design of industrial-strength analog circuits by means of genetic programming. In: Genetic Programming Theory and Practice II 2004: p. 121-42.
12. Udroiu I. Estimation of Erythrocyte Surface Area in Mammals. arXiv Preprint arXiv: 1403.7660; 2014.
13. Hodgson DR, Mckeever KH, McGowan CM. The Athletic Horse: Principles and Practice of Equine Sports Medicine. Virginia, Maryland: Elsevier Health Sciences; 2013.
14. Duck FA. Physical Properties of Tissues: A Comprehensive Reference Book. Bath, England: Academic Press; 2013.
15. Duck F. Optical properties of tissue including ultraviolet and infrared radiation. Physical Properties of Tissue. 1990. p. 43-71.
16. Kimme-Smith C, Hussain R, Durinckx A, Tessler F, Grant E. Assurance of consistent peak-velocity measurements with a variety of duplex Doppler instruments. Radiology 1990;177:265-72.
17. Thorne MR, Poopeing TL, Rankin RN, Steinman DA, Holdsworth DW. Use of an ultrasound blood-mimicking fluid for Doppler investigations of turbulence in vitro. Ultrasound Med Biol 2008;34:1163-73.
18. Raine-Fenning NJ, Nordin NM, Rammarine KV, Campbell BK, Clewes JS, Perkins A, et al. Determining the relationship between three-dimensional power Doppler data and true blood flow characteristics: An in-vitro flow phantom experiment. Ultrasound Obstet Gynecol 2008;32:540-50.
19. Zhou X, Kenwright DA, Wang S, Hossack JA, Hoskins PR. Fabrication of two flow phantoms for Doppler ultrasound imaging. IEEE Trans Ultrason Ferroelect Freq Control 2017;64:53-65.
20. Oates CP. Towards an ideal blood analogue for Doppler ultrasound phantoms. Phys Med Biol 1991;36:1433-42.
21. Hoskins PR, Loupas T, McDicken WN. A comparison of the Doppler spectra from human blood and artificial blood used in a flow phantom. Ultrasound Med Biol 1990;16:141-7.
22. Yalcin O, Ortiz D, Williams AT, Johnson PC, Cabrera P. Pressure and blood flow determine microvascular apparent viscosity. Exp Physiol 2015;100:977-87.
23. Wells RE Jr., Merrill EW. Influence of flow properties of blood upon viscosity-hematocrit relationships. J Clin Invest 1962;41:1591-8.
24. Mandal M. Rheology of blood: Biophysical significance, measurement, pathophysiology and pharmacologic therapy. Vol. S. Bihar, India: 2016. p. 2165-84.
25. Lowe GD. Clinical Blood Rheology. National Agricultural Library, USA: CRC Press; 1988.
26. Elblbesy MA, Hereba AT. Computation of the coefficients of the law model for whole blood and their correlation with blood parameters. Appl Phys Res 2016;8:1.
27. Erkan M, Koksal C. The relationship between shear rate and vessel diameter. Anesist Analg 2003;96:307.
28. Papatsoannou TG, Stefanakis C. Vascular wall shear stress: Basic principles and methods. Hellenic J Cardiol 2005;46:9-15.
29. Feddey TJ. The Fluid Mechanics of Large Blood Vessels. Vol. 1. Cambridge: Cambridge University Press; 1980.
30. Rabbly MG, Shupti SP, Molla MM. Pulsatile non-Newtonian laminar blood flows through arterial double stenoses. J Fluids 2014:2014: p. 1-13.
31. Nichols W, O’Rourke M, Vilachopoulos C. McDonald’s Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. Gainesville, Florida, USA: CRC Press; 2011.
32. Angelsen BA. A theoretical study of the scattering of ultrasound from blood. IEEE Trans Biomed Eng 1980;27:61-7.
33. Mo LY, Cobbold RS. A stochastic model of the backscattered Doppler ultrasound fluid. IEEE Trans Biomed Eng 1986;33:20-7.
34. Shung KK. On the ultrasonic scattering from blood as a function of hematocrit. IEEE Trans Sonics Ultrason 1982;29:327-30.
