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To my family and friends,
whose understanding and support
were very valuable
all these years...
Abstract

The efficacy of ultrasound-assisted thrombolysis as an adjunct treatment of ischemic stroke is being widely investigated [1]. Ultrasound facilitates the process of blood clot disruption, or thrombolysis [2]. The mechanisms of ultrasound-assisted thrombolysis are not well known. Our overall goal in this work is to elucidate the potential of one physical mechanism, thermal, that might be responsible for the ultrasound assisted thrombolysis.

For the theoretical modeling of ultrasound induced hyperthermia, the acousto-mechanical and thermal properties of clotted blood were measured in vitro. The density of porcine clots is $(1.06 \pm 0.01) \times 10^3 \text{ kg/m}^3$ and that of human clots is $(1.08 \pm 0.02) \times 10^3 \text{ kg/m}^3$. The measured values of the speed of sound in porcine clots are $(1.547 \pm 0.001) \times 10^3 \text{ m/s}$ at $20 \ ^\circ \text{C}$ and $(1.577 \pm 0.002) \times 10^3 \text{ m/s}$ at $37 \ ^\circ \text{C}$. The speed of sound in human clots is $(1.597 \pm 0.009) \times 10^3 \text{ m/s}$ at $20 \ ^\circ \text{C}$ and $(1.633 \pm 0.004) \times 10^3 \text{ m/s}$ at $37 \ ^\circ \text{C}$. The amplitude coefficient of attenuation in blood clots was determined from $120 \ \text{kHz}$ to $3.5 \ \text{MHz}$ at room temperature $(20 \pm 2) \ ^\circ \text{C}$. It ranged from $0.10$ to $0.30 \text{ Np/cm}$ in porcine clots and from $0.09$ to $0.23 \text{ Np/cm}$ in human clots. The magnitudes of specific heat and thermal conductivity of porcine clotted blood are $(3.2 \pm 0.5) \times 10^3 \text{ J/kg} \cdot \text{K}$, $0.55 \pm 0.13 \text{ W/m} \cdot \text{K}$, respectively. For human clotted blood, they are $(3.5 \pm 0.8) \times 10^3 \text{ J/kg} \cdot \text{K}$ and $0.59 \pm 0.11 \text{ W/m} \cdot \text{K}$.

The in vitro experiments conducted at two temperatures, $20 \ ^\circ \text{C}$ and $37 \ ^\circ \text{C}$, gave lower values for the ultrasound hyperthermia then the theoretically predicted ones. The thermal elevation in the human cranial temporal bone was below $1 \ ^\circ \text{C}$ at the
spatial peak intensity of 0.5 $W/cm^2$ and with 80% duty cycle in the frequency range between 120 kHz and 3.5 MHz. The ultrasound hyperthermia in porcine and human clots did not exceed $(0.33 \pm 0.06)$ °C at the same level of intensity and in the same frequency range. It increases with the increase of the ultrasound frequency. The results on the magnitude of the ultrasound hyperthermia in clotted blood will provide the necessary information for the numerical estimates of the contribution of the thermal mechanism to ultrasound thrombolysis.

**Key words:** ultrasound, hyperthermia, thrombolysis, clotted blood, ischemic stroke.
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Chapter 1

Introduction

“Stroke ranks number 3 among all causes of death, behind deceases of the heart and cancer in the US”.\(^1\) Aggregate direct and indirect lifetime cost (care, treatment and the lost of productivity) associated with first stroke is $57.9 billion [115]. Despite significant improvements in mortality rates from stroke during the past two decades, stroke still affects over 700,000 Americans each year [3,115]. Ischemic stroke, which accounts for 80 - 85% of all strokes, is caused by the sudden loss of blood flow to a brain region due to a thrombosis or embolism. Investigational therapies have focused on limiting the severity of ischemic injury (neuronal protection) and reducing the duration of ischemia (restoring blood flow). However, at present, the only therapy for ischemic stroke that is approved by FDA is the thrombolytic agent, recombinant tissue Plasminogen Activator (rt-PA) [117]. In 1995, the National Institute of Neurological Disorders and Stroke tissue plasminogen activator stroke study group reported that rt-PA reduces disability in patients with acute ischemic stroke. Recombinant t-PA is moderately effective in lysing thrombi in ischemic stroke patients and improves neurologic deficits if given within 3 hours after the onset of stroke symptoms [130]. Unfortunately, thrombolytics also can cause intracerebral hemorrhage.

Adjuvant therapies that lower the dose of rt-PA or increase its efficacy would rep-

\(^1\)Heart Disease and Stroke Statistics - 2006 Update. American Heart Association, Dallas, TX, 2006.
resent a significant breakthrough. Improved effectiveness or greater safety would provide a powerful impetus for physicians to administer rt-PA to a larger portion of the patients with ischemic stroke [131].

Recently, it has been demonstrated that ultrasound used as an adjuvant to rt-PA can increase thrombus dissolution in an in vitro model [5]. The overall goal in the University of Cincinnati medical ultrasound laboratory is to elucidate the physical mechanisms underlying ultrasound-assisted thrombolysis [131].

The central hypothesis of this dissertation is that low intensity ($\leq 0.5 \ W/cm^2$) pulsed ultrasound can be used to increase the temperature of a clot. Our interest in this thermal increase is motivated by the assumption that the exposure of a blood clot to ultrasound may significantly enhance rt-PA lysis, primarily via thermal mechanism. We assume that the exposure to ultrasound increases the temperature of the clot by less than a degree Celsius so that the enzymatic activity of rt-PA is enhanced [2, 130]. Successful completion of these studies should provide new information for the development of a strategy for improving acute stroke therapy.

The theoretical model of the thermal elevation in clotted blood induced by ultrasound insonification in spherical geometry was developed in this study in Chapter 2.

In order to proceed with numerical calculations of a thermal effect in the tissue insonified with ultrasound, the acousto-mechanical and thermal properties of clotted blood must be well characterized. We need to know the physical properties not only for the human clotted blood but for the porcine clotted blood as well since the first stage of verification of the efficacy of ultrasound as an adjunctive treatment of ischemic stroke can be carried out in an in vivo on pigs. We could not find the necessary information after a thorough analysis of the published data on these or relevant types of tissues presented in Chapter 2. Because of the lack of data on the physical properties of clotted blood in literature, we conducted the measurements of the thermal conductivity, the specific heat, the speed of sound, and the density
Chapter 1. Introduction

of human and porcine clots.

We also measured the magnitude of the amplitude attenuation coefficient, $\mu = \mu(f)$ (an amplitude absorption coefficient, $b$, plus a scattering term, $\sigma$) in blood clots in the frequency range of 120 kHz - 3.5 MHz at the room temperature and determined its frequency dependence.

In order to proceed with an experimental part of this work, we have chosen the appropriate experimental techniques and implemented it into the experimental setups. The analysis of all available methods and techniques for the measurements of physical properties of biological tissues is conducted in Chapter 3.

Experimental procedures and the description of experimental setups and samples are presented in Chapter 4.

The obtained results on the acousto-mechanical and thermal parameters of clotted blood were used to fulfill the numerical calculations of the spatial and temporal distribution of temperature elevation in blood clots. Those estimates along with the calculated values for the steady state temperature on the surface of cranial bone are presented in Chapter 5.

The direct measurements of temperature elevation in human skull and its dependence on frequencies (120 kHz - 3.5 MHz) of ultrasound will help to assess the suggestion that the big losses in acoustic beam lead to direct heating of the skull wall. Our purpose is to verify the extent of validity of this suggestion. The assessment of the transcranial transmission losses at 1 MHz was presented in Chapter 5.

We measured the temperature rise during the ultrasound exposure of blood clots in an in vitro model. The dependence of the ultrasound-induced thermal elevation is explored over three center frequencies (120 kHz, 1 MHz, 3.5 MHz) at the peak-to-peak pressure amplitude of 0.25 MPa. The dependence of ultrasound-induced thermal elevation on the central frequency will help to clarify the mechanism responsible for increased thrombolysis, and provide new data on the magnitude of ultrasound induced hyperthermia in blood clots and cranial bone, which may be
useful in the transcranial applications of ultrasound.

Experimental measurements of the ultrasound-induced thermal elevation in blood clots and on the surface of cranial bone were compared to the theoretical predictions. The analysis of the observed discrepancies between theory and experiment was conducted in Chapter 6.

1.1 General background for thermal bioeffects due to ultrasound exposure

We imply the thermal mechanism of the ultrasound bioeffect when we use the term a thermal bioeffect. By limiting the number of ultrasound bioeffects involved in the heating of biological tissues to the thermal bioeffects, we constrict the number of the physical mechanisms we are going to consider in the current work.

Different mechanisms of the ultrasound bioeffects may act on the biological tissues synergetically, which means that some effects produced by cavitation or streaming may occur and act more effectively under lower intensities of ultrasound when significant thermal elevation is present. The ultrasound propagating through the continuous biological media attenuates. In part it is caused by the absorption of the ultrasound energy and conversion it into heat. The other part of ultrasound attenuation is caused by scattering. For a homogeneous medium such as water, all of the attenuation comes from absorption of acoustic waves and the scattering is negligible [33]. If we consider the clotted blood with all its inhomogeneities (air bubbles, irregular molecular structure) except the absorption in the material of a clot, some attenuation of the acoustic energy occurs due to the scattering. It is understood that the thermal bioeffect depends on the acoustic properties of the insonified tissues. If the ultrasound absorption of the biological tissue is high we expect the significant heating of the surrounding areas. Quantitative estimates of a
Figure 1.1: The main mechanisms of interaction between biological tissues and ultrasonic wave passing through. Thermal, mechanical and “other” mechanisms are presented when tissues are exposed to ultrasound [19]. “Other” mechanisms include the direct excitation of neurons [56], a change of viscosity of tissue and a change of permeability of the cell membrane [38].

Sonic heating at any location can be easily made by means of the following formula [33]:

\[ \dot{Q} = 0.055 \cdot \alpha \cdot I_0 \]  \hspace{1cm} (1.1)

where the heating rate, \( \dot{Q} \), is given in calories per cubic centimeter of exposed tissue per second (\( cal/cm^3 \cdot s \)), \( \alpha \) is the absorption coefficient, depending on the ultrasound frequency and the type of tissue, \( I_0 \) is the local time averaged intensity in watts per square centimeter (\( Watts/cm^2 \)). For example, a typical value of the absorption coefficient for the soft tissue at a frequency of 3.5 MHz is 2 \( dB/cm \). If the local intensity is 0.1 \( W/cm^2 \), which is typical for both diagnostic and therapeutic ultrasounds, the
estimate, according to the formula above, gives the heating rate of 0.011 \( \text{cal/cm}^3 \cdot \text{s} \).

By taking into account that the typical values for the heat capacity of soft tissues vary within 10% that of water [30], we can estimate the heating rate for 1 \( \text{cm}^3 \) of a soft tissue [33]. It gives that the temperature rises with a rate of 0.011 \( ^\circ \text{C}/\text{s} \) or 0.7 \( ^\circ \text{C}/\text{min} \).

As time progresses, the new process starts to appear. The heat starts to flow into the regions with the lower temperatures. The total heating rate decreases and the temperature of the insonated region reaches saturation. There are two possible mechanisms responsible for such a heat transfer: a heat perfusion accomplished by a blood flow and a thermal conduction (or alternatively, thermal diffusion). The final temperature in the ultrasound exposed tissue is difficult to predict due to different conditions of the insonified tissues and many ultrasound and biological factors which have to be taken into consideration [19].

![Figure 1.2: Temperature elevation in a rat skull and liver exposed to CW ultrasound. A 12.7 mm diameter transducer was coupled to the scalp by gel. Because of motion, its effective diameter was 15 mm. The acoustic beam power at 2 MHz was 1 W. (Data reproduced from Carstensen et al. [57]).](image-url)
During the insonification of the hard tissue the resulting temperature elevation may exceed 1 °C, which is the typical upper limit for the soft tissue insonified by the diagnostic ultrasound. The results of the investigation of the sonication of a rat skull obtained by Carstensen et al. [57] show that the rate of the temperature elevation is 3 °C/W/cm².

The results of the thermal elevations in a mouse spinal cord during the ultrasound irradiation at 982 kHz were reported in [58]. The thermal elevations of 15.7°C and 4.3°C were reached after 10 s of insonification with the intensity of 100 W/cm² and duty cycles of 40 % and 10 % respectively. In the case of a CW insonification with the same intensity, a temperature rise in 16.2°C was observed after 4 s of sonication and 5.2°C after 1 s of sonication. When the intensity was 70 W/cm², the thermal elevations were 8.9°C after 7 s of sonification with a duty cycle in 40 % and 2.5°C after 10 s of sonication with a duty cycle in 10 %. With a CW insonification, the authors received the temperature rise in 9.4°C after 2.8 s and 3.8°C after 1 s of insonification.

### 1.1.1 Thermal index (TI)

In many diagnostic and therapeutic applications of ultrasound, it is necessary to know the expected magnitude of the thermal elevation in tissues irradiated by ultrasound. This information is intended to help to estimate the risk of the application of ultrasound. For these purpose, the concept of the thermal index (TI) was introduced by American Institute of Ultrasound in Medicine (AIUM) in collaboration with National Electrical Manufacturers Association (NEMA) in 1992.

The definition of TI may be given by the following relation:

\[
TI = \frac{W}{W_{eg}},
\]

where \( W \) is the power of the ultrasound wave during the tissue exposure, \( W_{eg} \) is a power necessary to cause a maximum temperature increase of 1 °C anywhere in the
beam [36]. It is supposed to be the upper limit of temperature rise in the object sonicated with the power. This limit can be approached but never exceeded in vivo. The difficulty with calculating the TI lies mostly in the estimation of $W_g$, the power giving a worst-case $1 \, ^\circ C$ rise. There are three tissue models developed for practical applications and three types of indices are used for different cases: for the soft tissue - TIS, for the bone-at-focus case - TIB and for the cranial application - TIC. The tissue models are based on the simple assumptions about the acoustic and thermal properties of tissue with the tissue attenuation coefficient of $0.3 \, dB/cm \cdot MHz$.

