EFFICACY AND COMPATIBILITY OF BLOOD-MIMICKING FLUID IN VASCULAR WALL-LESS FLOW PHANTOM FOR USE IN DOPPLER ULTRASOUND APPLICATIONS

AMMAR ANWAR KHALED OGLAT

UNIVERSITI SAINS MALAYSIA

2019

EFFICACY AND COMPATIBILITY OF BLOOD-MIMICKING FLUID IN VASCULAR WALL-LESS FLOW PHANTOM FOR USE IN DOPPLER ULTRASOUND APPLICATIONS

by

AMMAR ANWAR KHALED OGLAT

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

June 2019

Dedication

This work is dedicated to the

PROPHET MUHAMMAD ﷺ BIN ABDULLAH BIN ABDUL MUTTALIB,

HIS FAMILY AND COMPANIONS.

ACKNOWLEDGEMENT

"In the name of Allah, the Most Gracious, the Most Merciful"

My gratitude and praise to Almighty Allah for giving me the strength and patience to help me accomplish this scientific research. I admit, His Grace and Guidance, without which my career this far in my education ambition would have been fruitless.

I would like to extend my sincere appreciation and gratitude to the many individuals who helped make this work possible. I would like to express my deepest appreciation to my main supervisor, **Professor. Dr. Muhammad Zubir Mat Jafri,** for his patience, guidance, support, priceless instructions, valuable directions, encouragement academically and financially throughout conducting this research and encouragement that had a great role in accomplishing this thesis. I am also grateful to my co-supervisor **Dr. Nursakinah Suardi** for her kind concerns and supports throughout the entire journey of the project. I also want to extend my gratitude to my field co-supervisor **Dr. Mostafa Abdelrahman** for his advice, guidance, and encouragement during this research. I wish to thank the academic and non-academic staff of the School of Physics, USM for their assistant and support of this research.

Special appreciation is extended and goes to my parents, Anwar Oglat and Hayat Audat. I will not forget the contributions and prayers of my aunty (Munawar Oglat), my brothers, all my family members, research mates, friends and everyone that had given their help and support during this project session.

Ammar A. Oglat 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A-SCAN	Amplitude-scan
AL_2O_3	Aluminum Oxide
Ap_1	Power signal amplitude
BMF	Blood Mimicking Fluid
B-mode	Brightness-mode
CCA	Common Carotid Artery
c.f	Compared to the human
C-mode	Color-mode
C-Doppler	Color- Doppler
C powder	Carrageenan powder
CW	Continuous wave
DMA35	Density meter
d	depth (thickness) of sample
ECP	European Commission Project
ECA	External Carotid Artery
EDV or Vd	End diastolic velocity
ERV	Electronic rotational viscometer
FFT	Fast Fourier Transformer
GAMPT	German Society for Applied Medical Physics and Technology

HI	Hitachi Avious
ICA	Internal Carotid Artery
IMT	Intima-Media Thickness
IEC	International Electrotechnical Commission
KC	Konjac Carrageenan
K powder	Konjac powder
L_o	Entrance length
LV	Left ventricle
M-mode	Motion-mode
NIT	Non-invasive testing
PG	Propylene glycol
PEG	Polyethylene glycol
Pc	Potassium chloride
PC	Personal computer
PSV or Vp	Maximum velocities values or Peak systolic velocity
PE	Piezoelectric element
Pe	Pulse echo
PW-mode	Pulse wave-mode
PI	Pulsatility index
RBCs	Red blood cells
R_e	Reynolds number

RI	Resistance index
RF or rf	Radio frequency
S;D or S/D	Systolic-diastolic
S.T	Soft Tissue
SC	Silicon Carbide
TMM	Tissue Mimicking Material
ToF	Time of flight
t	Time shift
TAMV	Time-average maximum velocities
US	Ultrasound
VMM	Vessel Mimicking Material
Vm	Mean velocity
V_{max}	The maximum velocity
WBCs	White blood cells

LIST OF SYMBOLS

mPa.s	Millipascal times seconds
µm	Micrometer or micron
wt %	Weight percentage
dB/cm. MHz	Decibel per centimeter times Megahertz
m/s or m/sec	Meter per second
kg/m$^{\hat{}3}$	Kilogram per cubic meter
λ	Wavelength
g/cm^3	Gram per cubic centimeter
H	Hour
kg/ms	Kilogram per meter times second
v_s	Speed of sound
α	Attenuation coefficient
H_2O	Water
dp_l	Thickness of protective layer
F	Frequency
ΔF_T	Shift frequency or Doppler frequency
f_o	US probe frequency (Hz)

V	Blood velocity
Cosθ	Cosine angle of the insonation
2-d	two-dimensions
C	Speed sound in soft tissue which is equal to1540m/s
Hz	Hertz
MHz	Megahertz
KHz	Kilohertz
O_2	Oxygen
CO_2	Carbon dioxide
Mw	Molecular weight
ml/min	milliliter per minute
L/min	Liter per minute

