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## Blood Mimicking Fluid for Reproducing Rheological and Acoustical Blood Properties

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### Abstract

Blood tends to coagulate during therapies with extracorporeal circulation like dialysis or surgery with the use of heart-lung-machines. To study the effects of the incipient coagulation and to develop new and continuous supervising methods for blood therapies, a fluid mimicking rheological and acoustical blood properties with nearly the same amount of particles as blood is necessary. We enhanced the International Electrotechnical Commission Doppler blood mimicking fluid recipe to be used at physiological hematocrit values and to mimic incipient plasma coagulation and blood clotting. This paper presents the recipe, acoustical and rheological data of the refined blood mimicking fluid.

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**Keywords:** (Doppler) flow test object; blood-mimicking fluid; blood analogue; ultrasound phantom; hemodynamic studies

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### 1. Introduction

For many therapies it is essential to examine and monitor the properties of blood to assess the patient's condition. Especially during treatments with extracorporeal blood circulation, like surgery with heart-lung machines or dialysis, a vast number of non-physiological processes are triggered and must be supervised. Among others, this is blood coagulation due to the contact of blood with foreign surfaces. Only a few non-invasive, continuous measurement methods are available in therapy, e.g. to assess the relative blood volume, detect large clots, hemolysis, or small air bubbles, or measure blood pressure and temperature. To monitor the anticoagulation therapy and coagulation activity, complex, discontinuous and expensive laboratory blood tests must be performed.

At present there are not enough possibilities to continuously and non-invasively assess multiple blood parameters related to blood viscosity, blood volume and composition, or coagulation and simultaneously ensure patient safety and comfort. Measurands of interest are the hematocrit, rheological blood properties like the blood and plasma viscosity, the flow profile, or the detection of very small, emerging clots within the large number of red blood cells. For this measurement tasks it is worthwhile to advance ultrasound methods.

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A blood mimicking fluid (BMF) is needed for the systematic development of new ultrasound methods. The fluid shall represent rheological as well as acoustical properties of physiological human blood as closely as possible and consist of nearly the same amount of particles. Experiments with porcine or bovine blood are problematic for the systematic development of new measurement methods since blood samples can vary strongly inter- and intra-individually in their properties and the samples are not storable. The properties of BMFs can be adopted separately and in a well-defined way.

In the last 20 years a number of blood mimicking fluids have been suggested in the context of evaluating the performance of Doppler ultrasound equipment, resulting in an International Electrotechnical Commission (IEC) standard (IEC 61685:2001). Polyamide particles are used as scatterers, surfactant to disperse them in the liquid and water, glycerin and dextran to adjust viscosity, speed of sound and ultrasonic backscatter. We also used polyamide particles since the effective scatterer size is very close to that of blood, they are firmly dispersed within the solution, retain shape under mechanical stress (e.g. roller pumps), have narrow and specific size distributions (5  $\mu\text{m}$ , 20  $\mu\text{m}$ , 30  $\mu\text{m}$ , 40  $\mu\text{m}$  and 60  $\mu\text{m}$ ) and, most importantly, it has been extensively studied (i.e. Oates (1991), Rickey et al. (1995), Ramnarine et al. (1998), Lubbers (1999)). Other materials used as red blood cell mimics were cellulose particles with a diameter of approximately 20  $\mu\text{m}$  (Newhouse et al. (1982), Holdsworth et al. (1991), Frayne et al. (1993)), polystyrene microspheres with approximately 15  $\mu\text{m}$  to 30  $\mu\text{m}$  (Poots et al. (1986), Boote and Zagzebski (1988), Kimme-Smith et al. (1990)), or polysaccharide (Sephadex) particles with a diameter of 20  $\mu\text{m}$  to 50  $\mu\text{m}$  (Douville et al. (1983), McDicken (1986), Hoskins et al. (1989), Hein and O'Brien (1992)). Apart from that latex particles were used (Law et al. (1989)), oil droplets (Gill (1979)), silicon particles (Jorgensen et al. (1973)), silicon carbide particles (Giddens and Khalifa (1982)), a silicone derivative (Calil and Roberts (1985)), starch (5  $\mu\text{m}$  to 100  $\mu\text{m}$ ; Voyles et al. (1985)), talc particles (Lavandier et al. (1985)), or milk. With respect to polyamide particles all these alternatives show significant disadvantages which are larger effective scatterer sizes, a larger size distribution, short shelf life, and fewer reported applications in literature. As a plasma mimic a water – glycerol mixture, sometimes with dextran and a surfactant to disperse the red blood cell mimics, was used most often since viscosity and speed of sound can be adapted well to that of blood. Other base fluids or additives used to modify viscosity were machine cutting fluid (Rickey et al. (1995)), agar-agar or gelatin, and starch. They are not as durable and easy-to-handle as a water-glycerol mixture.