The index TI depends linearly on the acoustic power, which follows directly from the definition, and on the frequency because the heating as well as the coefficient of ultrasound absorption depend on frequency of ultrasonic wave. The direct measurements of the thermo-physical and acoustic properties of a particular type of tissue may give more precise values of a TI index and prevent, in such a way, the possible tissue damage due to overheating.

1.2 Ultrasound-assisted thrombolysis: unwanted or wanted thermal side effects?

For a healthy living being, the temperatures range from $36 \, ^\circ C$ to $38 \, ^\circ C$ is vitally acceptable. This range of temperatures provides conditions under which many chemical processes maintaining life are balanced and proceed with the normal rates [36]. A temperature of $42 \, ^\circ C$ is “largely incompatible with life”. More drastic changes, such as the denaturation of enzymes and other large molecules, happen at the temperatures above $45 \, ^\circ C$. Generally speaking, the human body is quite capable of recovering from such thermal changes. However, there are several organs which do not tolerate the thermal elevation well: the reproductive cells, the unborn fetus, and the central nervous system (brain and spinal cord) [36].

Ultrasound, producing the thermal elevation in tissue, affects the biological ob-
jects. The thermal mechanism is the main mechanism of interaction between ultrasound and a biological tissue during many therapeutic and surgical applications. The heat produced by nonacoustic means will cause the same response as the ultrasound does, provided that the thermal conditions are the same [33].

The rate of the process of the blood clot disruption increases during the ultrasound insonification [2, 5, 6, 7, 12]. Along with the increased thrombolysis, the ultrasound increases the temperature of the clot, blood vessels, and surrounding tissue including the soft tissues and bones. If we want to consider the possible consequences of the action of ultrasound insonification which we intend to use to enhance a thrombolysis, first we have to review the all known effects of the hyperthermia, produced by the other means. Below is a list of the following desirable effects in which the tissue temperature is maintained at a value from 40 to 45°C for a period of 5-30 minutes [31]:

- increased extensibility of collagen tissue;
- decreased joint stiffness;
- pain relief;
- relieves of muscles spasms;
- assistance in resolution of inflammatory infiltrates, edema, and exudates;
- increased blood flow.

Hyperthermia is being used increasingly for the treatment of cancer. In some applications, the entire body of a patient is maintained at a temperature of 41 – 45°C for several hours [31]. Some studies also showed that the ultrasound irradiation of a tumor in mice makes it disappear. Application of a hyperthermia to the treatment of cancer depends, partly, on the destructive capabilities of heat [34]. Elevated temperature causes a tissue death (necrosis) at the rate that depends on the type
of tissue and on the temperature. For example, when normal human skin tissue is maintained at a temperature of 45 °C, thermal death occurs in about 2.5 hours. At lower temperatures, the time required for thermal death is longer and at higher temperatures it is shorter. Specifically, a change of 1 °C in the temperature changes the time required for a thermal death approximately by about a factor of 2. This is true for cells and tissues and depends considerably on their type and physiological state [34]. It is not well understood why the body responds as it does to temperature elevation, although there are a number of possible contributing factors. From physics and chemistry we know that the rates of fluid flow, particle diffusion, and biochemical reactions are all very temperature dependent. Related to these facts are findings that rising the temperature affects membrane permeability, active transport processes, and metabolic rates. The temperature rise may also produce the changes in the functioning and integrity of cellular and subcellular structures [33].

Some of the therapeutic effects of heat are the consequence of vasodilation which, in turn, is brought about by various mechanisms. Among these is an interaction of blood vessels with histamine-like substances and bradykinin released by inflammatory reactions or by other means [31].

The changes described above, which occur in a living organism when its temperature is elevated by nonacoustic means, should also occur when the same temperature increase is produced by ultrasound. In contrast to the nonacoustic hyperthermia, the ultrasonic hyperthermia techniques offer advantages over the other methods of applying heat to a patient. With ultrasound, the heat is generated within the tissue rather than at its surface. The spatial distribution of heat also can be controlled by manipulation of the focused beams. As it might be expected, undesirable effects of temperature elevation can also occur, whether the heat is generated by ultrasound or by other means. There are several ultrasound bioeffects which are undesired. Ultrasound insonification can produce teratologic effects, subcellular chemical alterations and fetal death. A development of abnormalities in a fetus ex-
posed to ultrasound would be expected if, in applying ultrasound to a pregnancy, the temperature of the fetus were to be elevated by several degrees and maintained at the level for an extended period. However, significant heating of the fetus is very unlikely in any usual application of ultrasound in diagnosis or therapy.

### 1.3 Literature review on the mechanisms involved in blood clot disruption

According to the recent studies, there are three major mechanisms which may cause the ultrasound enhanced thrombolitic effect: thermal heating, microstreaming and cavitation. The synergetic effect of all of them may also take place during the ultrasound thrombolysis.

The thermal mechanism may contribute through the increased metabolic rates under the ultrasound exposure. For a typical biochemical reaction, catalyzed by
an enzyme, its rate increases with increasing temperatures until a peak value is reached, then declines as the enzyme becomes denatured. A temperature dependence of the kinetic rate of the chemical reaction is given by the following formula discovered by Arrhenius in 1889 [22]:

\[ \chi(T) = M \cdot e^{\frac{-E_a}{k_B \cdot T}}, \]  

(1.3)

where \( M \) and \( E_a \) are the pre-exponential factor and an activation energy for a given type of chemical reaction, \( \chi(T) \) is its kinetic rate, and \( k_B \) is the Boltzmann’s constant, \( T \) is the absolute temperature of solution. Thus, tissue metabolism may be speeded up or slowed by temperature [31]. The formula above gives the relation for the mass loss, \( m_l(T, t) \) for blood clots treated with rt-PA over some period of time \( t \) [118, 119]:

\[ m_l(T, t) = C \cdot e^{\frac{-E_{eff}}{k_B \cdot T}} \cdot A(t), \]  

(1.4)

where \( C \) and \( \Delta E_{eff} \) are the pre-exponential factor and the effective activation energy for the chemical reaction leading to the formation of the fibrin degradation products (FDP), \( A(t) \) is a function of time and the initial concentration of reactants, \( T \) is the absolute temperature of solution of chemical reactants. Thus, enzymatic activity and tissue metabolism may be speeded up or slowed by temperature. The change in mass loss \( \Delta m_l(T, t) \) due to a small temperature change \( \Delta T \) can be expressed in the following relation:

\[ \Delta m_l(T, t) = \frac{\partial m_l(T, t)}{\partial T} \cdot \Delta T = C \cdot e^{\frac{-E_{eff}}{k_B \cdot T}} \cdot \frac{E_{eff}}{k_B T^2} \cdot A(t) \cdot \Delta T, \]  

(1.5)

where \( \Delta T \ll T \). The temperature change \( \Delta T \) may be produced by any mean including ultrasound insonification.

Siddiqi et al. reported the results of the study of the increased permeation of fibrinolytic enzymes into a fibrin gel due to the pulsed ultrasound insonification at 1 MHz with intensity in 2 W/cm\(^2\) and 50 % duty cycle [67]. The authors attribute
this effect to the ultrasound induced cavitation. The degassing of the fluid, in which the sample of fibrin gel was immersed, significantly reduced the effect of fibrinolysis.

The increased penetration of the radiolabeled rt-Pa into clots during the 1 MHz ultrasound sonication was observed by Francis et al. [68]. They examined the effect of ultrasound insonification at 1 MHz on the spatial distribution of plasminogen activator between the clot and the surrounding fluid in vitro. The intensity of ultrasound was $4 \text{ W/cm}^2$.

The data obtained in [65] by Sakharov et al. prove the significant role of streaming and heating during the continuous wave ultrasound lysis of plasma clots at 1 MHz. The temperature dependence of lysis for two periods of exposure in a water tank is presented in Figure (1.4). The results of the conducted experiments in the temperature range between 37 and 43°C showed that the temperature increase in the clot of a few degrees produces a two-fold increase in the lytic rate. The least square fit to their results presented in Figure (1.4) shows exponential dependence between the clot lysis and the temperature in a water tank.

The analysis of the data obtained in their experiments in which the plasma clots where exposed to ultrasound shows that none of the known mechanisms can solely prevail in an in vivo. They attribute 30% of the effect of ultrasound-assisted fibrinolysis of plasma clots to heating and the other 70% of the final mass loss of clots to microstreaming. The authors tried to reduce the role of cavitation in the fibrinolysis by reducing the ultrasound frequencies and intensities.

The peak to peak pressure in the targeted area was 0.26 MPa and the corresponding time averaged intensity was $2.3 \text{ W/cm}^2$. They tried to separate the total synergistic effect from both heating and acoustic streaming by simulating the microstreaming with forceful stirring and heating by changing the temperature in the experimental tank. In order to eliminate microstreaming, they degassed the experimental cell with the sample of clot.
Figure 1.4: The temperature dependence of a plasma clot mass loss during the exposure of a clot to the different temperatures in a water tank. (Reproduced from Sakharov et al. [65]).

In [69], the authors presented the results of the investigation of the ultrasound-enhanced fibrinolysis at low kilohertz frequencies (27 - 100 kHz) at which the tissue penetration of enzymes (rt-PA) is better and heating is less. The results showed that the maximum of enhancement of fibrinolysis is reached at 27 kHz. The ultrasound irradiation with intensity in $1 \text{ W/cm}^2$ gave 26 % mass loss at the temperature of 37 °C. The fibrinolysis was directly proportional to the concentration of rt-PA. The 10 % duty cycle ultrasound insonification gave 60 % of lysis with the CW ultrasound. This fact is very important in diagnostic and therapeutic ultrasound because the pulsed ultrasound significantly decreases the risk of overheating of tissue during the ultrasound exposure.

According to [70], the most important factor significantly increasing ultrasound-enhanced thrombolysis is a transport rate of thrombolytic agents (rt-PA, urokinase...
etc.) into clots. The conducted studies showed that cavitation appeared to be an important physical mechanism in the ultrasound assisted thrombolysis because degassing the surrounding of clot fluid significantly reduced the ultrasound induced flow increase. Cavitation loosens the fibrous network of fibrin in clots and creates additional binding centers for fibrinolitic centers [70].

According to [81], the use of galactose-based microbubbles increases the ultrasound assisted fibrinolitic effect during the low intensity 2 MHz ultrasound exposure of the whole human clots with rt-PA or without it. The authors reported 31 % clot mass loss during the ultrasound insonification with the intensity $0.46 \, W/cm^2$ and in the presence of rt-Pa and microbubbles. From the results of in vitro studies, the authors concluded that cavitation and microstreaming are the main mechanisms responsible for the clot disruption and fragmentation. They may allow a deeper penetration of rt-PA into clot and enlarge, in such a way, the exposure of the fibrin network to rt-PA.
Chapter 2

Theoretical prediction of the temperature increase in a tissue due to the pulsed ultrasound exposure

2.1 Description of structure of clotted blood and bones and definitions of their acoustic and thermo-physical properties

The physical properties of clotted blood and cranial bone may be divided onto two groups: acoustical properties and thermophysical properties. Sound speed, amplitude absorption coefficient, backscattering coefficient belong to the acoustic properties, while density, specific heat, thermal conductivities and thermal diffusivity belong to the thermo-physical group of properties.

Before discussing each property, one remark has to be done. We assume in our further discussion that all properties are isotropic. It concerns both type of tissues discussed in this work: clotted blood and cranial bone. This assumption simplifies the type of geometry for many problems and allows us to consider the one dimensional case only.

The definition of the heat flow $q$ through the area $\vec{A}$ generated by a temperature gradient $\vec{\nabla}T$ is given by the following expression:
$q = \vec{A} \cdot K \cdot \vec{\nabla}T = A \cdot K \cdot \vec{n} \cdot \vec{\nabla}T, \quad (2.1)$

where $\vec{n}$ is the normal to the area $A$ and $K$ is a coefficient of thermal conductivity \[35\]. In one dimensional case, we can replace the temperature gradient by the ratio $\frac{\delta T}{\delta L}$, where $\delta L$ is the length of a sample, so that the heat flow in this case is $q = A \cdot K \cdot \frac{\delta T}{\delta L}$, and for the coefficient of thermal conductivity $K$, we have:

\[ K = \frac{q \cdot \delta L}{A \cdot \delta T}. \quad (2.2) \]

Specific heat \[1\], $C_v$ is defined by the following relation:

\[ C_v = \frac{1}{m} \cdot \frac{\partial q}{\partial T}, \quad (2.3) \]

where $m$ is the mass of the physical object, $\partial q$ is the amount of the absorbed or released heat caused by the change of the object’s temperature, $\partial T$.

The definition of the coefficient of thermal diffusivity, $\kappa$ can be given by the following expression:

\[ \kappa = \frac{K}{\rho \cdot C_v}, \quad (2.4) \]

The dimension of $\kappa$ in SI units is $\frac{m^2}{s}$, but for the practical reasons sometimes $\frac{mm^2}{s}$ is used instead.

The definition of the intensity absorption coefficient $\alpha$, along with the intensity attenuation coefficient $\gamma$, which is the sum of the intensity absorption coefficient $\alpha$ and the intensity scattering coefficient $\gamma_\sigma$, can be established through the expression for the spatial dependence of the time averaged intensity in a dissipating medium. In one dimensional case, we have:

\[ I(z) = I(0) \cdot e^{-\gamma z}, \quad (2.5) \]

for $z \geq 0$, where $z$ is the direction of propagation of a plane acoustic wave. This is\[1\]We use both terms in this notation: specific heat and heat capacity. Specific heat is the heat capacity per unit mass.
the general form of a solution for the spatial dependence of the intensity of acoustic wave in a dissipating medium due to viscous losses [60, 78]. If we ignore the contribution of scattering to the total losses of ultrasound wave, which is valid in practice for many biological tissues, then we obtain:

\[ I(z) = I(0) \cdot e^{-\alpha z}, \tag{2.6} \]

where \( \alpha \) is the intensity absorption coefficient.

The intensity absorption coefficient \( \alpha \) can be introduced as follows:

\[ \alpha = \frac{1}{z} \cdot \log \left( \frac{I(0)}{I(z)} \right). \tag{2.7} \]

It describes the decline in the acoustic intensity of ultrasound wave per unit length and is usually measured in \( \frac{\text{dB}}{\text{m}} \) or \( \frac{\text{dB}}{\text{cm}} \). The absorbed acoustic energy eventually converts into a thermal or electro-magnetic energy.