KEBERKESANAN DAN KESESUAIAN BENDALIR SEIRAS-DARAH FANTOM ALIRAN VASKULAR UNTUK DIGUNAKAN DALAM ULTRASOND DOPPLER

ABSTRAK

Alat ultrabunyi Doppler (AS) telah digunakan secara klinikal dan pra-klinikal selama beberapa tahun, dan sangat berperanan untuk mengesan aliran darah dalam arteri dan urat. Projek ini menunjukkan gambaran keseluruhan secara umum yang memberi tumpuan kepada pengukuran dan mengira pengaruh ciri fizikal (ketebalan) lapisan pelindung yang berstruktur dalam penyelidikan ultrasonik Persatuan Jerman Fizik Perubatan Gunaan dan Teknologi (GAMPT) bagi pengukuran kelajuan bunyi (v_s). Penyediaan dan pencirian BMF yang baru dengan kelikatan yang agak rendah, tepat, sesuai, dan mengambil masa yang singkat bagi penyediaan dengan cecair campuran ternari yang baru (air (H2O), propylene glycol (PG), dan polietilena glikol (PEG) 200Mw) dan zarah yang terserak (Poly (4-methylstyrene)) untuk menghasilkan kuasa backscatter yang sesuai berbanding dengan darah manusia yang tinggi dijelaskan. Tambahan, dalam kajian ini, mekanisme fabrikasi dan pencirian yang kuat (kukuh) dan vaskular yang elastik serta aliran fantom yang tidak berdinding (TMM dan VMM) yang menggunakan bahan yang kuat secara fizikal dipanggil Konjac (K), Carrageenan (C), dan gelatin (bovine kulit) berasaskan TMM untuk kadar aliran fisiologi tinggi dan mengesan masalah yang berkaitan dengan Doppler perubatan US yang dijelaskan. Teknik ultrasonik digunakan sebagai alat utama untuk pengukuran akustik (v_s, pelemahan (α), dan kuasa backscatter) BMF dan TMM. Pengukuran fizikal (kelikatan dan ketumpatan) diukur denagn menggunakan pelbagai alat. Sedangkan sistem US klinikal (Hitachi Avious (HI)) digunakan sebagai instrumen utama untuk memperoleh data dan menguji keserasian, keberkesanan dan pengesahan BMF, TMM, dan VMM. Hasilnya mendapati bahawa ukuran v_s dipengaruhi oleh ketebalan lapisan pelindung. Oleh itu, pengaruh ketidaktepatan telah dihapuskan dengan mengambil kira ketebalan lapisan pelindung. Walau bagaimanapun, ciri-ciri akustik dan fizikal BMF yang baru dan TMM sesuai dan dipersetujui dengan Suruhanjaya Elektroteknikal Antarabangsa (IEC) 61685 nilai standard. Juga, fantom aliran dan BMF baru boleh digunakan dan disimpan untuk jangka masa panjang (7-9 bulan) jika penjagaan fantom aliran vaskular yang tidak berdinding diambil. Di samping itu, corak aliran di dalam VMM telah berlaku dalam aliran parabola dan BMF adalah laminar (tidak bergolak). Setiap diameter saluran, kedalaman saluran, kadar aliran, halaju min, garis panduan halaju, peratus ralat halaju (%), indeks Doppler, corak spektrum Doppler, dan sudut Doppler dalam fantom aliran vaskular yang tidak berdinding yang dilakukan secara bergilir sebagai parameter diagnostik untuk imej ultrasonik dan mensimulasikan CCA normal, dan nilai-nilai adalah berada dalam julat CCA. Sebagai kesimpulan, BMF yang baru telah dihasilkan. Ia sesuai untuk kegunaan dalam fantom aliran vaskular tanpa dinding dan zarah yang terserak yang baru (poli (4-methylstyrene)) sesuai sebagai faktor yang mencerminkan darah buatan. Kecekapan TMM yang dibuat daripada KC dan gelatin dinilai dan ia sesuai untuk digunakan dan disimpan untuk jangka panjang dan menahan aliran yang oleh kebocoran BMF. Dalam projek ini, kedua-dua ciri akustik dan fizikal komponen fantom aliran Doppler (BMF, TMM, dan VMM) sesuai dengan ciri-ciri tisu manusia yang membuatkan ujian kelihatan signifikan. Dan, boleh digunakan untuk diagnostik mod-B dan kajian Doppler US in-vitro dengan frekuensi klinikal (5-13 MHz).

EFFICACY AND COMPATIBILITY OF BLOOD-MIMICKING FLUID IN VASCULAR WALL-LESS FLOW PHANTOM FOR USE IN DOPPLER ULTRASOUND APPLICATIONS

ABSTRACT

Doppler ultrasound (US) tools have been utilized clinically and pre-clinically since 1970s, and they were extremely responsible for the detection of blood flow in arteries and veins. This project shows a general overview focusing on the measuring and calculating the influence of the thickness of protective layer in ultrasonic probe of German Society for Applied Medical Physics and Technology (GAMPT) for the speed of sound (v_s) measurements. Preparing and characterizing new BMF with relatively low-cost, suitable viscosity, and less consuming time for preparation with both new adequate ternary mixture fluid (water (H_2O), propylene glycol (PG), and polyethylene glycol (PEG) 200Mw) and with a novel suspension scatter particle (Poly (4-methylstyrene)) to produce a suitable backscatter power comparable to the human blood were explained. Moreover, in this study, the mechanisms of fabrication and characterization a strong (robust) and elastic vascular wall-less flow phantom (TMM and VMM) utilizing a physically strong materials called Konjac (K), Carrageenan (C), and gelatin (bovine skin)-based TMM for high physiological flow rate and detection of the issues related to it by medical Doppler US were explained. The ultrasonic technique was utilized as a main apparatus for acoustical measurements (v_s, attenuation (α), and backscatter power) of BMF and TMM. The physical measurements (viscosity and density) were measured by various tools. Whereas the clinical US (Hitachi Avious (HI) model) system was used as a primary instrument for data acquisition and test the

compatibility, efficacy and validation of a BMF, TMM, and VMM. The results found that the v_s measurements influenced by the protective layer thickness of the probe. Thus, the influence of inaccuracy was removed by knowing the protective layer thickness and taking into consideration. However, the acoustical and physical properties of a new BMF and TMM were suitable and agreed with the International Electrotechnical Commission (IEC) 61685 standard values. Also, the new BMF and flow phantom can be utilized and storage for long-term (7-9 months) if the care of the vascular wall-less flow phantom is taken. In addition, the flow pattern inside the VMM has happened to be in the parabolic flow and the BMF was laminar (not turbulent). Each of vessel diameter, vessel depth, flow rate, mean velocity, velocity outline, velocity percentages (%) errors, Doppler indices, pattern of Doppler spectrum, and Doppler angles in the vascular wall-less flow phantom was carried out in turn as a diagnostic parameter for ultrasonic image and to simulate the normal CCA, and the values were closed to a normal range of CCA. As a conclusion, a new BMF has been developed. It was suitable for utilizing in vascular wall-less flow phantom and the new novel scatter particle (poly (4-methylstyrene)) was suitable as a reflecting factor in the artificial blood. The efficiency of TMM that made of KC and gelatin assessed and it was suitable to be utilized and storage for long-term and resist the flow rate of BMF leakage. In this project, BMF, TMM, and VMM be utilized for diagnostic B-mode and Doppler US in-vitro studies with clinical frequency (5-13 MHz).

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The ultrasound is the expression that describes the sound signal wave's frequencies above the domain of human hearing (>20 kHz) and their propagation in a medium. Ultrasonic vibrations (oscillations) are caused by some periodical disturbances of the flexible (elastic) medium event (compression and rarefaction), known by an alteration in its physical features, which happen simultaneously with disturbance. The local oscillations of the medium are emitted to neighbour regions throughout the waves propagation by the flexible medium, known by an alteration in medium density (Hangiandreou, 2003; Morkun et al., 2014). In the medical field of diagnostic US, the most common frequencies are used range between 2-10 or 2-15 MHz (Douville et al., 1983; Earle et al., 2016; Hangiandreou, 2003; Rumack et al., 2017). However, there is two main types of sound wave propagation, pulsed and continuous wave. These waves transmitted into the object utilizing a piezoelectric probe. Sound waves scattered and reflected in the blood as a portion of the backscattered power is detected by the examination in receiving process (Safari et al., 2008; Shung, 2015).