35. Sigel B, Machi J, Beiter JC, Justin JR. Red cell aggregation as a cause of blood-flow echogenicity. Radiology 1983;148:799-802.
36. Yuan YW, Shung KK. Ultrasonic backscatter from flowing whole blood. I: Dependence on shear rate and hematocrit. J Acoust Soc Am 1988;84:52-8.
37. Fährus R, Lindqvist T. The viscosity of the blood in narrow capillary tubes. Am J Physiol Lung Content 1931;96:562-8.
38. Thurston GB. The effects of frequency of oscillatory flow on the impedance of rigid, blood-filled tubes. Bioheorology 1976;13:191-9.
39. Singh S, de Trafford JC, Goss DE, Baskerville PA, Roberts VC. Ultrasound imaging of digital arteries. Clin Physiol Meas 1990;11:313-7.
40. Liespach D, Moravec S. Pulsatile flow of non-Newtonian fluid in
distensible models of human arteries. Biochimie 1984;21:571-86.
41. Moravec S, Liespach D. Flow investigations in a model of a
three-dimensional human artery with Newtonian and non-Newtonian
fluids. Part I. Biochimie 1983;20:745-59.
42. Mann KA, Deutsch S, Tarbell JM, Geselowitz DB, Rosenberg G, Pierce WS,
et al. An experimental study of Newtonian and non-Newtonian flow
dynamics in a ventricular assist device. J Biomech Eng 1987;109:139-47.
43. Thurston GB. Rheological parameters for the viscosity viscoelasticity
and thixotropy of blood. Biochimie 1979;16:149-62.
44. Sigelmann RA, Reid JM. Analysis and measurement of ultrasound
backscattering from an ensemble of scatterers excited by sine-wave
bursts. J Acoust Soc Am 1973;53:1351-5.
45. Yang P, Zhu H. Influence of Transducer Focus Position and Signal
Length in Backscatter Coefficient Measurement for Blood-mimicking
Fluid. In: Biomedical Engineering and Computer Science (ICBECES),
2010 International Conference on 2010. IEEE; 2010.
46. Twersky V. Acoustic bulk parameters in distributions of pair-correlated
scatterers. J Acoust Soc Am 1978;64:1710-9.
47. Angelsen BA. A Theoretical-study of the scattering of ultrasound from
blood. Model Identification Control 1981;2:225.
48. Atkinson P, Berry M. Random noise in ultrasonic echoes diffracted by
blood. J Phys A Math Nuel Gen 1974;7:1293.
49. Shung KK, Thieme GA. Ultrasonic Scattering in Biological Tissues.
USA: CRC Press; 1992.
50. Nassirii DK, Hill CR. The differential and total bulk acoustic scattering
cross sections of some human and animal tissues. J Acoust Soc Am
1986;79:2034-47.
51. Shung KK, Sigelmann RA, Reid JM. Scattering of ultrasound by blood.
IEEE Trans Biomed Eng 1976;23:460-7.
52. Chen J, Zagzebski JA. Frequency dependence of backscatter coefficient
versus scatterer volume fraction. IEEE Trans Ultrason Ferroelectr Freq
control 1996;43:345-53.
53. Shung KK, Kuo I, Cloutier G. Ultrasonic scattering properties of blood.
In: Intravascular Ultrasound. Springer; 1993. p. 119-39.
54. Campbell JA, Waag RC. Normalization of ultrasonic scattering
measurements to obtain average differential scattering cross sections for
tissues. J Acoust Soc Am 1983;74:393-9.
55. Wang SH, Shung KK. An approach for measuring ultrasonic
backscattering from biological tissues with focused transducers. IEEE
Trans Biomed Eng 1997;44:549-54.
56. Yuan YW, Shung KK. The effect of focusing on ultrasonic backscatter
measurements. Ultrason Imaging 1986;8:121-30.
57. Boote EJ, Zagzebski JA. Performance tests of Doppler ultrasound
equipment with a tissue and blood-mimicking phantom. J Ultrasound
Med 1988;7:137-47.
58. Azimi M, Kak AC. An analytical study of Doppler ultrasound systems.
Ultrason Imaging 1985;7:1-48.