In a general case, the process of propagation of an acoustic wave in a dissipating medium can be described by the dependence of acoustic pressure \( p(z) \) in the acoustic wave. In dissipating medium the pressure in a wave decays exponentially along with the amplitude of the particle displacement and intensity [61]. In one dimensional case we have:

\[ p(z) = P(z) \cdot e^{i(\omega t - qz)} = P_0 \cdot e^{-bz} \cdot e^{i(\omega t - qz)}, \tag{2.8} \]

where \( q \) and \( \omega \) a wave vector and a cyclic frequency of the acoustic wave, \( P(z) \) is the amplitude of a pressure of the acoustic wave at the point with a coordinate \( z \), \( P_0 \) is the pressure amplitude at \( z = 0 \).

The amplitude absorption coefficient \( b \) is defined by the following expression for the spatial dependence of the amplitude of a pressure of the acoustic wave in the absorbing medium with no scattering [13, 17, 41]:

\[ b = \frac{1}{z} \cdot \ln \left( \frac{P_0}{P(z)} \right). \tag{2.9} \]
The last formula is convenient in the experimental measurements of the coefficient of amplitude absorption, because we, usually, measure the pressure in the incident ultrasonic wave. The dimension of this coefficient in the metric system is $\frac{Np}{m}$ or $\frac{Np}{cm}$.

The relationship between an intensity absorption coefficient $\alpha$ and an amplitude absorption coefficient $b$ follows directly from definitions of both terms and the relations between the time averaged intensity and the amplitude of the pressure in the acoustic wave [17, 61]:

$$I = \frac{P^2(z)}{2 \cdot Z} = \frac{P^2(z)}{2 \cdot \rho \cdot c},$$

(2.10)

where $Z$ is the acoustic impedance of the medium, $\rho$ is the density of the medium, and $c$ is the sound speed in the medium. The last gives us the necessary relation:

$$\alpha \left[ \frac{\partial B}{m} \right] = 8.686 \cdot b \left[ \frac{Np}{m} \right].$$

(2.11)

The amplitude attenuation coefficient, $\mu$ includes both terms: coefficient of scattering, $\sigma$ and absorption, $b$:

$$\mu = \sigma + b.$$  

(2.12)

But in most practical applications, the absorption term dominates over the scattering term:

$$\frac{\sigma}{\mu} = \frac{\sigma}{\sigma + b} \approx 0.1 \div 0.3,$$  

(2.13)

so that the amplitude absorption coefficient $b$ and amplitude attenuation coefficient $\mu$ have practically the equal magnitudes:

$$\mu \approx b.$$  

(2.14)

A few words must be said about the assumption mentioned above for the ratio $\sigma/\mu$. First of all, it concerns a number of uniform media within the body which appears not to scatter the sound at all, at least at frequencies in the normal range of medical use. Then, Equality (2.13) is well held and the absorption in equal to the attenuation. Examples are amniotic fluid, aqueous humour, vitreous humour,
the lens of the eye (internal) and cyst liquids [83]. In more complicated cases, the contributions of absorption and scattering into the total attenuation must be found separately. There are two possible approaches for the evaluation of the ratio: direct and indirect.

Direct measurements of the absorption coefficient and scattering coefficient have been attempted for only a few tissues. The results from direct scattering measurements probably represent the most accurate estimates of the ratio that are currently available. It was found in [101], that the ratio $\sigma/\mu$ in the frequency range 3-7 MHz is about 2 % for a calf liver. The measurements of this ratio for human liver, muscle and blood in the frequency range 4 - 7 MHz were conducted in [100]. They gave approximately 19 %, 17 % and 0.3 % for the values of $\sigma/\mu$ in these tissues.

These data are very important as an additional verification of the values obtained from direct measurements of attenuation and absorption. The direct measurements of both ultrasonic amplitude attenuation and absorption coefficients in a bovine liver at low megahertz frequencies showed that they are not significantly different i.e. $b \approx \mu$ [103]. The results obtained in bovine liver samples at 1.1 and 3.3 MHz gave statistically indistinguishable values for attenuation and absorption. Although at 5.6 MHz a significant difference (equivalent to a value of 18 %) was observed.

The numerous indirect approaches involve the reduction of attenuation as well as scattering by homogenizing the tissue. It may be inferred from the results of these studies that the scattering in the whole beef liver is in the frequency range 1-10 MHz drops by 30% after the tissue was homogenized [104]. It was assumed in these studies, that the homogenizing process, whilst removing all scattering structures (down to the subcellular level), do not affect the absorption coefficient.

The other indirect estimates for the value $\sigma/\mu$ include modeling the frequency dependence of attenuation in liver matching of changes which take place in the levels of absorption, backscattering and attenuation when tissues decay or are fixed.
histochemically [98, 105]. The results show that the ratio $\sigma/\mu$ is about 1% in normal liver and 8-13% in fatty liver, over a 1-5 MHz frequency range.

Generally speaking, the value of 30% seems reasonable for the estimate of the upper limit values for the $\sigma/\mu$ ratio in a soft biological tissue at the frequency range between 0.1 and 5 MHz.

The physical properties of a whole blood are determined by the physical properties of its components. The absorption of ultrasound is determined mainly by two proteins contained in blood: hemoglobin (in red cells) and albumin in plasma (60% volume concentration in plasma) [45]. At least, this is true up to 1.2 MHz. Both proteins have similar molecular weight (70 000) and length to diameter ratio 5:1 and 9:1, respectively.

After coagulation, when the fibrinogen turns into fibrin and creates the netlike fibrous structure with the gas-filled voids, clotted blood looks like jellylike consistency. The main components of a formed blood clot are red cells, platelets and fibrin fibers. The size of red cells is about $7 - 8 \mu m$ and the size of platelets is about $2 - 4 \mu m$. Red cells and platelets are incorporated into the mesh of fibrin fibers. Fibrinogen, a precursor of fibrin, is a large, elongated (length/diameter ratio $\approx 12$) molecule with a neutral electric charge and a molecular weight of 330 000 units. The normal physiological levels of fibrinogen is $0.2 \div 0.4 g/100 ml$. The yield stress of whole blood appears to be caused by the presence of fibrinogen, and apparently, the sound velocity is determined by this factor. The diameters of fibrin fibers vary between 50 and $150 nm$ [70]. The presence of air inside the clots may significantly influence the magnitude of density and specific heat experimentally obtained. The evidence of the presence of the trapped gas in a clot was obtained after degassing the samples of clotted blood in the vacuum pump to the pressure $P = 2.3 kPa$ (a water vapor pressure at $20^\circ C$ is 17.5 mmHg). The density and specific heat of samples were measured before and after degassing. One of the possible explanations of the change of the clot density during insonification is the effect of squeezing the
clot. This means that sonication reduces the volume of the clot.

Bone is a complex hard specialized connective tissue with a calcified collagenous intercellular substance. Bones can be divided on two groups: long bones and membranous bone [17]. Long bones consist of a rigid dense external cortex, a middle layer of less dense cancellous (or spongy) bone, and an innermost marrow chamber containing adipose, blood, and blood forming cells. Membranous bones, such as the flat skull bones, consist of two layers of dense compact bone separated by a spongy inner layer called diploë.

Bone tissue possesses acoustic propagation properties greatly different from those of soft tissues. The magnitude of absorption coefficient for bones is one order of magnitude greater than that for the soft biological tissues. The acoustic impedance of bones is much greater than that of any other mammalian tissue [17]. Hence when reflected, the ultrasound wave can produce much higher thermal elevation in the region of interface between a bone and a soft tissue. In addition, a bone is sufficiently rigid to support the shear wave. When longitudinal waves from soft tissue strikes a bone interface at oblique incidence, shear waves may be created in the bone. These shear waves have absorption coefficients which are greater than those for longitudinal waves. Therefore, the shear waves are immediately converted
into heat near the surface of the bone [37].

### 2.2 Physical characterization of biological tissues

For the purpose of this study, we are interested in the particular types of the physical properties of biological tissues. We may divide them onto two big groups: acousto-mechanical and thermal properties.

![Diagram](image.png)

**Figure 2.3: Physical characterization of biological tissues.**
Sound speed, absorption, attenuation and density belong to the acousto-mechanical properties. Specific heat, diffusivity and thermal conductivity form the group of thermal properties. Schematically, it is shown in Figure (2.4). Although, the speed of sound in the clotted blood is not the subject of our interest, it will be determined for both types of clotted blood: porcine and human.

2.3 Analytical model for the ultrasound induced hyperthermia in a blood clot and a cranial bone

Absorption of ultrasound causes the thermal elevation in soft tissue and bones. By knowing the intensity of ultrasound at particular point and the physical properties of surrounding material, it is possible theoretically to calculate the expected thermal elevation in the material of interest. For this purpose we need, namely, the coefficient of acoustic absorption, density, specific heat and thermal conductivity of clotted blood and cranial bone.

In order to determine the spatial and the temporal dependence of temperature $T(\vec{R}, t)$ in the homogeneous medium insonified with the ultrasound, we have to solve the bio-heat transfer equation for this medium. The bio-heat transfer equation in the tissue characterized by thermal conductivity $K$, density $\rho$, and specific heat $C_v$ with no perfusion is given by the following equation [17, 86]:

$$\nabla^2 T - \frac{1}{\kappa} \cdot \frac{\partial T}{\partial t} = -\frac{2 \cdot b \cdot I}{K} = -4\pi \cdot \Psi(\vec{r}, t)$$

(2.15)

where $\kappa = \frac{K}{\rho C_v}$ is the thermal diffusivity of tissue, $b$ is the amplitude absorption coefficient in blood clot, $I$ is the intensity in the incident ultrasonic wave, $\Psi(\vec{r}, t)$ is the heat source density. We left only a radial term in the expression for the Laplacian operator, since, in our model shown in Figure(2.4), we consider the clot as an absorbing sphere of radius $a$ where the absorption of acoustic energy occurs only within the limits of blood clot:
\[ \Psi(\vec{r}, t) = \frac{bI}{2\pi K} \text{ for } r \leq a, \text{ and } \]
\[ \Psi(\vec{r}, t) = 0 \text{ for } r > a. \]  
(2.16)

The amplitude absorption coefficient \( b \) is defined by the following expression for the

![Figure 2.4: The geometry used to model the ultrasound heating of a spherical blood clot of radius \( a \) surrounded by a fluid and insonified with the ultrasound with the incident intensity of \( I \). Both, clot and fluid, are characterized by the same values of density \( \rho \), specific heat \( C_v \), and thermal conductivity \( K \). The absorption in a clot is \( b \).](image)

spatial dependence of the time averaged intensity of an acoustic wave in a medium with no scattering [17]:

\[ I(z) = I(0) \cdot e^{-2bz} \text{ for } z \geq 0 \]  
(2.17)

where \( I(0) \) is the initial intensity in ultrasound wave.

Due to heat conduction from the source of heat, there is the heat flow from it into the surrounding liquid. Either it concerns the insonified clot or temporal bone. In our model, we disregard the heat convection in a liquid or perfusion which may contribute to the loss of heat generated inside the insonified tissue.

Solution of Equation (2.15) with the homogeneous boundary conditions \( T(0, 0) = \)
0, \( T(\infty, 0) = 0 \) is given by the integral equation [121]:

\[
T(\vec{R}, t) = \int_0^t \int_{V_0} \Psi(\vec{r}, t) \cdot G(\vec{R}, t|\vec{r}, t) dV_0 d\tau,
\]

(2.18)

where volume \( V_0 \) implies the space within the boundaries of a clot. The Green’s function for Equation (2.15) is:

\[
G(\vec{R}, t|\vec{r}, t) = \frac{1}{2\pi^{1/2} x^{1/2} \tau^{3/2}} \cdot e^{-\frac{4(R - r)^2}{\pi \tau}} \cdot u(t)
\]

(2.19)

where \( u(t) \) is a step function. Then, we have for the temperature dependence:

\[
T(\vec{R}, t) = \int_0^t \int_{V_0} \frac{b \cdot I}{2\pi K} \cdot \frac{1}{2\pi^{1/2} x^{1/2} \tau^{3/2}} \cdot e^{-\frac{4(R - r)^2}{\pi \tau}} \cdot u(\tau) dV_0 d\tau =
\]

\[
= \frac{b \cdot I}{4\pi^{3/2} x^{1/2} K} \int_0^t \int_{V_0} \frac{1}{\tau^{3/2}} \cdot e^{-\frac{4(R - r)^2}{\pi \tau}} dV_0 d\tau, \text{ for } t \geq 0.
\]

(2.20)

In the case of the spherical symmetry of our problem, we may rewrite the expression for the spatial and temporal dependence of the temperature inside the clot:

\[
T(\vec{R}, t) = \frac{bI x^{3/2}}{\pi^{1/2} K} \cdot \frac{1}{R} \cdot \int_0^t \tau^{3/2} \cdot \exp \left\{ -\frac{R^2 + r^2 - 2Rr \cos \theta}{4\tau x} \right\} \cdot r^2 \cdot \sin \theta d\varphi d\theta dr d\tau.
\]

(2.21)

Since the error function is odd, i.e. \( \text{erf}(-\eta) = -\text{erf}(\eta) \), we may rewrite the final expression for the temperature rise in the following way:

\[
T(\vec{R}, t) = \frac{2bI x^{3/2}}{\pi^{1/2} K} \cdot \frac{1}{R} \cdot \int_0^t \tau^{1/2} \left[ \exp \left\{ -\frac{(a + R)^2}{4\tau x} \right\} - \exp \left\{ -\frac{(a - R)^2}{4\tau x} \right\} \right] d\tau + \frac{bI x}{K} \cdot \int_0^t \left[ \text{erf} \left( \frac{a - R}{2\sqrt{\tau x}} \right) + \text{erf} \left( \frac{a + R}{2\sqrt{\tau x}} \right) \right] d\tau
\]

(2.22)

At the center of a clot \((R \equiv 0)\), we have:

\[
T(0, t) = \frac{2bI x}{K} \cdot \int_0^t \text{erf} \left( \frac{a}{2\sqrt{\tau x}} \right) d\tau + \frac{2bI a}{K} \cdot \sqrt{x} \cdot \int_0^t \frac{\exp \left( \frac{a^2}{4\tau} \right)}{\sqrt{\tau}} d\tau.
\]

(2.23)
We may simplify the final expression for the temperature at the center of a clot:

\[
T(0, t) = \frac{bl^2}{K} \cdot \left[ 1 - \left( 1 - \frac{2\x \cdot t}{a^2} \right) \cdot \text{erf} \left( \frac{a}{2\sqrt{\x \cdot t}} \right) - \frac{2\sqrt{\x}}{a \cdot \sqrt{\pi}} \cdot \exp \left\{ - \frac{a^2}{4t\x} \right\} \cdot \sqrt{t} \right] = \\
= \frac{bl^2}{K} \cdot \left[ 1 - \frac{1}{\sqrt{\pi}} \cdot \sqrt{\frac{t}{\tau_h}} \cdot \exp \left\{ - \frac{\tau_h}{t} \right\} - \left( 1 - \frac{t}{2\tau_h} \right) \cdot \text{erf} \left( \sqrt{\frac{\tau_h}{t}} \right) \right],
\]

(2.24)

where \( \tau_h = \frac{a^2}{4\x} \) is the time constant for the heating.