Doppler US supplied an instrument which measures the blood speed and flow (Virginie Grand-Perret et al, 2018). It also utilized in the research field and clinical field investigations to quantify the range and influence of arterial disease (Auboire et al., 2017; Kenwright et al., 2015). In Doppler imaging of blood, the constant object is the probe, and the moving or shifting reflectors that produce the returning signal echoes are initially the red blood cells (RBCs). The Doppler frequency (Doppler-shift) is known as the variation between the frequencies of transmitted and received of US waves echoes (Ferreira et al., 2011; Hangiandreou, 2003; Mehra, 2010). The shade of gray refers to the amplitude of each speed part. The higher amplitude, the brighter speed shown on the screen. By agreement, the flow toward the probe is presented above the zero-speed baseline, while the flow far away from the scrutiny is displayed below the zero-speed baseline. Similarly, Color (C)-Doppler is shown as a red color indicated flow toward the probe, while the other blue color stated the flow far away from the scrutiny (Wood et al., 2010).

Blood that precisely mimics real blood systems in all its properties is often referred to as blood mimicking fluid (BMF). It consists of blood-mimicking materials, chemical fluid and powder materials are occasionally mixed to mimic physical, acoustical and chemical properties. The advantage of items that used in a BMF preparation that can construct it with general acoustic, particles, and physical properties. Furthermore, BMF is applied to compare the act of US systems for the practice of Doppler US technicians, to allow comparison of backscatter properties of Doppler US and to evaluate it in a Doppler flow test object or diagnostic techniques (Kenwright et al., 2015; Oates, 1991; Ramnarine et al., 1998; Thorne et al., 2008; Yoshida et al., 2014; Yoshida et al., 2012; Zhou et al., 2017).

Flow phantoms constitute a significant appliance for the development of Doppler US mechanism for examining the blood flow. In making actual flow conditions, it is popular to fit the arbitrary geometries that closely resembles the in-vivo geometries. The acoustical features of the different ingredients of the flow phantom must correspond the acoustical features of human blood, tissue, and the vessel which identified explicitly by the International Electrotechnical Commission (IEC) 1685 standard 1999 (Grand-Perret et al., 2018; Santos et al., 2017; Zhou et al., 2017).

Typically, wall-less vessels are preferable to be used in preventing mismatch problems in acoustic properties between tissue mimicking material (TMM) and the vessel wall. It allows VMM to be suitable since the TMM direct link to the BMF, and reduce or remove the Doppler artifacts (Browne, 2014; Grand-Perret et al., 2018; Meagher et al., 2007; Ramnarine et al., 2001; Rickey et al., 1995). Also, to manufacture more sophisticated geometries, utilizing a lost-material casting mechanism is required (Virginie Grand-Perret, 2018). However, during use of wall-less vessels, the TMM its prone to changes because of exposure to water or air, the action of breaking, inconsistency with the BMF, and leakage problems (Meagher et al., 2007; Watts et al., 2007).

1.2 Motivation and Problem Statement

As recognized, BMF is used for applications in all flow phantom and with all sort of pumping mechanism. A BMF applies for Doppler flow test objects since its proper to describe it. Currently, there are some commercially available BMF as test objects for Doppler US applications. However, the main drawbacks for commercial BMFs presently being used are high cost, extended time for preparation and is not customizable for specific applications (Grand-Perret et al., 2018; Kenwright et al., 2015; Ramnarine et al., 2001; Ramnarine et al., 1998; Thorne et al., 2008; Zhou et al., 2017). Thus, there is need to prepare BFM to be adequate with relatively low cost, less consuming time of preparation, and to be customizable for clinical in-vitro (large vessels diameters) applications without effect on the blood flow velocity or other measurements. Even though most common scatter particles (polystyrene, sephadex, and nylon) that are used in preparation BMF have a suitable acoustical and physical properties. The possibility of the particles to clot during flow is high. Because the items have particle sizes more than red cells sizes in the blood, such as 10 µm, 20 µm, 30 µm, and 70 µm, and these large particles combined during flow (Kenwright et al., 2015; Law et al., 1989). Therefore, preparing a new BFM with novel suitable to scatter particle as suspension material will be of vital importance.

Furthermore, the main drawback of the two hydrogel materials (KC-based TMM) that it is not fit for long-term storage and easy to deteriorate (Zhou et al., 2017). The TMM of wall-less flow phantoms made of KC its prone to changes with short-term because of exposure to water or air, the action of breaking, inconsistency with the BMF, and leakage problems during high flow rate due to rupture of the TMM. Moreover, TMM items require refrigeration to prevent spoiling. Thus, to avoid the leakage the BMF throughout the TMM with over time, fabrication of a suitable vascular wall-less flow phantom for evaluation and estimation of high physiological flow rate pattern and to be qualified for long-term storage without refrigeration at room temperature is required. This to maintain the stable operation when exposed to the full high flow rates and produce TMM with appropriate elastic properties to obtain biologically worth diameter changes per pulse cycle. Moreover, the primary purpose of this work is to study and gain the understanding of the response for the properties (BMF, TMM and VMM) to B-mode and Doppler US tools. This study will clarify whether this new BMF will be responsible as a reflecting factor of ultrasonic waves for in-vitro diagnostic studies (standard CCA), evaluating the velocity % errors as a trial target for the flow phantom. Therefore, developing a new BMF and vascular wall-less flow phantom for Doppler US applications is necessary.

1.3 Research Objectives

The fundamental objectives of this research are:

a) To prepare and characterize a new artificial blood or BMF for clinical in-vitro Doppler test object.
b) To fabricate and describe a robust and elastic vascular wall-less flow phantom made of TMM and VMM as a test object (in a design to mimic the human CCA) to be qualified for long-term utilize and storage with high physiological flow rate at room temperature without refrigeration.
c) To examine and study the efficacy, compatibility, and validation of a new BMF and vascular wall-less flow phantom using B-mode and Doppler US (HI) imaging technique.

1.4 Scope of the Research

This research study is concentrated in the protective layer thickness of A-scan ultrasonic transducer influence on the v_s measurements of the new BMF samples. The measuring and calculating its thickness are required since it is a challenge in the project. Furthermore, preparation of a suitable BMF using a flow phantom and examine its acoustical and physical features by applying A-scan, B-mode, and Doppler US. BMF made with distilled water 70.0 wt %, PG 5.0 wt %, PEG (200 Mw) 25.0 wt %, and poly (4-methylstyrene) 0.8 wt % will be characterized and considered with five parameters to the v_s, density, viscosity, α, and backscatter power. Mixture fluid samples criteria will be chosen base on physical and acoustical properties obtained from mixture fluid and compared with IEC standard values. Moreover, this research is focused on fabricating a robust and elastic vascular wall-less flow phantom of both TMM and VMM, with only three acoustical parameters will be considered, which is the v_s, backscatter power, and α. Examine the validation and investigation of the compatibility of a new BMF, and vascular wall-less flow phantom will be done using B-mode and Doppler US Hitachi Avius (HI) for in-vitro research studies.