The BMF in the IEC standard focusses on a strong match between acoustic properties of blood and BMFs, mainly density, whole fluid / blood viscosity, effective particle / scatterer size, ultrasound backscatter, acoustic velocity and attenuation. However, they do not match in the number of particles per unit volume and can therefore not mimic the flow or turbulence correctly or different stages of the incipient blood coagulation with changes in plasma viscosity and formation of very few and very small clots.

To refine the existing Doppler blood mimicking fluid to the tasks of developing new ultrasound methods, we adapted the recipe from IEC 61685:2001 by changing the particle content and plasma composition, and measured its properties. The rheological and acoustical properties of the fluid can be adjusted in a medical range of interest. This paper presents the recipe for the new blood mimicking fluid and its properties measured under no-flow conditions.

## 2. Material and Methods

### 2.1 Composition of the BMF

We used the following composition for the blood mimicking fluid (all percentages by volume):

- 0 % to 40 % 5  $\mu\text{m}$  polyamide particles (Orgasol 2001 UD NAT 1, PA12, Arkema GmbH,  $\rho=1.03 \text{ g/cm}^3$ )

- 0 %, 1 % or 2 % of polyamide particles in sizes 20  $\mu\text{m}$ , 40  $\mu\text{m}$ , or 60  $\mu\text{m}$  (Orgasol 2002 D NAT 1, 2002 ES4 NAT 3, 2002 ES6 NAT 3, all PA12, Arkema GmbH,  $\rho=1.03 \text{ g/cm}^3$ )
- 0.13158 ml/m<sup>2</sup> surfactant per particle surface (Anti-Terra 250 (BYK-Chemie GmbH),  $\rho=1.0 \text{ g/cm}^3$ )
- Plasma:
  - 91.26 % of plasma water ( $\rho=0.9982 \text{ g/cm}^3$ )
  - 8.74 % of plasma glycerol ( $\rho=1.2613 \text{ g/cm}^3$ )

The blood mimicking fluid was used at room temperature. The quantity of surfactant was 31 % of the ratio used in the IEC standard to lower the viscosity at the same time by dispersing all particles. The water-glycerol-ratio of plasma equals the ratio used in IEC standard without dextran. A spherical shape of the polyamide particles was assumed to calculate the amount of surfactant needed.

The constituents perform the following tasks: polyamide particles with a diameter of 5  $\mu\text{m}$  act as red blood cell mimics and 20  $\mu\text{m}$ , 40  $\mu\text{m}$ , or 60  $\mu\text{m}$  particles as small, emerging clot analogues. Surfactant suspends the polyamide particles in the liquid but also raises viscosity. Water, glycerin and dextran mimic the blood plasma. Water ensures fluidity, glycerin adapts speed of sound and influences plasma viscosity to some extent, and dextran raises viscosity. Antifungal agent was used for biological stability. More should be added after using the fluid for experiments. The high particle content and consequently high amount of surfactant largely increased plasma viscosity, which is why we did not use dextran (150 kDa). For experiments where physical properties of the plasma shall be changed dynamically then a mixture of water, glycerol and dextran can be added during the experiment to the blood mimicking fluid.

## 2.2 Preparation of the BMF

The beaker with degassed water and a stir bar with circular cross-section was placed on a magnetic stirrer under a vacuum pump. Surfactant was added, and the mixture degassed until foaming ceased. Half cups of Orgasol were added one by one and the mixture degassed after each addition until all the Orgasol particles were suspended and the foam disintegrated. Glycerol and antifungal agent were added last, the mixture degassed adequately and sieved through a 100  $\mu\text{m}$  sieve.