In the limiting case of zero thermal conductivity, when \( \x = 0 \) and \( K = 0 \), Equation (2.24) transforms into the following expression for the temperature inside the clot:

\[
T(\vec{R}, t) = \frac{2 \cdot b \cdot I}{\rho \cdot C_v} \cdot t,
\]

(2.25)

which corresponds to a simple adiabatic heating of a clot by ultrasound. Similarly, for small value of time, Equation (2.24) also reduces to Equation (2.25) representing the adiabatic case.

Equation (2.24) gives the values for the steady state temperature increase at the center of the blood clot after a long period of insonification (\( t = \infty \)):

\[
T(0, \infty) = \frac{b \cdot I \cdot a^2}{K}.
\]

(2.26)

The temperature elevation at the center of bone disk produced by ultrasonic insonification with the intensity \( I_{SPTA} \) can be calculated with the help of following formula [122]:

\[
T_{\text{bone}}(0, 0, t) = \frac{1}{8\pi \cdot K_{ck}} \int_0^\infty q_V(r, z, t) \cdot F(r, t) \, dV,
\]

(2.27)

where \( q_V(r, z, t) \) is the volume rate of heat generation from the absorption in skull bone characterized by the coefficient of thermal conductivity, \( K_{sk} \), \( V \) is the cylindrical volume of the skull disk exposed to ultrasound, and \( F(r, t) \) is a function proportional to the temperature rise from a small volumetric heat source. Since perfusion is neglected in our model, this function reduces to 2 for large values of time [57]. We also assume that the heating of the skull bone occurs within the limits of \(-3 dB\)
beamwidth $W$. The geometrical model used in this case is depicted in Figure (2.5).

![Figure 2.5: The geometry used to model the ultrasound heating of a bone disk of radius $a$ and a height $h$ surrounded by the fluid characterized by density $\rho_W$, specific heat $C_W$, and thermal conductivity $K_W$ and insonified by the ultrasound with the incident intensity of $I$. Bone tissue has the density $\rho_{sk}$, the specific heat $C_{sk}$, and the thermal conductivity $K_{sk}$. The absorption in the material of cranial bone is $b$.](image)

Then, the steady state temperature elevation is given by the integral:

$$T_{ST}(0, 0, \infty) = \frac{1}{4\pi \cdot K_{sk}} \int_{a}^{W \over 2} \int_{0}^{h} q_v(r, z, \infty) \frac{dSdz}{r},$$

(2.28)

where integration is carried out over the ultrasound beam radius $a$ and the height of the bone disk $h$. Finally, after some manipulations, we have the expression for the temperature $T_{ST}$ at the center of bone disk:

$$T_{ST}(0, 0, \infty) = \frac{\gamma I_{SPTA} \cdot W}{8 K_{sk}} \cdot \left[ 1 - e^{\exp\{-2b_{sk}h\}} \right],$$

(2.29)

where the coefficient $\gamma$ accounts for the portion of acoustic energy absorbed by the skull wall after the reflection of the ultrasonic wave, and $b_{sk}$ is the coefficient of absorption in the skull wall.

In order to compare our theoretical prediction of ultrasound hyperthermia with those measured experimentally in vitro, we estimated the contribution of the re-
flection of acoustic energy from the interface between either blood clot, or cranial bone, and water. The coefficients of reflection for both types of clots were estimated from the acousto-mechanical properties of human and porcine clots [123]. The density of human and porcine clots is $1.076 \cdot 10^3 \text{ kg/m}^3$ and $1.058 \cdot 10^3 \text{ kg/m}^3$, respectively, and the speed of sound is $1.59 \cdot 10^3 \text{ m/s}$ for both types of clots. The intensity reflection coefficient from the interface between human or porcine clot and water is $5.2 \cdot 10^{-3}$ and $4.0 \cdot 10^{-3}$, respectively [124]. Therefore, the reduction of amplitude due to reflection from the clot surface may be ignored. Similarly, the transmission coefficients for sound passing through a layer of bone were estimated as a function of frequency [124]. The values for the density of human skull, $1.7 \cdot 10^3 \text{ kg/m}^3$, and for the speed of sound, $3.36 \cdot 10^3 \text{ m/s}$, were taken from the literature [13, 18]. The intensity transmission coefficients, $\gamma$, for the three ultrasound center frequencies (0.12, 1.0, 3.5 MHz), are 0.34, 0.40, 0.82, respectively. Thus, we included this reduction of acoustic energy in Equation (2.29). For our calculations of the temperature increase in clotted blood, we assume that the density, specific heat, and thermal conductivity of human clots were $\rho = 1.08 \cdot 10^3 \text{ kg/m}^3$, $C_v = 3.48 \cdot 10^3 \frac{\text{ J}}{\text{ kg} \cdot \text{ K}}$, $K = 0.59 \text{ W/m} \cdot \text{ K}$, respectively, and in porcine clots were $\rho = 1.06 \cdot 10^3 \text{ kg/m}^3$, $C_v = 3.23 \cdot 10^3 \frac{\text{ J}}{\text{ kg} \cdot \text{ K}}$, $K = 0.55 \text{ W/m} \cdot \text{ K}$, respectively [123]. Note that these physical properties were measured at 20 °C. The ultrasound amplitude absorption coefficients for each frequency were previously measured by Nahirnyak et al. and are utilized for the calculations presented here [123]. For our calculations, we ignored the contribution from scattering in the amplitude attenuation coefficient. Direct measurements of both ultrasonic amplitude attenuation and absorption coefficients in soft tissues at low megahertz frequencies have demonstrated that scattering can be neglected [100, 103]. The density, specific heat, and thermal conductivity values used for cranial bone are $\rho_{sk} = 1.7 \cdot 10^3 \text{ kg/m}^3$, $C_{SK} = 1.59 \cdot 10^3 \frac{\text{ J}}{\text{ kg} \cdot \text{ K}}$, $K_{SK} = 1.16 \text{ W/m} \cdot \text{ K}$ [13, 25, 31]. The values for frequency dependent absorption coefficients are presented in Figure (2.6) and Table (5.8) [8, 25, 83, 92]. A spatial
peak, time average intensity, $I_{SPTA}$, of 0.4 $W/cm^2$, was identical for all frequencies. However, for the theoretical calculations of the temperature increase, the spatial average, time average intensity, $I_{SATA}$, was used for each frequency. By using the numerical calculations of the field produced by unfocused and focused transducers, an estimate of the spatial average, temporal average of each transducer was performed [126, 127]. Since the $-3\,dB$ beamwidths were different for each frequency (0.12, 1.0 and 3.5 MHz), the $I_{SATA}$ values were estimated to be 0.39, 0.31, 0.14 $W/cm^2$, respectively, for the human clots with an average diameter of $6.7 \pm 0.7\,mm$ and 0.38, 0.29, 0.12 $W/cm^2$ for the porcine clots with an average diameter of $7.8 \pm 0.5\,mm$. Numerical calculations and theoretical estimates were made using MATLAB (MathWorks, Inc., Natick, MA) and Mathematica (Wolfram Research, Inc., Champaign, IL).

There is one other assumption we have to make in the further discussion. We may neglect the variation of values $\rho$, $C_v$, $b$ with temperature in the temperature range between 20 °C and 37 °C. The uncertainty in the final calculations brought by the variation of these parameters does not exceed 10-15% [125].
2.4 Need for measurements of acousto-mechanical and thermal properties of clotted blood

Before proceeding with further discussion, we would like to analyze the published results on the acousto-mechanical and thermal properties for the biological tissues similar to those considered in this work. The found results compiled from literature are presented in Tables (2.1) and (2.2).

According to the report from the Laboratory of Applied Physics of the University of Washington, the calculated absorption coefficient for a whole blood clot at 1 MHz was $0.01 \text{ Np/cm}$ [32]. The absorption coefficient varies with frequency slightly less than the first power of frequency. According to [17], the absorption coefficient in a cortical bone is of order of $1.5 \text{ Np/cm}$ at 1 MHz.

There is a very comprehensive study of acoustic properties of a human skull in [44]. The study found some differences in density, sound speed, and attenuation between the inner, outer tables of skull and diploë. There was no significant dispersion of the phase velocity of acoustic waves of $2.42 \times 10^3 \text{ m/s}$ in the frequency range extending from $500 \text{ kHz}$ up to $1.4 \text{ MHz}$. The diploë layer doesn’t contribute much into the total absorption of sound wave. The attenuation in deploë ranges from 2.1 to 4.1 $\text{ Np/cm}$, primarily, due to scattering of acoustic waves. The amplitude absorption coefficients for cortical skull bones at 1 MHz were $1.67 \text{ Np/cm}$ and $2.15 \text{ Np/cm}$ for inner and outer tables respectively. The total losses of the transcranial ultrasound transmission in the sample of a skull of 1 cm thick were $24 \text{ dB}$ at 1 MHz and $12.5 \text{ dB}$ at $500 \text{ kHz}$. In the 6 mm thick sample the losses were 14 and 8.5 dB, correspondingly.

Huter has measured the acoustic absorption coefficient of specimens of fresh human skull in the frequency range of 0.6 to 3.5 MHz at temperatures between $25^\circ \text{C}$ and $30^\circ \text{C}$ [25]. These measurements show that the absorption coefficient of bone exhibits a quadratic dependence upon frequency (the classical viscous type) to a-
proximately 2 MHz, followed by a transition to a lower power dependence at higher frequencies. The acoustic amplitude absorption coefficient per unit path length is of order of $1 \text{Np/cm}$ at 1 MHz, approximately, an order of magnitude greater than that of most soft tissues at the same temperature and frequency. Carstensen et al. reported 85 % losses during the transmission of the ultrasound wave through the mouse skull of thickness of 0.5 mm. This includes 75 % loss due to conversion of the acoustic energy into heat and 9 % loss due to reflections and scattering. On the basis on the experiments conducted, the attenuation coefficient of mouse skull is of the order of $5 \text{Np/cm} \cdot \text{MHz}$ for the experimental frequency range of 2.5-3.6 MHz [57].

Low frequency ultrasound penetration through the skull has also been investigated. Akiyama et al. observed transmission of 40 % of ultrasound at 211.5 kHz [71]. Behrens et al. reported only 0.1 $\text{dB}$ transtemporal ultrasound attenuation at

Figure 2.6: Transcranial attenuation of ultrasound in a human skull vs. frequency. The experimental data were compiled from [8, 25, 73, 74, 75].
33.3 kHz in a human skull [72]. The use of low frequency ultrasound during the transcranial treatment of the ischemic stroke has undoubted advantage because it allows to increase the intensity of ultrasound for more effective thrombolysis, while at the same time helps to avoid the intensity level at which tissue can overheat [70].

As it was mentioned previously, there is a lack of data on thermo-physical and acoustic properties of clotted blood in literature. For numerical estimates of the thermal elevation in blood clots during ultrasound insonation, we need to know the density, specific heat, and coefficient of ultrasonic absorption for human and porcine clots. We also need to know the magnitude of the ultrasound intensity at the location of a blood clot.
Table 2.1: Thermo-physical properties of human skull, human and porcine blood and their components compiled from the literature [17, 24, 28, 29, 30, 31, 32, 39, 44, 46, 52, 53, 58].

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Density, $g/cm^3$</th>
<th>Specific heat, $J/g \cdot K$</th>
<th>Thermal conductivity, $W/m \cdot K$</th>
<th>Thermal diffusivity, $m^2/s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bone (cranium, skull)</td>
<td>$1.81^{[13]}, 1.7^{[18]}, 1.61^{[13]}, 1.85^{[61]}$</td>
<td>$1.256^{[28-30]}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Bone (diploë, cancellous)</td>
<td>$1.3^{[17]}, 1.74^{[44]}$</td>
<td>$1.62^{[17]}, 1.59^{[31]}$</td>
<td>$0.58^{[17]}$</td>
<td>$0.28 \times 10^{-6}^{[17]}$</td>
</tr>
<tr>
<td>3. Bone (ivory, cortical)</td>
<td>$1.7^{[17]}, 1.87^{[17]}, 1.91^{[44]}$</td>
<td>$1.3^{[13]}, 1.59^{[17]}$</td>
<td>$2.3^{[17]}$</td>
<td>$0.85 \times 10^{-6}^{[17]}$</td>
</tr>
<tr>
<td>4. Whole human blood</td>
<td>$1.06^{[13]}, 1.05^{[17, 31]}, 1.60^{[28-30]}$</td>
<td>$3.894^{[28-30]}, 3.61^{†}[13], 3.62^{[17]}, 3.84^{[13]}, 3.64^{[31]}$</td>
<td>$0.488^{[24]}, 0.55^{[17]}, 0.514^{†}[13], 0.484 - 0.491^{[13]}, 0.509-0.530^{[52]}, 0.506^{[39]}, 0.549^{[31]}$</td>
<td>$0.14 \times 10^{-6}^{[17]}, 1.19 \times 10^{-7}^{[13]}, 1.43 \times 10^{-7}^{[31]}$</td>
</tr>
<tr>
<td>5. Human blood plasma</td>
<td>$1.03^{[58]}, 1.027^{[13]}$</td>
<td>$3.93^{[13]}, 3.93^{[58]}$</td>
<td>$0.582^{[39]}, 0.572^{[13]}, 0.571^{[52]}, 0.599^{[58]}$</td>
<td>$1.21 \times 10^{-7}^{[13]}, 1.48 \times 10^{-7}^{[58]}$</td>
</tr>
<tr>
<td>6. Isolated fibrin fiber</td>
<td>$1.3^{[32]}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. Serum</td>
<td>$1.026^{[13]}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. Human red blood cells</td>
<td>$1.093^{[13]}, 1.084-1.091^{[46]}$</td>
<td>$3.21^{[53]}$</td>
<td>$0.482^{[13]}, 0.45^{[53]}$</td>
<td>$1.30 \times 10^{-7}^{[53]}$</td>
</tr>
<tr>
<td>9. Porcine blood plasma</td>
<td>$1.023^{[46]}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10. Porcine red blood cells</td>
<td>$1.087^{[46]}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$†$ - Calculated values
### Table 2.2: Acoustical properties of human bones, human and porcine blood and some of their components compiled from the literature \([9, 13, 14, 17, 18, 25, 42, 44, 25, 45, 47, 32, 36, 55, 59, 51, 54, 61]\).