1.5 Thesis Organization

The thesis is arranged into the following chapters. Chapter one describes and explains the background of this study, motivation and problem statement, research objectives, importance, scope of study and thesis organization. This chapter highlights and focused on the critical point of the research study. Chapter two provides a comprehensive literature review and theoretical background on past studies. Related researches on BMF, the protective layer thickness of probe, vascular wall-less flow phantom (TMM and VMM), acoustical properties of TMM, anatomy, and physiology of CCA, gear pump, Doppler US technique, and diagnostic US protocols. Chapter three explains the apparatus (instruments), materials utilized in this research study, and the methodology such as experimental arrangement, setup, protocol, and data acquisition. Chapter four present the the result and discussion of the data findings and analysis obtained in this research study. Chapter five, present the conclusion and recommendations for further research were given.

CHAPTER 2

THEORY AND LITERATURE REVIEW

2.1 Introduction

At recent time, there is a rising number of literature review related to Doppler US applications (BMF, TMM, and VMM) research as a result of continued research interest. Indeed, the number of publications on Doppler US has grown recently. Many of radiologists and Medical Physicists are focused on in-vitro applications such as BMF, TMM, and VMM with enhanced applications and properties.

2.2 Artificial Blood

The blood considers non-Newtonian (fluids are fluids that show a fixed viscosity and does not depend on the flow rate and the previous parameters) when the viscosity rely on shear rate (Nichols et al., 2011). Usually, viscosity depends on the shear rate when tiny vessels of arterial were measured. Further, during the movement of erythrocyte and when the US beam scattered from every red cell it will combine, and then the Doppler US signal becomes clear. Nevertheless, the essential drawback of Doppler signal is the noise, which was the output of different varieties of the numeric and geometric order of the sample volume within the scattering elements (Hoskins et al., 1990).

The diverse types of BMFs are contained in the literature and are revised by P. Hoskins et al. (1990). A BMFs usually have a scattering particles material suspended in a fluid such as nylon, polystyrene, starch, and Sephadex (Grand-Perret et al., 2018; Ramnarine et al., 1998; Yoshida et al., 2014; Zhou et al., 2017). The viscosity degree of BMF is suitable for a Newtonian fluid, and it does not build on the shear rate. Blood work as a Newtonian fluid that deals with the prime (large) vessels of the body (Pedley, 1980). However, to obtain a suitable BMF, the acoustical and physical properties of BMF should be close to that of IEC standard with constant values (Table 2.1).

BMF which imitate the properties of human blood such as density, α, scattering, the V_s and viscosity are commercially available, the ranges of prices of the commercial BMF are different depending on the volume of required blood. Moreover, commercialized BMF is not customizable because it is made for wide markets and specific implementation (Grand-Perret et al., 2018; Kenwright et al., 2015; Ramnarine et al., 2001; Ramnarine et al., 1998; Thorne et al., 2008; Zhou et al., 2017).

Table 2.1 Specifications of the BMF defined as the IEC standard (Yoshida et al., 2014).

Acoustical and physical properties of BMF	**Values**
Viscosity (×10³ Pas)	4.0 ± 0.4
Attenuation [(dB/cm/MHz)]	≤ 0.1
Acoustic speed (m/s)	1570 ± 30
Density ($\times 10^3$ kg/m^3)	1.050 ± 0.040

Furthermore, Law et al. (1989) investigated that the BMF properties such as physical and acoustical properties, diverse according to room temperature, humidity, and atmospheric. Many of physical properties, acoustic properties, and other measurements with different methods applied to develop general BMF. The different types of mixture fluids and scatters particle materials used in BMF preparation have been explained in this chapter. Artificial or mimic blood is prepared as a particles suspension in fluids. The diameter of these particles is close to that of real human's RBCs, even though some have a larger diameter than the real human's RBCs (Grand-Perret et al., 2018; Law et al., 1989; Zhou et al., 2017).

In general, the BMF using in-vitro hemodynamic styles of Doppler US should have identical properties like real human blood and easy to prepare. The RBCs (erythrocytes) are responsible for the blood scattering in the US, although the blood consists of RBCs (erythrocytes), platelets (thrombocytes) and leukocytes (white blood cells (WBCs)) and why was that?

The components of human blood are responsible for significant sides of the Doppler signal. Real blood cells (RBCs) of a suspension material (erythrocytes), platelets (thrombocytes) and leukocytes (WBCs). Due to the presence of comparable low numbers of platelets (250000-350000) and leukocytes (6000-11000), it is typically supposed that the RBCs (erythrocytes) (5000000-6000000) are responsible for the blood scattering in the US. The average diameter of the erythrocyte is 7 microns, which is less than the US wavelength (ʎ) (0.2-0.5 mm). Thus, the single erythrocyte works as a point scatter, whose combined influence is indicated 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 (Burns, 1987; Oglat et al., 2018). Based on this view, the best fluid to use is the blood itself. In contrast, there are some obstacles and disadvantages in using blood and its ingredients, such as the possibility of biohazard. RBCs is easily damaged in-vitro because the expiration date of blood is limited (about 120 days), the practice in-vivo is not ethical and not safe, and this limits the use of blood as a norm fluid in long-range studies of measurement and quality monitor. Thus, the attention must be taken to reduce the hazard (Samavat et al., 2006).

2.3 Physical Properties of Blood Mimicking Fluid (BMF)

2.3.1 Density

Density is recognised as one of the fundamental factors in BMF because it defines the quantity of mass per unit volume. The particles materials used in BMF preparation should be able to remain suspended (not float or precipitate) inside a mixture fluid, because it is good to stay neutrally buoyant, even at minimum speeds.

The density of particles should be approximately 1.05±0.04 g/cm^3 (1.01- 1.09 g/cm^3) close to the human blood density as IEC values (Grand-Perret et al., 2018; Kenwright et al., 2015; Ramnarine et al., 1998; Samavat & Evans, 2006; Yoshida et al., 2014; Zhou et al., 2017).