The fluid is not neutrally buoyant but as do red blood cells Orgasol particles drop. We waited at least 24 h before using the BMF because some of the fluid diffuses into the Orgasol particles. Before using it for experiments the mixture was degassed again. This process can alter the particle proportion within the mixture leading to a higher particle amount the longer the mixture is degassed. Microbubbles within the mixture strongly influence attenuation, backscattering and to some extent viscosity.

## 2.3 Measurement methods

Viscosity of the BMFs was measured with a plate-plate-rheometer (Anton Paar MCR 502, CP60). Speed of sound and the attenuation coefficient were measured in pulse-echo-mode at 2 MHz center frequency under no-flow conditions (Fig. 1). Ten measurements were performed, moving the transducer 500  $\mu\text{m}$  upward after each measurement. For speed-of-sound measurement the arrival time and for attenuation measurement the amplitude of the first or second maximum were recorded. Speed of sound (Eq. 1) and attenuation (Eq. 3) were calculated with MATLAB (R2016b) using a least squares fit (*polyfit*) of first order:

$$\Delta t = c \Delta x \quad (1)$$

$$p = p_0 \exp^{-\alpha x} \quad (2)$$

With  $A \propto p$  and  $A_0 \propto p_0$  follows

$$\lg(A(f)) = -\frac{\alpha(f)}{\ln(10)} \Delta x + \lg(A_0(f)) \quad (3)$$

with  $\Delta t$  and  $\Delta x$  being the time and path difference to the echo measured at first position,  $A$  and  $A_0$  being the echo amplitudes and  $p$  and  $p_0$  the pressure amplitudes from the echos in the current and first position, and  $c$  and  $\alpha$  being the speed of sound and attenuation, respectively. Uncertainties were calculated with the error estimation structure given as an output argument by MATLAB *polyfit*. Uncertainties of the parameters resulting from the fit were much smaller than the standard deviation between different measurements.

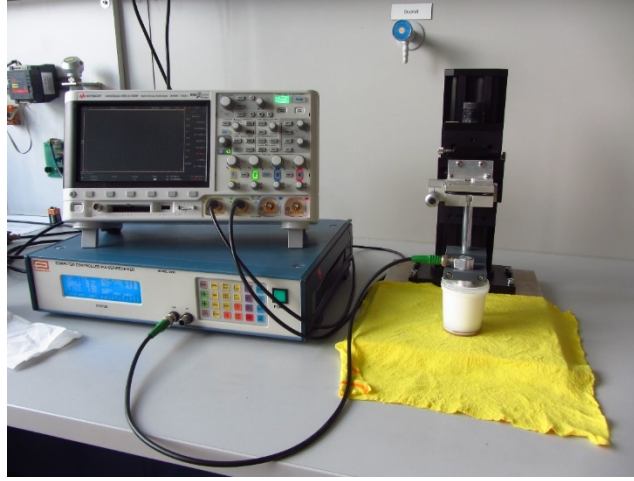


Fig. 1 Experimental setup for speed of sound and attenuation measurement.

Density was measured with a pycnometer (25 ml). The particle content was determined by filtering out water under vacuum and using a drying balance (Moisture Analyzer MA 30, Sartorius AG). However, since the discrepancy to the calculated particle content was always less than 1 % the theoretical values are used in this paper.

### 3. Results and Discussion

#### 3.1. Comparison to blood

Rheological and acoustical properties are close to that of standard values of human blood at physiological hematocrit concentrations (Tab. 1). Effective scatterer size, density and hematocrit are in very good agreement with blood. Viscosity, speed of sound and the attenuation coefficient strongly depend on the content of polyamide particles, increasing their values largely at higher particle amounts. Best agreement with blood properties can be achieved at lower particle amounts. The increased speed of sound is due to a higher speed of sound in polyamide particles than red blood cells. The increase in the attenuation coefficient is due to the unequal impedance differences between polyamide particles and BMF plasma and red blood cells and blood plasma.