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Speed of sound, (m/s)</th>
<th>Attenuation (\mu, \quad Np/cm)</th>
<th>Absorption (b, \quad Np/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bone (cranial)</td>
<td>(3.36 \cdot 10^3[18]), (2.65 \cdot 10^3[42])</td>
<td>(2.5[9]), (0.9) at (0.8) MHz ([18]), (2.53) at (1) MHz ([36]), (2.48[42])</td>
<td>(1.0) at (1) MHz ([25])</td>
</tr>
<tr>
<td>2. Bone (diploë, cancellous)</td>
<td>(3.022 - 3.289 \cdot 10^5) ([13])</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Bone (cortical)</td>
<td>(2.9 \cdot 10^3[44]), (2.65 - 3.05 \cdot 10^3) ([13]) (\text{(outer)}), (2.903 - 3.258 \cdot 10^3) ([13]) (\text{(inner)})</td>
<td>-</td>
<td>(1.5) at (1) MHz ([17]), (1.67-2.15) at (1) MHz ([44])</td>
</tr>
<tr>
<td>4. Whole human blood</td>
<td>(1.59 \cdot 10^3) ([13]), (1.54 \cdot 10^3) ([51])</td>
<td>(0.017) at (1) MHz ([36]), (0.014-0.018) at (4.8) MHz ([13]), (0.013) ([13]), (0.053) at (2.8) MHz ([47]), (0.02) at (1) MHz ([61])</td>
<td>(0.02) ([45]), (0.004) ([59]) at (1) MHz ([59])</td>
</tr>
<tr>
<td>5. Human blood plasma</td>
<td>(1.53 - 1.55 \cdot 10^3) ([51])</td>
<td>(0.0066) at (1.7-15) MHz ([13]), (0.007) at (1) MHz ([61])</td>
<td>(0.008) at (1) MHz ([45])</td>
</tr>
<tr>
<td>6. Red cells</td>
<td>-</td>
<td>-</td>
<td>(0.003) at (1) MHz ([45]), (0.04) ([54])</td>
</tr>
<tr>
<td>7. Isolated fibrin fiber</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. Human serum</td>
<td>(1.53 \cdot 10^4) ([13])</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9. Porcine blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10. Human blood clot</td>
<td>(1.59 - 1.61 \cdot 10^3[14]), (1.59 \cdot 10^3) ([55])</td>
<td>(1.22-1.38) at (4.5-4.8) MHz ([14]), (0.45) at (7.5) MHz ([55])</td>
<td>(0.07) ([14]), (0.01) ([32]) at (1) MHz</td>
</tr>
</tbody>
</table>

\(\dagger\) - Calculated values
Chapter 3
Definitions and approaches

3.1 Transducer characterization and attenuation measurements technique

An acoustic beam diverges due to the phenomenon of diffraction. It happens, primarily, at distances larger than the focal distance of a transducer. This may significantly increase the magnitude of experimental errors and make the interpretation of experimental data significantly complicated. In order to proceed with the experiments on ultrasonic attenuation, firstly, we have to evaluate the significance of this phenomenon for the every experimental frequency.

There are three major factors contributing to the total divergence of the acoustic beam: the natural divergence of a beam produced by the piston transducer, diffraction on the opening of the acoustic window in the sample holder and diffraction of the acoustic wave on the clot sample. As a result of such a distortion of acoustic field, there is a need in the diffraction correction to the results on the attenuation measurements.

We may neglect the effect of diffraction of the acoustic beam on the acoustic windows of the sample holder if the distance $\Delta L$ between a hydrophone tip and the plane of the opening diameter, $d$, is much less then the ratio $d^2/\lambda$ [84]:

$$\Delta L \ll \frac{d^2}{\lambda}, \quad (3.1)$$
Figure 3.1: The aperture of acoustic beam $\omega$ in the near zone $N$ of a circular piston transducer is given by the ratio $\frac{D^2}{\lambda}$. The acoustic beam diverges in the far field due to diffraction of a beam.

where $\lambda$ is the acoustic wavelength, $d$ is the diameter on which the diffraction occurs: it is either the diameter of the window in a sample holder or the diameter of the clot in the case of a small wavelength. The diameter of the window in the sample holder was 70 mm and the diameter of a clot was about 7 – 9 mm. All calculations were made under the assumption that the temperature of water is 21 °C and the speed of sound in water is 1483 m/s.

To evaluate the beam width divergence at the different frequencies, namely at 120 KHz, 1 MHz and 3.5 MHz, we will use the following equation [27]:

$$\alpha = 2 \cdot \arcsin \left( 0.15 \cdot \frac{\lambda}{d} \right).$$  \hspace{1cm} (3.2)

As it is seen from the results presented in Table (3.1), the divergence of the acoustic beam is negligible for all frequencies, i.e. $W \approx A$. The change in the value of ultrasound intensity in this case less than 12 % and is greatest at 120 kHz.

The detailed calculations of the systematic errors caused by the effect of the diffraction on the clot sample are beyond the limits of our study. Our experiments
Table 3.1: Calculations of the spatial structure of the ultrasonic beam created by the unfocused cylindrical piston transducer in water. $\Delta L$ is equal to 0.9 cm.

<table>
<thead>
<tr>
<th>Frequency $f$, MHz</th>
<th>$D$, mm</th>
<th>$c$, m/s</th>
<th>$\lambda$, mm</th>
<th>$D^2/4\lambda$, mm</th>
<th>$\alpha$, degrees</th>
<th>$N$, mm</th>
<th>Beam width $W$, mm</th>
<th>$A$, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>61.4</td>
<td>1482</td>
<td>12.4</td>
<td>76</td>
<td>11.8</td>
<td>73</td>
<td>14.7</td>
<td>16.6</td>
</tr>
<tr>
<td>1.0</td>
<td>25.4</td>
<td>1482</td>
<td>1.48</td>
<td>109</td>
<td>3.4</td>
<td>108</td>
<td>6.3</td>
<td>6.9</td>
</tr>
<tr>
<td>3.5</td>
<td>19.0</td>
<td>1482</td>
<td>0.42</td>
<td>213</td>
<td>1.3</td>
<td>213</td>
<td>4.7</td>
<td>5.0</td>
</tr>
</tbody>
</table>

showed that the errors brought by this factor do not exceed 5%.

Figure 3.2: Directionality of the normalized intensity in the focal planes of the piston transducers of the different diameters, $D$. The transverse beamwidth corresponds to the $-3\, dB$ drop in the amplitude of the axial beam intensity.

The calculations of the lateral profile of the ultrasonic beam in the far field of the unfocused circular piston transducers were made on the basis of the following formula [84, 60]:

$$...$$
\[
\frac{I_\theta}{I_0} = \left[ \frac{2 \cdot J_1(ka \cdot \sin\theta)}{ka \cdot \sin\theta} \right]^2,
\]

where \( J_1(ka \cdot \sin\theta) \) is the first order Bessel function of the first kind, \( k \) is a wavevector of acoustic wave, \( a \) is the radius of a piston transducer, \( I_\theta \) is the azimuthal intensity of acoustic wave in a focal plane, \( I_0 \) is the axial intensity of acoustic wave in a focal plane, \( \theta \) is the azimuthal angle. These calculations presented in Table (3.1) help to evaluate the uniformity of acoustic power across the sample of clot.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.3.png}
\caption{Determination of a focal zone and a near field of the acoustic beam produced by the unfocused circular piston transducer with a diameter of \( D \). The focal zone of the beam corresponds to a \(-3\, dB\) drop in the axial beam intensity around the last axial maximum. The position of the last maximum gives the near field distance, \( N \). (See Appendix B1)}
\end{figure}

The calculations of the axial parameters of the ultrasonic beam were made on the basis of the following formula [19]:

\[
\frac{I_z}{I_0} = \sin^2\left\{\left[(a^2 + z^2)^{1/2} - z\right] \cdot \frac{k}{2}\right\},
\]  

(3.4)

where \(k\) is a wave vector of acoustic wave, \(a\) is the radius of a piston transducer, \(I_\theta\) is the azimuthal intensity of acoustic wave in a focal plane, \(I_0\) is the axial intensity of acoustic wave in a focal plane, \(\theta\) is the azimuthal angle, and \(z\) is the distance from the face of transducer.

Table 3.2: Theoretical estimates for a near field distance, a focal zone and a focal depth of acoustic beam for the ultrasound transducers used in experiments. We used the technical note from Panametrics to calculate the parameters of acoustic beams [27].

<table>
<thead>
<tr>
<th>Transducer</th>
<th>Effective diameter (D), mm</th>
<th>Frequency (f), MHz</th>
<th>Focal length (N = \frac{D^2}{4\lambda}), mm</th>
<th>(F)-number, N/D</th>
<th>Focal zone (F_z), mm</th>
<th>Beam width (W), mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>61.4</td>
<td>0.12</td>
<td>76</td>
<td>1.2</td>
<td>101</td>
<td>15.7</td>
</tr>
<tr>
<td>-</td>
<td>25.4</td>
<td>1.0</td>
<td>109</td>
<td>4.3</td>
<td>145</td>
<td>6.5</td>
</tr>
<tr>
<td>MD3483</td>
<td>19</td>
<td>3.5</td>
<td>213</td>
<td>11.2</td>
<td>284</td>
<td>4.9</td>
</tr>
</tbody>
</table>

These calculations presented in Table (3.2) help to evaluate the uniformity of acoustic power across the volume of a clot sample and choose the proper transducer for experiments. In all our cases, the focal depth of the transducers are much greater than the dimensions of the clot samples. This guarantees that the variation of the pressure of acoustic field over the distance equal to the size of a clot along the axis of transducer is negligible.
3.1.1 Methods of measuring attenuation (absorption) in soft tissue

There are four main groups of methods to measure the ultrasound attenuation in the media which are relevant for the application in soft biological tissues [18]:

- transient method with diffraction correction;
- thermoelectric method;
- backscattering method;
- interferometric and reverberation methods.

All methods have their advantages and disadvantages. Some of them are suitable for investigation only in liquids, some are useful for the investigation of attenuation in all types of biological tissues. We shall briefly analyze them in the further discussion.

All types of the absorption measurement techniques can be divided on two major groups: narrowband and broadband techniques. In the narrowband technique, the measurements are being made at a single frequency. In the broadband technique, the ultrasound pulses frequency spectrum is utilized to determine the absorption in the material. Transient and backscattering experimental systems use the pulse transmission method to measure ultrasound attenuation. This approach is suitable for in all types of biological tissues including hard, soft and liquid materials in most practical cases. It is based on the transmission of the burst or pulse of ultrasound propagating in the investigated substance. There are two configurations in this method: two-transducer configuration and one transducer-reflector configuration. If the transmitted pulse is being registered with the same transducer after reflection from the plane interface and propagation back through the specimen, it is called the echo-pulse method [83].

The transient methods can be divided on two types: with a fixed path and with
a variable path. In the first case the distance between transmitter and receiver is constant. Short bursts of sound are transmitted through the sample and received, either with a separate transducer aligned coaxially with the transmitter or with the transmitting transducer after the pulse has been reflected by a plane interface and propagated back through the specimen. In the second case the distance between transducer and receiver continuously changes during the experiment. Variable path method can give the absolute value of the coefficient of attenuation but requires additional corrections to the results due to diffraction losses. This method is not suitable for the experiments in non-fluid media [83]. All fixed path method can be divided on two types: substitution technique and insertion technique. In substitution technique, the transducer and receiver are mechanically linked and placed in two different media. They can move only simultaneously: one through the test liquid and the other through the reference liquid. This method is widely used to investigate the acoustic properties of blood. The insertion technique is most widely used method for the investigation of attenuation in both soft and solid biological tissues. The final magnitude of the attenuation is given by the logarithm of the ratio of the received signals when the tissue is present between the transmitter and receiver and when a reference medium only is present. The insertion technique may be used with the broadband pulse. Both two-transducer and one transducer-reflector configurations are in common use. The sound pulses are converted upon reception to a spectrum of transmitted acoustic frequencies, and the variation of the attenuation with frequency is determined from the logarithm of the ratio of the spectra obtained when the tissue is present in the beam and when it is absent.

The narrowband class of techniques also includes various instruments that employ continuous ultrasound waves and make use of the resulting resonances. These include interferometric and reverberation methods.

Ultrasonic interferometers may be configured with variable path or variable frequency and the resonant path may be between two transducers or one transducer
and a reflector. A wavelength, and hence a sound speed, are obtained from the distance, or the frequency difference, between resonance peaks. The variation in the strength of the resonance with distance, or the bandwidth of the resonance, can be used to determine the attenuation coefficient. These methods are not absolute and require calibration with a liquid of known properties. It is necessary because the diffraction effects and the side wall reflections contribute significantly to the measurement. This method is used mainly to cover the frequency range 0.2-10 MHz.