59. Mo LY, Cobbold RS. A unified approach to modeling the backscattered
Doppler ultrasound from blood. IEEE Trans Biomed Eng 1992;39:450-61.
60. Shung KK, Yuan YW, Fei DY, Tarbell JM. Effect of flow
disturbance on ultrasonic backscatter from blood. J Acoust Soc Am
1984;75:1265-72.
61. Browne JE, Watson AJ, Hoskins PR, Elliott AT. Validation of a
sensitivity performance index test protocol and evaluation of colour
Doppler sensitivity for a range of ultrasound scanners. Ultrasound Med
Biol 2004;30:1475-83.
62. Sato M, Ishida H, Konno K, Komatsuda T, Furukawa K, Yamada M,
et al. Analysis of refractive artifacts by reconstructed three-dimensional
ultrasound imaging. J Med Ultrasound (2001) 2006;33:11-6.
63. Saffman P. The lift on a small sphere in a slow shear flow. J Fluid Mech
1965;22:385-400.
64. Dintenfass L. Blood Viscosity. University of Sydney: Springer Science
& Business Media; 1985.
65. Kenwright DA, Laverick N, Anderson T, Moran CM, Hoskins PR.
Wall-less flow phantom for high-frequency ultrasound applications.
Ultrasound Med Biol 2015;41:890-7.
66. Ho CK, Chee AJ, Yu BY, Tsang AC, Chow KW, Yu AC, et al. Wall-less
flow phantoms with tortuous vascular geometries: Design principles
and a patient-specific model fabrication example. IEEE Trans Ultrason
Ferroelectr Freq Control 2017;64:25-38.
67. Hein IA, O’Brien WD Jr. A flexible blood flow phantom capable of
independently producing constant and pulsatile flow with a predictable
spatial flow profile for ultrasound flow measurement validations. IEEE
Trans Biomed Eng 1992;39:1111-22.
68. Douville Y, Johnston KW, Kassam M, Zuech P, Cobbold RS, Jares A,
et al. An in vitro model and its application for the study of carotid
Doppler spectral broadening. Ultrasound Med Biol 1983;9:347-56.
69. Hoskins PR, Anderson T, McDIcken WN. A computer controlled flow
phantom for generation of physiological Doppler waveforms. Phys Med
Biol 1989;34:1709-17.
70. McDiCeken WN. A versatile test-object for the calibration of ultrasonic
Doppler flow instruments. Ultrasound Med Biol 1986;12:245-9.
71. Hoskins PR. Measurement of arterial blood flow by Doppler ultrasound.
Clin Physiol Meas 1990;11:1-26.
72. Brody WR, Meindl JD. Theoretical analysis of the CW Doppler
ultrasonic flowmeter. IEEE Trans Biomed Eng 1974;21:183-92.
73. Tortoli P, Valigimigli F, Guidi G, Pignoli P. Clinical evaluation of a
new anti-aliasing technique for ultrasonic pulsed Doppler analysis.
Ultrasound Med Biol 1985;15:749-56.
74. Oglat AA, Matjafri M, Suardi N, Oglat MA, Abdelrahman MA,
Oglat AA, et al. A review of medical doppler ultrasonography of blood
flow in general and especially in common carotid artery. Journal of
Medical Ultrasound 2018;26:3.
75. Oglat AA, Matjafri M, Suardi N, Oglat MA, Oglat AA, Abdelrahman MA,
et al. A new blood mimicking fluid using propylene glycol and their
properties for a flow phantom test of medical doppler ultrasound.
International Journal of Chemistry, Pharmacy and Technology
2017;2:220-31.
76. Oglat AA, Matjafri MZ, Suardi N, Oglat MA, Abdelrahman MA, Oglat
AA, et al. Chemical items used for preparing tissue-mimicking
material of wall-less flow phantom for doppler ultrasound imaging. J
Med Ultrasound 2018; [In press].
77. Oglat AA, Matjafri MZ, Suardi N, Abdelrahman MA, Oglat MA,
Oglat AA, et al. Anew scatter particle and mixture fluid for preparing
blood mimicking fluid for wall-less flow phantom. J Med Ultrasound
2018; [In press].