Table 1 Properties compared

	Blood	BMF with higher particle amount	Standard BMF (IEC 61685)
Hematocrit	HK 37 to 54, Dialysis patients HK 25 to 45	HK 1.82 to 40	HK 1.82
Effective scatterer size	5.5 $\mu\text{m}$	5 $\mu\text{m}$	5 $\mu\text{m}$

Density	(1,020 to 1,050) kg/m <sup>3</sup>	(1,042±0,004) kg/m <sup>3</sup>	1,037 kg/m <sup>3</sup> (-0.22 kg/(m <sup>3</sup> °C))
Whole blood viscosity	3.52±0.49 mPas (men, at HK 45.6) 3.18±0.48 mPas (women, at HK 42.2)	Minimum 4.19 mPas	4.0±0.4 mPas
Temperature	37 °C	Room Temperature (ca. 22 °C)	22 °C
Attenuation	0.17 - 0.24 dB/(MHz cm)	(1.33±0.47) dB/cm; HK 20, 2 MHz (1.70±0.52) dB/cm; HK 25, 2 MHz (2.14±0.30) dB/cm; HK 30, 2 MHz	Preferably below 0.1 dB/(MHz cm)
Speed of Sound	1,570±30 m/s	(1,621.8±3.7) m/s at HK 20 (1,648.5±3.2) m/s at HK 25 (1,673.1±3.7) m/s at HK 30	(1,570±30) m/s (+5 m/(s °C))

### 3.2. Properties of the Blood Mimicking Fluid

The blood mimicking fluid can be stored for at least one year without changing its properties. After extended storage it must be stirred well, degassed and sieved again before use. The polyamide particles are dispersed within the fluid and do not change in shape or size during storage or use of the BMF in tube systems, i.e. with roller pumps (Fig. 2).

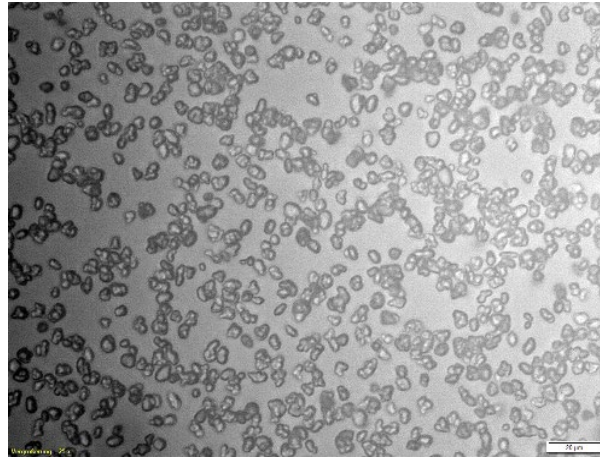


Fig. 2 Polyamide particles dispersed in the blood mimicking fluid.

Blood is a non-Newtonian fluid at small shear rates and Newtonian at shear rates higher than approximately 200/s. This behavior is reproduced by the blood mimicking fluid (Fig. 3). At a particle amount of 30 Vol% the non-Newtonian behavior above shear rates of 200/s is not reproduced correctly in our rheometer measurements. Most likely this is an artefact and due to the measurement method with a plate-plate-rheometer (plasma is removed from the BMF due to the centrifugal forces, effectively raising the particle content and therefore the viscosity).

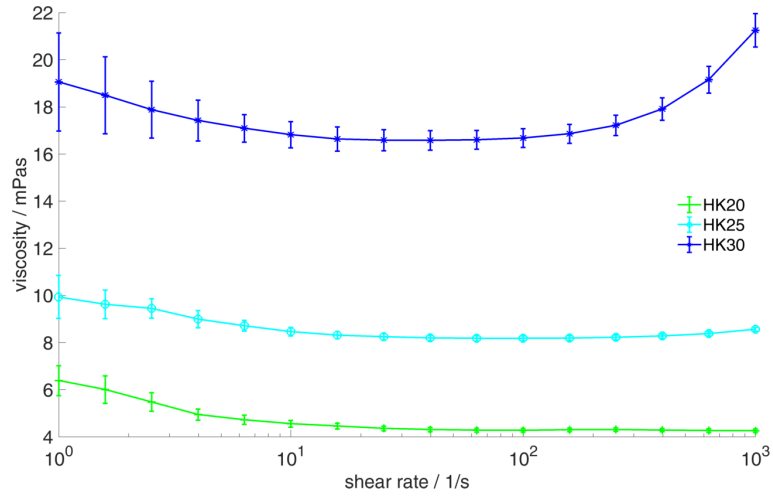


Fig. 3 Viscosity of blood mimicking fluids with particle contents 20, 25, and 30 Vol%.