Reverberation methods make use of the fact that, if a relatively large, almost lossless, resonant container holding the test liquid is excited into vibration, then the rate of decay of the amplitude of vibrations when the sound source is turned off is primarily determined by the attenuation coefficient of the liquid. The evaluation of the acoustic absorption in the medium enclosed in the resonator is being made on the basis of the measurements of $Q$-factor for the resonance curve of the resonator. Diffraction effects are absent, but wall losses require correction by the use of a reference liquid that has the same acoustic impedance as the test liquid, and absolute values of attenuation are not obtained. The operating frequencies of reverberation methods are generally below 1 MHz.

For in vivo absorption studies, the transient thermoelectric technique is most convenient. The time rate of change of temperature recorded by a thermocouple embedded in the biological tissue exposed to a long ultrasonic pulse is proportional both to the ultrasonic intensity and to the absorption coefficient of the medium [40]. This relationship may be used to determine the local value of absorption coefficient in biological material.

The other technique which may be used for an in vivo measurement of attenuation in tissue is based on the assumption that the frequency dependences of attenuation and scattering are the constant simple functions. This method neglects diffractional scattering and assumes directional isotropy of the backscattering and other propagation properties, and the use of multiple frequencies. The latter method is
essentially a reconstruction approach that assumes constant simple functions for the frequency dependencies of attenuation and scattering. For automated attenuation compensation in medical pulse-echo imaging it appears to be useful to assume a simple monotonic relationship between attenuation and scattering. The measurement of a backscattering coefficient, the assumption about directional isotropy of this coefficient, and simple relation between scattering and attenuation are the main principles of the backscattering method for the measurements of attenuation.

In our experiments, we are going to use a narrowband insertion transient method with a diffraction correction and with a fixed path which is the most suitable for the samples of clotted blood we have for the in vitro experiments.

### 3.1.2 Main mechanisms of ultrasonic attenuation in soft tissue and bones

The absorption of acoustic energy by the surrounding medium causes a temperature increase in soft tissue and an increase of internal energy of molecules in a tissue. The macroscopic description of the absorption in fluids includes the pressure in the medium and its density. If the excess pressure, \( p_e \), and excess density, \( \rho_e \), in the sound wave are in phase, there is no absorption in the medium. This case can be expressed by the following relation:

\[
p_e = Const \times \rho_e. \tag{3.5}
\]

When there is a phase shift between these two values, the medium absorbs the sonic energy. From microscopic point of view, the following three factors may be expected to produce a time lag between pressure and density in a sound wave: shear viscosity, heat conduction and heat radiation [26]. Let us consider each factor independently. The coefficient of absorption due to shear viscosity, \( b_v \), is given by the following formula [26]:
\[ b_v = \omega \cdot \left( \frac{\rho_0}{2 \cdot \zeta \cdot \omega_v} \right)^{1/2} \cdot \left( \frac{1}{(1 + \omega^2/\omega_v^2)^{1/2}} - \frac{1}{1 + \omega^2/\omega_v^2} \right)^{1/2}, \]  

(3.6)

where \( \omega \) is the circular frequency of the acoustic wave, \( \omega_v = c^2 \cdot \rho_0 / \zeta \) is the characteristic frequency for the given medium, and \( \rho_0 \) is the equilibrium density of a fluid. The relation between the constant \( \zeta \) and the coefficient of the shear viscosity of the medium \( \eta \), is:

\[ \zeta = \frac{4}{3} \cdot \eta. \]  

(3.7)

In the most practical cases \( \omega_v \gg \omega \). We can evaluate the magnitude of \( \omega_v \), for example, for a whole blood. The coefficient of shear viscosity for the whole blood, \( \eta \) is \( 4.0 \cdot 10^{-3} \text{ Pa} \cdot \text{s} \), the sound speed \( c \) is 1590 m/s, the density \( \rho \) is \( 1.06 \cdot 10^3 \text{ kg/m}^3 \). The coefficient \( \zeta \) is \( 5.3 \cdot 10^{-3} \text{ Pa} \cdot \text{s} \), and the characteristic frequency \( \omega_v \) is of order of \( 0.7 \cdot 10^{12} \text{ s}^{-1} \). In all applicable cases \( \omega_v \gg \omega \), which allows us to simplify the previous expression (Equation (3.7)). Thus, the coefficient of absorption in a whole blood due to shear viscosity becomes:

\[ b_v \approx \omega^2 \cdot \left[ \frac{\zeta}{2 \cdot \rho_0 \cdot c^3} \right]. \]  

(3.8)

The numerical value for the pressure-absorption coefficient \( b_v \) at 1 MHz in a whole blood is of order of 0.02 m\(^{-1}\). Ultrasonic absorption by any aqueous solution of polypeptide may be related to any or all of four possible mechanisms: proton transfer, helix-coil transition, salvation, and relaxation of the shear viscosity [18].

Relaxation refers to the finite time required for a medium to establish new thermodynamic equilibrium after the external changes of state. There are different processes, including reversible and irreversible, which may contribute to the phenomenon of relaxation: a chemical reaction, a phase transition, a molecular vibration, and an excitation of molecular internal energy states [76]. For example, in air it is mainly the vibration of oxygen and nitrogen molecules, in seawater it is
the dissociation of boric acid and magnesium sulfate molecules. The changes of the internal state are accompanied by the absorption of energy with a characteristic period of time called a relaxation time.

Carstensen and Schwan showed that the acoustic properties of blood are closely correlated to proteins the blood contains in both red cells and plasma, namely hemoglobin and albumin [54]. The absorption of the ultrasonic wave occurs due to the molecular phenomena in them. According to [59], the attenuation of the ultrasonic wave in erythrocyte suspension is determined not only viscosity mechanism but multiple relaxation mechanisms due to chemical and structural relaxations in solutions.

Thermal diffusion is the other factor contributing to the acoustic absorption. In general, the contribution of this effect into total absorption of ultrasonic wave is negligible for liquid and liquidlike materials [18]. It is true when the wavelength of a bulk acoustic wave becomes comparable with the mean free path of molecules in medium and direct heat transfer from the wave to the molecular momentum takes place through a relaxation mechanism.

Additional losses of acoustic energy occur when the sonicated soft tissue radiates energy in the form of electromagnetic waves. This process can be described by the Stefan-Boltzman law, which gives the relationship between the temperature of tissue and the intensity of the electro-magnetic radiation:

\[ I_r = \sigma \cdot T^4. \]

where \( \sigma = 5.67 \cdot 10^{-8} \, \text{W/m}^2 \cdot \text{K} \) is a Stefan-Boltzman constant, \( T \) is the absolute temperature of the insonicated tissue. We may estimate the significance of this effect by comparing the radiated intensity with the incident intensity. If the thermal elevation in the tissue does not exceed a few degrees, the magnitude of the intensity radiated by the physical body \( I_r \) has the value of order of \( 0.5 \cdot 10^{-7} \, \text{W/m}^2 \), which is by 11 orders less then the incident intensity. Analogously to many cases, we may
disregard the contribution of heat radiation to the total ultrasonic absorption.

The experimental evidence indicates that in most fluids the absorption is caused primarily by viscosity and heat conduction, while the effect of heat radiation is negligible. In liquids, viscosity plays the major role. In gases, on the other hand, viscosity and heat conduction come into play both about equally but are often overshadowed by molecular phenomena [26]. About 19% of the attenuation of ultrasound in blood at normal haematocrit and over a frequency range 0.7 - 4 MHz cannot be accounted for by absorption by the blood proteins, and has to be regarded as being due to the presence of intact cells [106]. Rough estimates of the absorption due to viscous (and thermal) interaction between the cells and the surrounding fluid appear to account for something in the region of 10% and the remainder, only somewhat less than 1%, can be attributed to longitudinal wave scattering [107].

Using muscle myofibrils and collagen fibrils as structures for heart muscle and skin respectively, O’Donnell and Miller have also attempted to estimate the inhomogeneity losses for these organs. Their approximate results were about 60% of published values of attenuation in both cases, and demonstrate that, for many soft tissues, the attenuation due to this mechanism is not to be neglected [107].

When the biological medium in which the ultrasonic wave propagates contains the air bubbles, the other factor contributing to the ultrasound absorption has to be taken into account [18]. Gas bubbles presented in a liquid exert a marked influence on the propagation characteristics of ultrasound in the medium. Since some biological components such as lung, blood stream as well as the formed blood clots normally contain gas “bubbles”, we must consider quantitatively the effects of gaseous inclusions on the magnitude of the coefficient of absorption of ultrasound. When the bubble dimensions are small compared with the acoustic wavelength, i.e. \( R_r \ll \lambda \), several physical processes become important in contributing to the dissipation of acoustic energy [90]. The time rate of dissipation of acoustic energy by a bubble is greatest when the resonant frequency of the acoustic field is equal to
that of the bubble. The radius of a resonant bubble $R_r$ is given by the following expression:

$$R_r = \frac{1}{\omega_r} \cdot \left\{ \left( \frac{3 \gamma_g \cdot P_0}{\rho_0} \right) \cdot \left( \frac{g_r}{\epsilon} \right) \right\}^{1/2},$$

(3.10)

where

$$g_r = 1 + \frac{2 \sigma}{P_0 \cdot R_r} \cdot \left( 1 - \frac{1}{3 h_1} \right),$$

(3.11)

$$\epsilon = 1 + \frac{3(\gamma_g - 1)}{2\Phi \cdot R_r} \cdot \left[ 1 + \frac{3(\gamma_g - 1)}{2\Phi \cdot R_r} \right],$$

(3.12)

$$\Phi = \left( \frac{\omega_r \cdot \rho_g \cdot c_p}{\kappa_g} \right)^{1/2},$$

(3.13)

$\omega_r$ is the resonant frequency for the gas bubbles, $\gamma_g$ is an adiabatic constant of the gas in a bubble ($\gamma_g = 1.4$ for $O_2$, $N_2$, air), $P_0$ is the ambient static pressure, $R_r$ is the mean radius of the bubble, $\rho_0$ is the density of the liquid, $\rho_g$ is the density of the gas, $\sigma$ is the surface tension, $h_1 = \gamma_g / \epsilon$, $c_p$ is the specific heat of the gas at constant pressure, and $\kappa_g$ is the thermal conductivity coefficient of the gas. The quantity $h_1$ lies between unity (isothermal case) and $\gamma_g$ (adiabatic case). Consequently, $g_r$ is always positive. As an example of the magnitudes of the quantities involved, a resonant frequency of 1 MHz for an air bubble in water corresponds to a bubble radius of approximately 3 $\mu$m.

When the dimension of the gas bubbles across the volume of tissue is uniform, the acoustic pressure-absorption coefficient $b_g$ is given by the following formula:

$$b_g = \frac{p \cdot n_r \cdot v}{4} \cdot \frac{3 \gamma_g P_0 / R_r^2 + \omega^2 \rho_0}{\left[ \frac{1}{4\pi R_r} \cdot \left( \rho_0 \omega^2 - \frac{3p \gamma_g P_0}{\epsilon R_r^2} \right) \right]^2 + p^2 \omega^2},$$

(3.14)

where $n_r$ is the concentration of the gas bubbles of the given radius $R_r$. The expression for the amplitude absorption coefficient $b_g$ at the resonant frequency simplifies:

$$b_g = \frac{n_r \cdot v}{4p \omega_r^2} \cdot \left( \frac{3 \gamma_g \cdot P_0}{R_r^2} + \omega_r^2 \rho_0 \right),$$

(3.15)
The parameter $p$ is equal to the sum of the thermal $p_t$, radiation $p_r$, and viscous dissipation $p_v$ parameters. The radiation dissipation parameter $p_r$ is given by the following expression:

$$p_r = \frac{\omega^2 \cdot \rho_0}{4\pi \cdot v},$$  \hspace{1cm} (3.16)

and the viscous dissipation parameter $p_v$ by:

$$p_v = \frac{\eta}{\pi \cdot R^3_r},$$  \hspace{1cm} (3.17)

where $\eta$ is the coefficient of shear viscosity of the fluid. The expression for the thermal dissipation parameter $p_t$ has two limiting cases. When $2\Phi R_r \leq 2$, it is equal to:

$$p_t \approx P_t \cdot \frac{\gamma_g - 1}{\gamma_g} \cdot \frac{(2\Phi \cdot R^2_r)^2}{30},$$  \hspace{1cm} (3.18)

where

$$P_t = \frac{3\gamma_r \cdot P_0 \cdot g_r}{4\pi \cdot R^3_r \cdot \omega_r \cdot \epsilon}.$$  \hspace{1cm} (3.19)

If $2\Phi R_r \geq 5$, then:

$$p_t \approx P_t \cdot \left[ \frac{1 - 1/\Phi R_r}{1 + 2\Phi R_r/3(\gamma_g - 1)} \right].$$  \hspace{1cm} (3.20)

In the previous discussion, we considered only the bubbles of one size. In reality, we have to sum up the contribution to the total acoustic absorption from the bubbles of all radii.

Equations (3.11) - (3.20) for attenuation due to gas bubbles were used by Dunn and Fry to model the level and frequency dependence of attenuation in lung [97]. The calculated magnitude of the amplitude absorption coefficient at 1 MHz was 5.7 Np/cm at the concentration of bubbles $n_r = 4.4 \times 10^3 \ cm^{-3}$ with the mean radius of 0.3 mm. The presence of air-tissue interfaces in the inflated lung accounts for such a high ultrasonic attenuation in this organ [83]. There is an agreement in published data that the presence of intact cells in the blood is responsible for only a small component of the attenuation, and that most of this is due to viscous relative
motion losses [108].

Attenuation coefficients of ultrasound in bone may be between 2 and 20 times those in soft tissues, and there is a disagreement regarding the frequency dependence of the attenuation. It was noted that the frequency dependence of attenuation in bone follows a similar relationship for the attenuation in a grain-like structures, when attenuation is dominated by scattering [25]. The compilation of the published data on the coefficient of attenuation in a human skull gives the power law dependence with the index of 2 between attenuation and frequency in the frequency range between 300 kHz and 3 MHz (see Figure (2.5)).

3.2 **Thermal diffusivity and specific heat apparatus: methods, approaches, technique, optimization**

In our approximation for the temperature range 20°C - 38°C, we assume that the specific heat and thermal conductivity do not depend on the temperature. The last assumption was justified by the experimental measurements of the specific heat of the same sample of clotted blood at different temperatures. The variation in the value of the specific heat did not exceed 1%.