The density of particles should allow them to be suspended in the fluid especially when the particles are flowing along the tube or vessel. Some researchers reported that the main problems related to the densities of particles happened when the density is less or much larger than the fluid density. For instance, Figure 2.1 Shows polystyrene scatter particles precipitated in the bottom because its density (1.050· g/ cm^3) is greater than mixture fluid (1.030· g/ cm^3) (Hoskins et al., 1990; Ramnarine et al., 1998; Yoshida et al., 2012). However, several previous studies measured the density of BMF via pycnometer tool (Hoskins et al., 1990; Kimme-Smith et al., 1990; Law et al., 1989; Oates, 1991; Raine-Fenning et al., 2008; Ramnarine et al., 1998; Samavat & Evans, 2006; Thorne et al., 2008; Yoshida et al., 2014; Yoshida et al., 2012; Zhou et al., 2017).

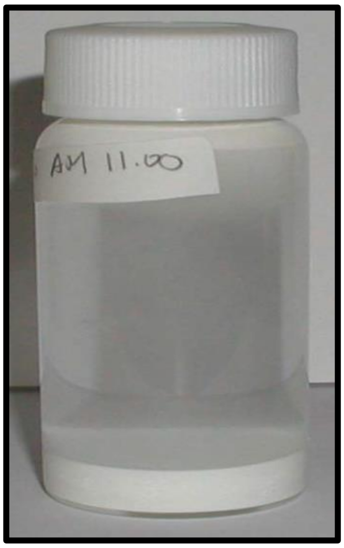

Figure 2.1 Appearances of the BMF samples in glass jars after 24H.
Liquid density: 1.030· g/ cm^3, particle diameter:5 microns. particles settles on the bottom of glycerin and water-soluble silicone oil (Yoshida et al., 2012).

2.3.2 Particle Size and Concentration

Erythrocytes (human red cells) are concave on both sides (Figure 2.2). Particles that are used in BMF are mostly spherical and with a diameter in the range between 5-8 µm to mimic real human red cells (Hodgson et al., 2014; Udroiu, 2014). For example, polystyrene microspheres with particle diameters 5-30 µm (Kimme-Smith et al., 1990; Tanaka et al., 2012; Yoshida et al., 2014; Yoshida et al., 2012), 5–20 µm for nylon (Grand-Perret et al., 2018; Law et al., 1989; Oates, 1991; Raine-Fenning et al., 2008; Ramnarine et al., 1998; Samavat & Evans, 2006; Thorne et al., 2008; Zhou et al., 2017), and 20–70 µm for Sephadex (Hoskins et al., 1990).

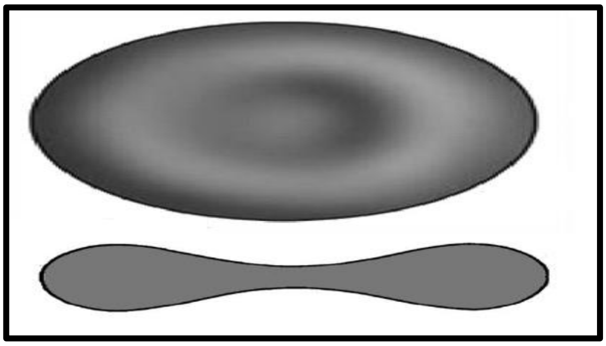

Figure 2.2 Erythrocyte surface and cross-section (Udroiu, 2014).

2.3.3 Viscosity

The viscosity of the fluid is a fundamental feature of a liquid, it is linked to the inner friction by the force and it against the proportional movement between layers gliding past one another. The blood viscosity is considered non-Newtonian in-vivo in the small arteries and Newtonian in-vitro because it is anomalous (Yalcin et al., 2015). The main factors that influenced non-Newtonian blood viscosity include temperature, RBCs aggregation, RBCs deformability, shear rate, plasma viscosity, and the hematocrit. Newtonian fluids are fluids that show a fixed viscosity and does not depend on the flow rate and the previous parameters. In other words, Newtonian fluids have a direct relationship between shear rate and shear stress. For example, glycerol, H_2O, blood plasma, and ethanol are Newtonian fluids (Mandal, 2016). The viscosity of the real blood has an immediate effect on the velocity in the vessel, especially in small vessels. The velocity increases with a higher viscosity fluid, and this occurs before the beginning of turbulence, and it has an advantage of a flow test object (Wells Jr, Merill., 1962).

In another study, they found that the dependent on shear rate happen slightly (Ercan et al., 2003; Papaioannou et al., 2005). Pedley (1980) stated that blood with an amount of viscosity measured under situations of high shear rate (4 mPa.s) is considered Newtonian in large vessels. The measurements of dynamic viscosity for overall human blood with high shear rate were reported as 3.5–4.5 mPa.s at 37 ºC and with different shear rate 2.25-4.5 mPa.s (Elblbesy et al., 2016; Mandal, 2016). Moreover, it found that the viscosities of fluids relied on their molecular weight, and are proportional to each other (Yoshida et al., 2014).

Changes of shear rate depend on several conditions, for example; the size of vessels; blood stream velocity. However, several researchers, they measured and calculated the kinematic viscosity and then converted it to dynamic viscosity of mixture fluid in the BMF by U-tube viscometers (Kimme-Smith et al., 1990; Tanaka et al., 2012; Yoshida et al., 2014; Yoshida et al., 2012; Zhou et al., 2017).

2.4 Acoustical Properties of Blood Mimicking Fluid (BMF)

2.4.1 Speed of Sound (v_s) and Attenuation (α)

The v_s in BMF and TMM is usually 1540 m/s (Browne et al., 2004; Grand-Perret et al., 2018; Ramnarine et al., 2001; Ramnarine et al., 1998; Yoshida et al., 2014; Zhou et al., 2017). The acoustic speed in the BMF should be identical (the same ranges to the tubes (VMM and TMM) to prevent refraction artifacts (Zhou et al., 2017). The refraction artifacts can be noticed when using tubes with a high velocity of sound (Sato et al., 2006). For example, Ramnarine et al. (1998) investigated that the speed of BMF in draft IEC 1685 standard was reported to be 1570 ± 30 m/s. This vast range permits the speed to correspond wall vessel, blood and the TMM of a flow test object. However, the rate of v_s and attenuation have been studied for human blood (Duck, 2013), and found that the attenuation of the BMF must be less than 0.1 dB/cm MHz, and this recommended from the draft of IEC 1685 standard (Browne et al., 2004). Therefore, to reduce inhomogeneity of the sound scope into the tube (VMM), the attenuation of the BMF should be minimal.