Speed of sound and attenuation depend most strongly on hematocrit rather than the amount of particles with larger diameters or their size (Fig. 4, Table 2). Measurements were performed within the hematocrit range 20 to 30 since this is closest to blood therapies. The measurement setup is not sensitive enough for detecting an influence on scatterer size or on the amount of larger scatterers since it is static, and the particles drop. This leads to an inhomogeneous particle distribution and larger measured attenuation coefficients and speeds of sound as well as larger differences of measured values between different measurement sessions. Depending on the execution of attenuation and speed of sound measurements (i.e. time between stirring and measurement, time between measurements in different distance, quality of degassing), these values can vary up to 0.5 dB/cm and 20 m/s or even more.

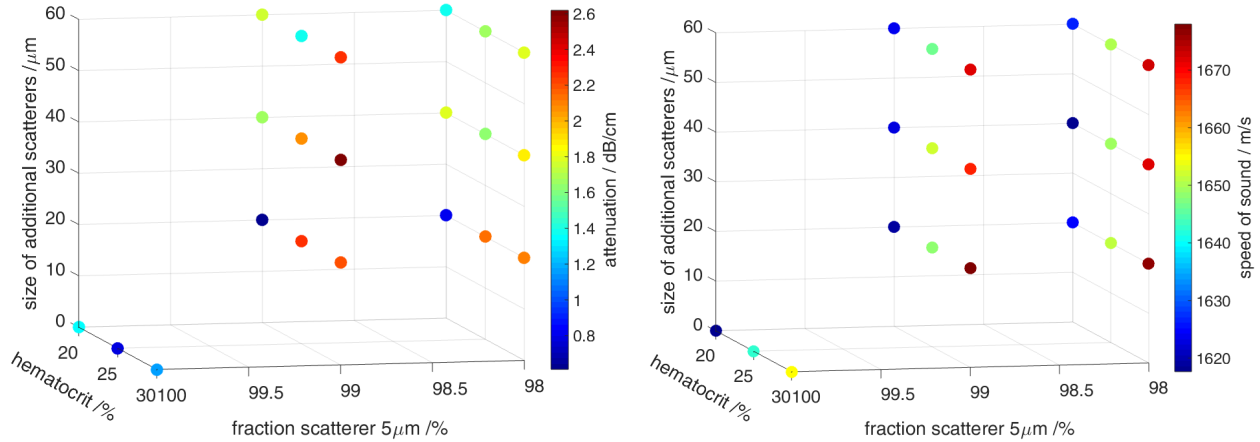


Fig. 4 Attenuation and Speed of sound for different compositions of blood mimicking fluids.

Table 2 Attenuation coefficient and speed of sound values of blood mimicking fluids.

Hematocrit (%)	5 $\mu\text{m}$ particles (%)	Size of other scatterers ( $\mu\text{m}$ )	Speed of sound (m/s)	Attenuation Coefficient at 2 MHz (dB/cm)
20	100	0	1,617.64	1.345
20	99	20	1,618.95	0.612
20	98	20	1,624.69	0.805

20	99	40	1,623.14	1.666
20	98	40	1,617.87	1.780
20	99	60	1,623.37	1.741
20	98	60	1,626.86	1.387
25	100	0	1,642.44	0.781
25	99	20	1,648.55	2.245
25	98	20	1,651.03	2.120
25	99	40	1,651.65	2.080
25	98	40	1,649.42	1.619
25	99	60	1,646.70	1.382
25	98	60	1,649.79	1.676
30	100	0	1,654.62	1.162
30	99	20	1,677.98	2.190
30	98	20	1,676.57	2.105
30	99	40	1,667.92	2.618
30	98	40	1,671.85	1.891
30	99	60	1,671.60	2.258
30	98	60	1,672.75	1.789

#### 4. Conclusion

The adapted blood mimicking fluid can be used for hematological experiments instead of using animal or human blood. The incipient blood coagulation can be mimicked by either adding small amounts of larger particles (20  $\mu\text{m}$ , 40  $\mu\text{m}$ , 60  $\mu\text{m}$ ) or changing the plasma viscosity by adding dextran. Rheological and acoustical properties of the blood mimicking fluid can be adjusted in a wide range. Since no liquid can represent all blood properties perfectly, the fluid is an adequate compromise to be used at higher hematocrit values. The fluid could also be of interest in food, chemical, pharmaceutical or biotechnological industries.

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