3.2.1 **General approach to the experimental techniques**

Direct calorimetric measurements using calibrated $E$-type thermocouples (Omega Engineering, Inc., Stanford, CT) were performed to determine the specific heat of the human and porcine thrombi against the specific heat of a standard fluid, saline [0.9% water solution of sodium chloride]. The experimental setup included two Styrofoam® containers with the insulating caps and with the E-type thermocouples (Omega Engineering, Inc., Stanford, CT) inserted in them. Two digital thermometers HH 506-R (Omega Engineering, Inc., Stanford, CT) were used to measure the temperatures.
The following standard method of mixtures was followed to determine the specific heat of the sample clots in saline held at an initial temperature of $7 \pm 1^\circ C$ and combined with a second aliquot of saline at room temperature $21 \pm 1^\circ C$ [121]. Two regular commercially available Styrofoam® 380 mg containers with the protecting lids were used to decrease heat flow from and to clot samples during calorimetric measurements. The samples of porcine or human clots with a total mass, $m_{clots}$, were placed into the first Styrofoam® container and immersed in an isotonic saline solution with mass, $m_{saline}$, and cooled to the temperature $7 \pm 1^\circ C$ in a refrigerator. After that all content of the first cup was added and mixed with saline with mass, $M_{saline}$, at room temperature, $22 \pm 1^\circ C$ in the second container. By knowing the final temperature of the mixture $T_{final}$, one can calculate the specific heat $C_v$ of the samples of human or porcine clots on the basis of the following formula:

$$C_v = \frac{C_{saline} \cdot [M_{saline} \cdot (T_1 - T_{final}) - m_{saline} \cdot (T_{final} - T_2)]}{m_{clots} \cdot (T_{final} - T_2)},$$

(3.21)

where $T_1$ is the initial room temperature, $T_2$ is the initial temperature of refrigerated clots.

The value of specific heat of saline solution with 0.9% concentration of NaCl were found on the basis of formulas given in [20] and the temperature dependence of the pure water given in [21]. The results of those calculations are shown in Table (3.3). We used $C_{saline} = 4.175 \, J/g \cdot K$ for the value of specific heat of saline at $22^\circ C$.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>22</th>
<th>25</th>
<th>30</th>
<th>37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific heat, J/g · K</td>
<td>4186.6</td>
<td>4180.0</td>
<td>4175.7</td>
<td>4174.5</td>
<td>4173.3</td>
<td>4172.1</td>
<td>4171.7</td>
</tr>
</tbody>
</table>

The general approach in designing the experimental setup for the thermal con-
ductivity measurements includes the analysis of the type of tissue we are going to investigate, its mechanical properties, the choice of the method, a range of temperatures in the future experiments. The cost and the desired accuracy must be also taken into consideration [23, 24]. For the direct measurements of the thermal conductivity of the clotted blood samples, we used the steady state one dimensional method. The advantage of this method is its simplicity in design and in interpretation of results, low cost, sufficient accuracy.

The formation of the blood clot implies the contraction of blood during the process of coagulation and the final decrease of the volume of the clot with respect to the initial volume of the blood. This means that the volume of a sample cell can not be fixed and should vary and be adjustable.

The direct measurement of thermal conductivity needs a creation of the thermal gradient across the sample in a steady state heat transfer process. The cylindrical geometry of the cell and one dimensional heat transfer model are suitable for our case. The horizontal orientation of a cell eliminates possible experimental errors during the calibration of a cell with water and ethanol due to a presence of thermal convection.

According to the one-dimensional Fourier-Biot heat conduction equation, the coefficient of thermal conductivity $K$ can be calculated as follows [24]:

$$K = -\frac{q \cdot \delta L}{S \cdot \delta T},$$

(3.22)

where $\delta L$ is the distance between two points in the volume of specimen where the temperature measurements were conducted, $\delta T$ is the magnitude of the temperature gradient, $S$ is the cross-sectional area. In order to measure the magnitude of the heat flow $q$ through the specimen, a supplementary substance is usually used. The magnitude of the temperature gradient should not exceed 1-2 degrees Celsius. Under this condition we can neglect the dependence of the coefficient of the thermal conductivity on temperature.
3.2.2 Optimization of experimental methods

There are two facts which should be noticed, which can improve the accuracy of the experiments on the thermal conductivity. First, we have to keep a control on the heat flow through the sample of clot by choosing the appropriate material for the pistons. Too big heat flow from the hot tank to the cylindrical cell causes the big temperature increase at one end of the clot sample. The choice of proper material for pistons (e.g. stainless steel) allows monitoring the heat flow through the experimental sample with the desired accuracy.

Second, we have to choose the proper substance for the comparative agent. The following expression can help to optimize the design of the experimental apparatus and chose the appropriate substance to measure the thermal gradient in a comparative method:

\[ K = K_s \cdot \frac{\delta T}{\delta L} \cdot \frac{\delta L_s}{\delta T_s}, \quad (3.23) \]

where \( \delta T_s \) is the temperature difference between two points in the supplementary substance, \( \delta L_s \) is the distance between these two points. As it may be seen from the formula above, the best accuracy in the measurements can be achieved if the thermal conductivity of the known substance and the expected magnitude of the thermal conductivity of the clotted blood have the same order. Distilled water was very suitable as a supplementary substance.

The third important factor influencing the effective measurements of thermal conductivity is the choice of the material for a sample cell. The thermal conductivity of the material for a sample cell must be significantly less then the expected magnitude of the coefficient of thermal conductivity under investigation. The last increases the heat flow in the axial direction and prevents the radial heat flow. The proper material for the sample cell with the additional thermal insulation makes the heat flow through the comparative substance and the substance with the unknown coefficient of thermal conductivity practically equal.
Chapter 4
Experimental procedures

4.1 Spatial characteristics of the used transducers

The actual acoustic field produced by an ultrasound transducer needs to be measured prior further experimentation. The main characteristics of each transducer were measured in a water tank filled with degassed and deionized water at the room temperature. The acquisition system included a hydrophone, generator, oscilloscope, positioning system, and LabView software as described in Chapter 4, Section 4.2. The results of those measurements are presented in the table below.

Table 4.1: The measured acoustic characteristics of the transducers used in experiments on the acoustic attenuation. Sound speed in water at 20 °C is 1482.3 m/s.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Type</th>
<th>Model</th>
<th>Effective diameter, mm</th>
<th>Near field, mm</th>
<th>Focal zone, mm</th>
<th>Transverse beam width, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 kHz</td>
<td>Unfocused</td>
<td>Custom design</td>
<td>61.4</td>
<td>74</td>
<td>&gt;120</td>
<td>22</td>
</tr>
<tr>
<td>1.0 MHz</td>
<td>Unfocused</td>
<td>Custom design</td>
<td>25.4</td>
<td>94</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>3.5 MHz</td>
<td>Focused</td>
<td>MD3483, Diagnostic Sonar</td>
<td>19</td>
<td>93†</td>
<td>75</td>
<td>2.5</td>
</tr>
</tbody>
</table>

† - For the focused transducer, it's called a focal distance (length) [15].

The axial profile of a 3.5 MHz transducer is presented in Figure 4.1(a). The lateral scan of acoustic pressure in the focal plane is presented in Figure 4.1(b).
proper alignment of ultrasound transducer, the acoustic beam is axially symmetric at the focal plane of the transducer. The 3D-mapping allows us to verify the spatial distribution of the acoustic field and the alignment of a water tank, positioning system and transducer.

Figure 4.1: Examples of the axial (left) and transverse (right) acoustic field profiles for the focused 3.5 MHz transducer. The -3 dB drop in the amplitude of acoustic pressure marks the limits of a beam width and the focal zone of a transducer.

The measurements of nonlinear effects were also conducted. The purpose of those measurements was to determine the range of magnitudes of acoustic pressure where nonlinearity was absent. We found that nonlinear effects are not present at the pressures below 0.18 MPa in amplitude.

Acoustic field mapping helps to evaluate the uniformity of the acoustic field created by ultrasound transducer, its axial symmetry and the quality of the alignment of entire system. The example of a 3D profile of acoustic field in the focal plane of
a 1 MHz transducer is shown in Figure (4.2). The distribution of acoustic pressure demonstrates good symmetry with respect to the transducer axis and uniformity of acoustic field produced by the ultrasound transducer.

### 4.2 Apparatus for the measurements of ultrasonic attenuation in blood clots and cranial bone

We exploited a insertion loss method or through-transmission method to measure the magnitude of a coefficient of amplitude attenuation [83]. All measurements were conducted in a tank with degassed and deionized water at the temperatures between 18°C and 21°C. The basic installation included a function generator (Model 33250A, Agilent Technologies, Inc., Palo Alto, CA), a power amplifier (Model 1502A, Amplifier Research, Souderton, PA) and an oscilloscope (LT 584, LeCroy Corp., Chestnut Ridge, NY). The controller system included the posi-
tioning system with a Velmex stepping motor controller NF 90 series and a MAC computer with the LabView data acquisition software installed on it. The general block-scheme of the experimental setup is shown in Figure (4.3).

Figure 4.3: General block diagram of the apparatus used in measurements of amplitude coefficient of attenuation in clotted blood by transient method.

The sample holder consisting of a plastic frame, with an opening filled with saline solution and sealed from both sides with the tegaderm films and with the prepared clot at the center, was placed in the focal plane of the calibrated ultrasonic transducer. The hydrophone placed immediately behind the clot registered the magnitude of the peak-to-peak pressure of the acoustic field. The diameter of the opening was 70 mm for the experiments at 120 kHz and 40 mm for measurements at 1 MHz and 3.5 MHz. A sheet of absorbing material (Aptflex F28, Precision Acoustics Ltd., Dorset, UK) was placed at the far wall of the water tank to prevent the reflection from it. For each frequency, a different pair of hydrophone and transducer was used. The types of transducers and hydrophones employed in our experiments are
presented in the Table (4.2).

Table 4.2: Types of transducers and hydrophones used in the experiments on the measurements of ultrasound attenuation in blood clots.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Transducer</th>
<th>Hydrophone</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 kHz</td>
<td>Unfocused, Custom Design, Sonic Concept, Inc., WA</td>
<td>TC4038, RESON, Inc., Goleta, CA</td>
</tr>
<tr>
<td>1.0 MHz</td>
<td>Unfocused, Custom Design</td>
<td>HP 0.5 mm Interchangeable Probe, Precision Acoustics Ltd., UK</td>
</tr>
<tr>
<td>3.5 MHz</td>
<td>Focused, MD3483, Diagnostic Sonar, Ltd., UK</td>
<td>HP 0.2 mm Interchangeable Probe, Precision Acoustics Ltd., UK</td>
</tr>
</tbody>
</table>

The plane acoustic wave generated by transducer propagated in the water tank and was registered by a hydrophone fixed to the holder of Velmex system with a stepping motor controller (NF 90 series, Velmex, Inc., Bloomfield, NY). The first step of the experiments included aligning the main axis of transducer and hydrophone with the coordinate axis of the positioning system. Subsequently, hydrophone was placed at the focus of the transducer and the peak-to-peak pressure in the focal plane was measured. Two factors were taken into account in order to determine the limits for the lateral scan in a focal plane of the transducer: the size of a clot and the width of an acoustic beam produced by transducer. In each case, the lesser size was chosen. In every individual experiment we used a clot sample prepared as described in Chapter 4.

4.2.1 Measurement techniques for transcranial attenuation

An acrylic tank with dimensions of $68 \times 55 \times 55 \text{ cm}^3$ filled with deionized, degassed water diminishing the acoustic attenuation was used to measure the coefficient of ultrasonic attenuation through skull wall in vitro at $20 \pm 2^\circ\text{C}$. The dry adult human skull was preliminary degassed in a special vessel filled with water and connected to a vacuum pump (Model 8803, Welch® Vacuum Technology Inc., Skokie, IL). The
thickness of the skull wall in the region of the temporal bone varied between 4 and 5 mm. A photograph of the experimental setup is shown in Figure (4.4).

Figure 4.4: Experimental setup for measurements of transcranial attenuation.

A 1 MHz unfocused piezoelectric transducer was used to generate an ultrasound beam and a hydrophone (Model TC4038, RESON, Inc., Goleta, CA.) was employed to measure the acoustic pressure of ultrasound after it passed through the temporal bone of the skull.

A computer-controlled positioning system (Velmex, Inc., Bloomfield, NY) was used to control the position of the hydrophone in the axial and focal planes of the transducer, including the far field region and the location of the middle cerebral artery, or MCA (see Figure(4.5)).

In order to determine the effect of angular rotation of the skull around the spinal chord on the attenuation of ultrasound through the skull, the measurements of the
attenuation were carried out for different angular positions of the skull wall with respect to the acoustic axis of transducer.

### 4.2.2 Attenuation measurements in blood clots at 120 kHz, 1 MHz, and 3.5 MHz

In order to determine the value of the amplitude attenuation coefficient, we have to find the change in the magnitude of acoustic pressure created by the ultrasound transducer caused by introduction of a clot sample into the space between a transducer and hydrophone. For this purpose we measured the acoustic pressure in a focal plane in two cases: free field case and when the clot is introduced in the space between the transducer and hydrophone. At 120 kHz, we used a custom-made 61.4 mm in diameter transducer (Sonic Concepts, Inc., WA) and a 4 mm in diameter hydrophone (TC4038, RESON, Inc., Goleta, CA). The calibration factor of the hydrophone in the vertical directivity at 120 kHz was 0.282 MPa/V. Three samples of porcine clots were used in order to determine the average magnitude of coefficient of attenuation. The diameter of the acoustic window in the acrylic...
sample holder was 70 mm.

We used the same technique previously described for the measurements of attenuation at 1 MHz. The custom-made unfocused transducer had the radiating aperture (effective diameter) of 25.4 mm. The HP Precision Acoustic hydrophone had a 0.5 mm tip. The calibration factor of the hydrophone was 0.021 MPa/mV at 1 MHz. The opening in an aluminum sample holder had a diameter of 40 mm.