Several researchers have measured both the acoustic speed and attenuation of the BMF by Pe signal technique (Boote et al., 1988; Hoskins et al., 1990; Kimme-Smith et al., 1990; Oates, 1991; Oliveira et al., 2016; Ramnarine et al., 1998; Samavat & Evans, 2006; Thorne et al., 2008; Ping Yang et al., 2010; Yoshida et al., 2014; Yoshida et al., 2012; Zhou et al., 2017). Through a comprehensive literature review, the studies measured and calculated the v_s, attenuation through the fluid and the solid samples via measuring the time of flight (ToF) or time shift, t, of the signal wave pulse from the reflection waves by applying Equations 2.1, 2.2a, and 2.2b, respectively (Grit, 2009; Halim et al., 2014; Kenwright et al., 2014; Ramnarine et al., 1998) :

$$v_s = \frac{2\,(d)}{t} \qquad 2.1$$

Where v_s is the speed of sound of the sample, d *is* sample depth (distance), t is the time of flight of the signal wave pulse from the reflection wave.

$$\alpha = \frac{1}{-D}\text{In}\frac{Ap2}{Ap1} \qquad 2.2\ (a)$$

$$\alpha = \frac{2x(dB/cm)}{-D}\text{In}\frac{Ap2}{Ap1} \qquad 2.2\ (b)$$

Where α is the attenuation coefficient of sample, $-D$ is the difference in distance (depth) of sample in mm, Ap_1 is the power signal amplitude at frequency f and x, y positions through the sample (amplitude of transmitted signal wave), Ap2 is the power signal amplitude at frequency f and x, y position through the sample (amplitude of received signal wave), and dB/cm is a constant value and equal to 8.686.

2.4.2 Effect of Particles Distribution on the Velocity Profile

Saffman (1956) found that when RBCs distribute in large vessels, the particle moves towards the center of the vessel was due to its force. Kenwright et al. (2015) also reported that it is not suitable to use large particles in small vessels because the particles inability to take over a small proportion of the diameter and which may have effects on both particles distribution and the velocity profile. Though, the effect can be an unnoticeable one. Furthermore, it is important to utilize particles that are tiny and similar to red blood cells (Ramnarine et al., 1998; Thorne et al., 2008; Yoshida et al., 2014). To make sure that the concentration of particle is rising even for the small sample volume in the most narrowing focused Doppler beam. It provides a BMF that may probably be helpful at greater frequencies (non-Rayleigh scattering increase due to the high proportion of diameter to wavelength for large 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 (Hoskins et al., 1990; McDicken, 1986).

2.4.3 Backscattering Pattern of Particles

One of the critical features of a possible suspension in the BMF is constancy and the US backscatter. When using the backscatter power of BMF in a Doppler flow test, the object should be entirely described, steady, and reproducible (Ramnarine et al., 2001; Ping Yang & Zhu, 2010). On the other hand, other researchers, Ramnarine et al. (2001); Ramnarine et al. (1998); and Ping Yang & Zhu (2010) indicated that measurements of penetration deepness and sensibility are essential because it should be similar backscatter from the BMF and flow human blood. This backscatter should be recognized by the draft IEC 1685 (Grand-Perret et al., 2018; Ramnarine et al., 1998). The relationship between backscattered power and the particles are proportional (linearity relationship). Thus, when the particles clotting, the backscattered power increase. This influence is recognized for blood (Law et al., 1989; Ramnarine et al., 1998).

Fast Fourier Transformation (FFT) produces a frequency scope performance of an amplitude obtained in the time range. An immediate measurement and calculation of the separate FFT would be very impressive. Via agents of FFT, it is probable to resolve a time unsteady signal wave into the frequency ranges contained herein and then measure the backscatter power. However, in the previous five decades, the FFT was measured and calculated manually by several algorithms, and it was taken more efforts and consuming time for the backscatter power measurements (Cooley et al., 1965). Currently, there are signal wave processing instrumentations that permit the quick and ease calculation by FFT, like the situation utilized in the current project (Ramnarine et al., 2001; Ramnarine et al., 1998; RUFO et al.).

2.5 Blood Mimicking Fluid (BMF) Components (Ultrasound Scatter Particles and Mixture Fluid Items) and Preparation Methods

2.5.1 Blood Mimicking Fluid (BMF) with Orgasol (nylon) Particles

BMF based on the use of the smooth powder of Orgasol (nylon) suspended in a mixture of H_2O and glycerol used as an alternative to blood (Oates, 1991). The BMF that made of Orgasol was named as home-made blood because it was the most common artificial blood used in the precious studies (Grand-Perret et al., 2018). However, there is a disadvantage of applying nylon particles even though it has densities and diameters close to the red blood cell. Because these particles aggregate or clotting at low shear rates (small vessels) to give non-Newtonian manner which is physical features of real blood (Kenwright et al., 2015). Figure 2.3.

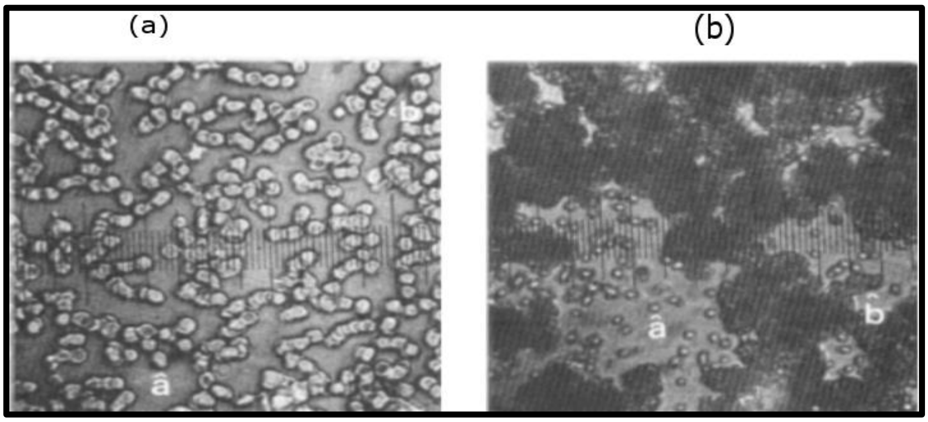

Figure 2.3 (a) Erythrocytes showing: a single cell and b, an aggregate of cells. (b) Nylon particles are showing: a single particle and b, an aggregate of particles. Small divisions equal 2.2 microns (Oates, 1991).

Also, Ramnarine et al. (1998) have used different diameters of nylon particle such as 5 µm, 10 µm, and 20 µm with 1.03 g/cm³ as density. The reliance of α, the speed of sound, and backscatter power with nylon particle diameter, at a constant particle concentration by weight 1.82% is tabulated in Table 2.2. 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 particles scattering of 5, 10 and 20 µm diameter explained in Figure 2.4 as a mission of scattering particle concentration.

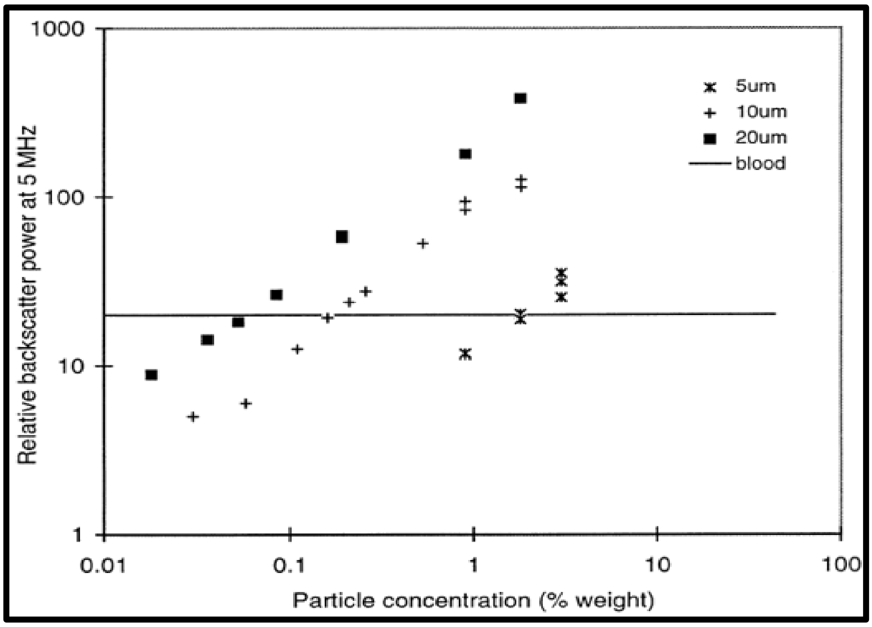

Figure 2.4 Regular flow from BMF with relative backscatter power plotted versus nylon particle concentration for particles of diameter 5, 10 and 20 µm (Ramnarine et al., 1998).

Table 2.2 Physical and acoustic properties of recommended BMF compared to whole human blood (Grand-Perret et al., 2018; Kenwright et al., 2015; Ramnarine et al., 1998).

Properties	**IEC 1685 Draft specifications**	**Human blood (37°C) red blood cells**	**Recommended BMF (22°C) Orgasol™ (nylon)**
Scattered size (µm)		7	5
Hematocrit (% volume)		45	<5
Density (kg/m^3)	1050 ± 40	1053	1037 ± 2
Viscosity (mPa s)	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)	c.f human blood	4 x 10 $^{-31}$	c.f human blood
Fluid properties	Newtonian	non-Newtonian	Newtonian

2.5.2 Blood Mimicking Fluid (BMF) with Polystyrene Particles

Numerous studies used polystyrene as a scattering material for BMF preparation since the polystyrene density, and particle size is close to the red blood cells. The BMF was examined with polystyrene microspheres for the Doppler flow phantom of medical ultrasonic diagnostic tools. The liquid is a mixture of glycerol and degassed H_2O in the proper ratio to give a gravity density of 1.043 g/cm^{-3}. This specific density was chosen to minimize deposition of the third element of the BMF. Polystyrene microspheres with 30 µm as a particle size. The polystyrene microspheres provide scattering from the liquid; their concentration and size distribution was chosen to provide an equal level of backscatter and to the actual blood. The V_S of this liquid was 1546 m/s, and the attenuation coefficient was 0.1 dB/cm. MHZ (Boote & Zagzebski, 1988; Yoshida et al., 2014).

Also, Tanaka et al. (2012) have achieved BMF with density 1.05 g/ml that density it value determined in the IEC standard, so, PEG (400), glycerin-aqueous-solution- and water-soluble silicone oil used as a mixture fluid- dispersed with polystyrene microsphere particles (Figure 2.5). The BMF was prepared based on the Scheffé and Urick equations. The speed of the sound of this liquid (BMF) was 1570 m/s, the viscosity was 4.0 mPa.s, density was 1.050 g/cm^3, and the attenuation coefficient was < 0.1 dB/cm. MHz. In addition, this BMF has been desired for experiment Doppler ultrasonic diagnostic instrument.

Figure 2.5 Appearances of the BMF samples in glass jars. Polystyrene particles stay suspended in the mixture fluid after 2 weeks (glycerin and water-soluble silicone oil) (Yoshida et al., 2012).

2.5.3 Blood Mimicking Fluid (BMF) with Sephadex Particles

When the density of particles more than the surrounding liquid, the settling (precipitate) may happen. This can surely be visible at low speeds for particles like a Sephadex (Ramnarine et al., 1998). Non-biological blood (artificial or chemical blood) has been studied briefly (Law et al., 1989). What the BMF prepared with powder material (Sephadex) could be a scattered particle and suspended in H_2O.

The Sephadex particle has been studied as a scatte material of the BMF (Douville et al., 1983; Hein et al., 1992; Hoskins et al., 1989; McDicken, 1986). According to the previous studies, the Sephadex density and size are much greater than the real red cells, the backscattered and other measurements of Sephadex are different from human blood such as (20-70 µm) at a concentration of l.5 g/L (Douville et al., 1983) and the particle density is 1.7 g/cm^3. Moreover, they aggregate or clotting at low and large shear rates can give non-Newtonian manner. However, several types of scatter particles and mixture fluids were used for BMF preparation as shown in Table 2.3.

Table 2.3 Common scatter particles items used in BMF and their preparations methods.

Scatter Material	**Materials and Method**	**Applications**	**Physical and acoustical properties**	**References**
• Orgasol (nylon)	The norm solution was made by mixing pure H_2O, surfactant, dextran 185000D, and glycerol 83.86%, 0.9%, 3.36%, 10.06% respectively, with 1.82% 5,10, and 20-µm Orgasol particles by weight, this mixing was done by magnetic stirrer then filtered the BMF to eliminate any residual mass clumps, this filtering is done through a 32 µm sieve. Ultimately, before measuring the acoustical and physical properties of BMF, it was degassed by vacuum pump technique until removal of the air bubbles.	(1) For the vessel flow phantom. (2) For Doppler US tool in the test object by using artificial blood. (3) For inspecting the influence of 'red blood cell density.' (4) For studying the thermal and acoustic properties (therapeutic study) of High Intensity Focused US (HIFU). (5) To discovering the problems of identifying tumours (swelling of a part of the body) by the medical US. (6) For evaluating the influence of transducer focus position and signal length in backscatter coefficient measurement for BMF.	Physical and acoustical properties were agreed on the IEC values.	(Grand-Perret et al., 2018; Ho et al., 2017; Liu et al., 2008; Oates, 1991; Raine-Fenning et al., 2008; Ramnarine et al., 2001; Samavat & Evans, 2006; Thorne et al., 2008; Yoshida et al., 2014; Zhou et al., 2017)
• Polystyrene microspheres	Prepare a BMF with the acoustic speed and density with polystyrene particles as a scattered material suspension in the mixture fluid made of water-soluble silicone oil.	Using Doppler US for measurement of blood flow	Physical and acoustical properties were agreed on the IEC values.	(Boote & Zagzebski, 1988; Tanaka et al., 2012; Yoshida et al., 2014; Yoshida et al., 2012)

2.6 Chemical Items Used for Preparing Blood Mimicking Fluid (BMF)

PG is an organic liquid, in-expensive, not evaporating and stable with time. The acoustical properties of PG were suitable as a TMM item, and it was with suitable acoustic speed 1513 m/s and its density of 1.036 g/ml. PG was used to produce oil gel by mixing it with ethylene glycol, Kondo et al. (2002) were used PG for making TMM. Moreover, Wang et al. (2002) were used PG as a contrast media in Computed Tomography (CT) applications. In Optical Coherence Tomography (OCT), the PG was played an important role as a contrasting agent for scanning and diagnose gastrointestinal (GI) tract in-vivo. In other words, because the PG has no side effect or toxicity, so can use as a contrast agent in the human.

PEG is a polyether element, with several applications from industrial processing to the medical field. PEG is also called polyethylene oxide (PEO), which relies on its molecular weight. PEG was utilized several times in BMF preparation for the Doppler applications. The PEG has several molecular weights 200-600 g/mole which relating to the viscosity, so it was used to mix with other glycols such as water-soluble silicon oil to be as a suitable mixture fluid then mixing it with polystyrene as a scattering particle. The density and V_S of PEG independent on their molecular weight and its density of 1.124 g/ml (Yoshida et al., 2014; Yoshida et al., 2012).

Poly (4-methylstyrene) has quite identical chemical structures to the polystyrene but the differences in the methyl group of Poly (4-methylstyrene). However, a Poly (4-methylstyrene) were used as an oxidative factor at elevated (high) temperature (McCreath et al., 2015). In addition, Bonaccorsi et al. (1993) were used a poly (4-methylstyrene) as dispersants small particles in a medium (coal-water mixtures), to make the connection between the scatter's properties of this category of chemical polymers.

2.7 Gelatin-based Tissue Mimicking Material (TMM)

Gelatin is a strong (avoid TMM rupturing) powder item, consist of a large molecular mass of the water-soluble proteins, gelatin exist in collagen with a density of 1.3 g/ml. This protein found in the animal's skins, bones, and cartilages. Furthermore, there are several factors influence on the gelatin features, such as the collagen type, age source of the animal (Gómez-Guillén et al., 2011; Johnston-Banks, 1990). There are several applications of gelatin such as in pharmaceutical, classical food, cosmetic, photographic and phantoms fabrication. The application depends on the gel-forming features. Gelatin is low in cost and very strong item (Gómez-Guillén et al., 2011).

TMM was made by gelatin-silica-based phantom for both modalities of ultrasonic (US) and photoacoustic (PA) scan imaging (Titus et al., 2018). Gelatin-silica was constructed with diverse additives to mimic actual optical and acoustic features. Gelatin- silica was in spherical particles (40 mm) shape to avoid unwanted speckle reflection, and with proper acoustical properties for simulating required properties of real soft tissues (S.T) (Cook et al., 2011).

The gelatin was mixed with graphite powder in many ratios, and alcohol with Madsen group. The p-propyl and p-methyl benzoic acid were used to prevent microorganism's growth. However, changing the graphite powder ratio in the gelatin (scattering coefficient), the attenuation changed from 0.2 to 1.5 dB/cm at 1MHz. They reported that changing the ratio of n-propanol in H_2O at room temperature changes the acoustic velocity from 1520 to 1650 m/s. These items were used to get high stability near-room temperature with long-term (over a period of 4 months) (Culjat et al., 2010; Madsen et al., 1978).

For improving the TMM stability, gelatin-based TMM is advanced by utilizing gelatin and alginate. However, for reducing the microorganism's growth, the bath disinfectants were used. And, to improve the thermal stability up to room temperature (25 $^{\circ}$C) the calcium chloride ($CaCl_2$) was added. The acoustical properties of the material were 1520 m/s and 0.12–0.5 dB/cm. MHz, respectively (Bamber et al., 1996; Bush et al., 1983; Culjat et al., 2010).

The main drawback of the US perfusion scan is that there are not many flow phantoms that could be utilized to evaluate these mechanisms. That's why some researchers have fabricated tiny linear tube, and the others have used dialysis cartridges. However, George et al. (2018) investigated and fabricated a technique utilizing traditional gelatin mold around a polymer network. The gelatin flow phantom was mixed with scattering particles of graphite which was used to simulate and mimic the scatter in real S.T. The small channels were similar to a VMM and were fixed by the resin. Moreover, the BMF was pumped through the channels then the gelatin phantom was evaluated to display the perfusion and V_S in BMF.

2.8 A-scan Ultrasonic Technique

In A scan-mode, the α, backscatter power, and SS measurements in biological items can be done via PE/US emission mode. The acoustical measurements of items are specified like a proportion of distance travelled through the ultrasonic signal wave and traveling of the Tof (Buniyamin et al., 2017; Halim et al., 2014; Nowak et al., 2013).

2.8.1 Protective Layer of Ultrasonic Transducer

US probe made of piezoelectric crystal layer, and then a Protective layer above the piezoelectric crystal layer as a coat to protect it. The presence of the protective layer increases attention to its influence during the determination of ultrasonic speed (Buniyamin & Halim, 2017; Halim et al., 2014). The influence of this protective layer on ultrasonic speed measurements has been debated by Nowak & Markowski (2013) in their research on ultrasonic speed measurement way, they found that ultrasonic signal wave will be generated then travel through the protective layer of probe before going to the medium or region of interest (experiment specimen). Moreover, also it was reported that the protective layer of the probe with a fixed thickness would produce the overvaluation of the present T ToF of measurement with fixed time delay (Norli, 2007; Sarvazyan et al., 2005).

Halim et al. (2014) investigated that for precise ultrasonic speeds measurement, the influence of the protective layer must be completely removed through the measurement by following Equation 2.3.

$$dp_l = \frac{(\frac{t1}{t2}\,da2) - da1}{2(1 - \frac{t1}{t2})} \qquad 2.3$$

Where dp_l 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 of acrylic plate (40 mm), t_2 is the time of flight of acrylic plate (80 mm).

Thus, to measure the V_S with taken in consideration of the thickness of the protective layer, applying Equation 2.4.

$$V_S = \frac{2\,(d + dpl)}{t} \qquad 2.4$$

Where V_S is the speed of sound, d is the sample depth, dp_l is the thickness of protective layer, and ultimately t is the time of flight.