The focused piston transducer (MD3483, Diagnostic Sonar, Ltd., UK) with the diameter of 19 mm and the hydrophone with diameter of the tip in 0.2 mm (HP Interchangeable Probe, Precision Acoustics, Ltd., UK) were used in the experiments at 3.5 MHz frequency.

The calibration factor of the hydrophone was 0.021 MPa/mV at 3.5 MHz. We used the same sample holder with a diameter of window of 40 mm for the fixation of sample in a water tank.

4.3 Density measurements of the human and porcine blood clots

4.3.1 Preparation of samples

Whole blood clots were prepared by aliquoting 1.5 – 2 ml arterial porcine or venous human blood into the 8-mm inner diameter glass tubes. The whole blood for the clot samples was collected for an entire year. We used the commercially available vacutainers (BD Vacutainer™, Franklin Lakes, NJ) with dimensions 11.2 × 100 mm² to form a clot. After pouring the blood into the vacutainers, they were placed into a water tank at 37 °C and incubated there for three hours. They were then placed in a refrigerator at temperatures between 7 °C and 9 °C for three days before the experiments began. Additional aliquots of blood from each pig or human were used to obtain a complete coagulation panel from Antech Diagnostics (Chicago,
IL), including D-Dimer, A-PTT, fibrinogen and prothrombin time testing, as well as a Complete Blood Count. A decision to use or reject the resulting clots was made based both on the test results and the clot appearance. Most pigs used as part of this study were found to be slightly anemic, with hematocrits in the range 25-35%. Only donors with values in the range $10 - 900 \, ng/ml$ for the D-Dimer test, 10-25 seconds for A-PTT, and $250 - 700 \, mg/dl$ for the fibrinogen concentration and 9-13 seconds for prothrombin time were considered to be acceptable. The resulting clots are normally dark red in color, roughly cylindrical in shape with an average diameter of $7 - 10 \, mm$, and the typical mass of each clot was about $0.5 \, g$. Human blood clots were slightly darker in color than the porcine ones. The typical look of the prepared clot is shown in Figure (4.6).

![Image of a blood clot](image)

**Figure 4.6:** Whole blood clots were prepared from either fresh porcine or human blood by aliquoting 1.5 or 2.0 ml into 10 ml glass tubes (BD Vacutainer™, Franklin Lakes, NJ) with dimensions $11.2 \times 100 \, mm^2$, immersing the tubes in a $37 ^\circ C$ water bath for three hours and storing the clots at the temperature around $7 - 9 ^\circ C$ for at least three days prior to assessment of the properties, which ensured complete clot retraction. The typical size of the clot samples was about $7 - 10 \, mm$ in diameter and the typical mass of each clot was about $0.5 \, g$. 
4.3.2 Experimental technique in density measurements

For the measurements of clot density, the volume of the clots was determined using a fluid displacement method in standard vacutainer tubes (BD Vacutainer™, Franklin Lakes, NJ) [16]. For the density measurement experiments, we additionally graduated two standard vacutainers and put graduation marks on their sides. The uncertainty of the volume measurement was 0.08 $cm^3$. The first stage of measurements included weighing the vacutainer filled with saline and determining the level of liquid in a tube. The vacutainer was partially filled with 0.9 % solution of saline and weighed initially with a standard laboratory scale (E200, Mettler Toledo Ltd., Columbus, OH), with an uncertainty of 0.01 $g$. After that blood clots were immersed in fresh saline, and the measurements of a total weight and a level of liquid in vacutainer were repeated. Each clot was clean up with napkin from the remains of serum before the experiment. The difference in weight of the vacutainer with and without the clots, and the change in liquid levels in the tube determined the mass and volume of the clots, respectively. All measurements were conducted in the room temperature range: $22 \pm 2^\circ C$. In every experiment a batch of 7-12 clots were used in order to increase precision of the measurements. The average value of clot densities and their standard deviations were found after 21 experiments with human clots and 30 experiments with porcine clots. For the preliminary calibration procedures, we used the tabulated value of 1.0044 $g/cm^3$ for the density of saline at 22 $^\circ C$. The total uncertainty in the density measurement was 0.01 $g/cm^3$ for porcine clots and 0.02 $g/cm^3$ for human clots. A total of 202 clots from 28 human subjects and a total of 336 porcine clots from 38 pigs were employed for the density measurements. Thus, the influence of physiologic variability and diet was captured in the standard deviation of the density measurements.
4.3.3 Degassing of the clot samples

In order to verify the hypothesis about the influence of the gas content of the samples of clotted blood on their physical properties, we performed the degassing of the clot samples and measured the properties of degassed clots. For this purpose, we placed the freshly prepared clot samples into the beaker filled with the saline solution and put the beaker with clots into the vacuum vessel with the tightly fitted lid and connected to the vacuum pump (Model 8803, Welch® Vacuum Technology Inc., Skokie, IL). All procedures were performed at the room temperatures. The duration of the degassing was about 75-85 minutes. The lowest pressure reachable in the vacuum vessel was 0.002 MPa. The initial phase of degassing was accompanied with a very intensive bubbling on the surface of clots and in the surrounding saline. This justifies the suggestions about the presence of the significant gas content in the formed clots.

4.4 Sound speed in clotted blood

We used an insertion echo pulse method to measure the speed of sound in clotted blood. A polystyrene cuvette of 4.5 ml capacity and dimensions $12.5 \times 12.5 \times 46 \text{ mm}^3$ (Curtin Matheson Scientific, Inc. Baltimore, MD) was immersed into a water tank and positioned within the focus of a 3.5 MHz transducer. Ultrasonic pulses were generated with a functional generator (Agilent 33250, Agilent Technologies, Inc., Palo Alto, CA), amplified with an amplifier (AR150LA, Amplifier Research, Souder ton, PA), monitored with an oscilloscope (LeCroy LT372, LeCroy Corp., Chestnut Ridge, NY). The cuvette and 3.5 MHz transducer were immersed in a temperature controlled water bath. The water tank was connected to the water thermostat (EX111, Neslab Instruments, Inc., Newington, NH) consisting of a bath circulator and microprocessor controller. Experiments were conducted at two temperatures: 20 °C and 37 °C. The choice of the working frequency was determined by two fac-
tors. First, the resolution of the ultrasound transducer had to be small enough to cover the area equal or less then the dimension of the cuvette with a sample of blood clot, which was about $5 - 7 \text{ mm}$. This dictates the lower frequency limit. On the other hand, the choice of too high frequency is limited by the attenuation in clot and cuvette walls.

We were measuring the flight times of the echo pulses reflected from the front faces of the near and far walls of a cuvette. The pulse repetition period for all experiments was $10 \text{ ms}$ which corresponds to a PRF of 100 Hz. The pulse duration was $0.9 \mu\text{s}$.

First, we measured the time delays between echoes from the front and back walls of the cuvette when it was filled with distilled water. By knowing the sound speed in pure water at the given temperature, we determined the distance traveled by ultrasonic pulse. Then, we measured the flight times when the blood clot was squeezed into a cuvette and the echo-pulse passed through the clot twice on the way from and back to the ultrasound transducer. The speed of sound in a clot was calculated by dividing the distance travelled by the ultrasound pulse in a cuvette by the difference in the flight times of the reflected pulses. Three clot samples were used in every measurement in different type of clotted blood. For validation purposes, the cuvette was filled with ethanol and the measurements of the sound speed were carried out. The obtained results are in good agreement with the tabulated values for ethanol [21].
4.5 Thermal conductivity and specific heat measurements

4.5.1 Calorimetric technique in the specific heat measurements

Direct calorimetric measurements using calibrated $E$-type thermocouples (Omega Engineering, Inc., Stanford, CT) were performed to determine the specific heat capacity of the human and porcine clots relative to the specific heat of a standard fluid, physiological saline [0.9 % water solution of sodium chloride]. The experimental setup included two $236\ ml$ Styrofoam® containers with the insulating lids, which contained $E$-type thermocouples (Omega Engineering, Inc., Stanford, CT). Two digital thermometers (Model HH 506-R, Omega Engineering, Inc., Stanford, CT) were used to measure temperature within the containers throughout the experiment. For the measurements of the mass of blood clots and saline, we used the standard scale (E200, Mettler Toledo Ltd., Columbus, OH). The batch of 7-12 prepared clots was placed in the first Styrofoam® container filled with $20-30\ ml$ of saline and cooled down to the temperature of $7\pm1\degree C$ in the refrigerator. The other Styrofoam® container filled with approximately the same amount of saline was fixed inside a $15\times20\times20\ cm^3$ Styrofoam® box for the better thermal insulation. The temperature of saline in this container was around $21\degree C$. Then the content of the first container was added to the saline in the second container and the equilibrium temperature of the mixture was recorded. The uncertainty in the temperature measurements was $0.06\degree C$ and the uncertainty in the measurements of the clot mass was $0.01\ g$. The propagated error for the specific heat measurements in this case was $0.3\cdot10^3\ J/kg\cdot K$. The accuracy of the measurements of the specific heat was determined eventually by the random variation of the position of the thermocouple tip in the cup. This systematic error gave the main contribution into the uncertainty in the determination of the equilibrium temperature and, as a result, in the heat capacity of the blood clots. In order to improve the results of the
measurements of the specific heat, we increased the number of clots involved in experiments. A total of 202 human and 336 porcine clots were utilized for the specific heat capacity measurements. We estimate the total uncertainty of the specific heat measurements to be on the order of $0.76 \, J/kg \cdot K$ for human clots and $0.46 \, J/kg \cdot K$ for porcine clots. We followed the same procedures in the experiments with the degassed clots.

### 4.5.2 Experimental setup for the thermal conductivity measurements

The comparative steady state method was employed for determination of the thermal conductivity of whole blood clots. The experimental setup for the measurements of the thermal conductivity of the clotted blood is shown in Figure (4.7). The horizontal thermal gradient was created in the cylindrical experimental cell holding both the sample of clot and the exemplary substance with respect to which we determined the magnitude of the thermal conductivity. The sample of clot was placed in one chamber of the cylindrical cell which was separated by the copper membrane from the other chamber of the same cell filled by water. The magnitude of the thermal gradient across the entire cell did not exceed $5 \, ^\circ C$. Two chamber cylindrical cell was made of polyethylene-terephthalate glycol (PETG). The dimensions of the cell were $11.2 \times 55 \, mm^2$ for the part holding a blood clot and $11.2 \times 35 \, mm^2$ for the part containing water. The copper membrane separating two chambers provided a good thermal contact between two cells. The size of separating membrane was $16.4 \times 1.27 \, mm^2$. Two Plexiglas tanks $25.4 \, cm \times 25.4 \, cm \times 25.4 \, cm$ in size filled with water were used as thermal baths providing the stable temperature gradient across the known substance (water/ethanol) and the samples of a clotted blood. Two stainless steel pistons provided the directed heat flow from the hot tank to the cold one through both substances: a comparative substance (agent) and the samples of clotted blood. The temperature difference between tanks was chosen
under the condition that it will give only a $1 - 2 \, ^{\circ}C$ temperature drop on the sample of a clot alone. Under this condition we can neglect the thermal dependence of the thermal conductivity. Four hypodermic $T$-type thermocouples (Omega Engineering, Inc., Stanford, CT, gauge 30, o.d. 294 $\mu m$) and two digital thermometers HH506-R (Omega Engineering, Inc., Stanford, CT) were employed to measure the temperature drops across the samples. Six LuerLock® connectors were attached to the experimental cell as ports for positioning thermocouples and bleeding air (4 along the chamber containing clot and 2 along the chamber containing water. The thermocouples were fixed in the caps of LuerLock® valves so that their tips could reach the center of cylindrical cell. Two stainless steel pistons provided good thermal flow from the hot water tank to the cold water tank. The experimental design allowed us to change the position of the thermocouple tips across the sample of clot and measure the temperature drop at the different points in the clot. We had three fixed positions of the thermocouple in the cell with a clot sample. The chamber for a sample of clot had five fixed positions with different separation between the thermocouple tips: $32 \, mm$, $25.5 \, mm$, $19.3 \, mm$, $12.5 \, mm$, and $6.4 \, mm$. The measurements were made in all five positions in order to reduce the errors due to inhomogeneity of a clot and a presence of air cavities in the volume of clot. The distance between the tips of thermocouples in the chamber with water was $19.2 \, mm$. The accuracy of the digital thermometers was about $1.0 \, ^{\circ}C$. The readings of both thermocouples were recorded by means of the software supplied by Omega Engineering and analyzed in Excel spreadsheets. There was the experimental protocol established especially for the measurements of thermal conductivity. It included the following procedures. First, we need to give some time for a system to reach a steady state. When the system reached the steady state, the temperature distribution across the cell does not depend on time any more, and we have a steady heat flow from the hot tank through the cylindrical cell to the cold water tank [35]. In order to make the estimates for the time necessary for the system to reach a steady
state, we need to solve Fourier’s heat transfer equation for a cylindrical geometry:

\[
\kappa \left( \frac{1}{\rho} \frac{\partial}{\partial \rho} \left( \rho \frac{\partial T}{\partial \rho} \right) + \frac{1}{\rho^2} \frac{\partial^2 T}{\partial \varphi^2} + \frac{\partial^2 T}{\partial z^2} \right) = \frac{\partial T}{\partial t}, \tag{4.1}
\]

where \( \kappa \) is the thermal diffusivity of the sample, \( T \) is a temperature, \( t \) is a time, \( \rho, z, \varphi \) are the cylindrical coordinates. Due to a thermal insulation of the experimental cell and due to its cylindrical shape, the equation above in this particular problem can be simplified to the following one dimensional form:

\[
\kappa \frac{\partial^2 T}{\partial z^2} = \frac{\partial T}{\partial t}, \tag{4.2}
\]

where \( z \) is the coordinate along the axis of a cylindrical cell. The general solution of this equation with appropriate boundary conditions is:
\[ T(\rho, \varphi, z, t) = T_0 + \left( \frac{T_1 - T_0}{l} \right) \cdot z + 2 \left( \frac{T_0 - T_1}{\pi} \right) \cdot \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n} \times \sum_{n=1}^{\infty} \frac{(-1)^{n}}{n} \cdot \exp \left\{ -\frac{\pi^2 n^2 \cdot \kappa \cdot t}{l^2} \right\} \cdot \sin \left( \frac{n\pi l}{l} \cdot z \right) \cdot \sin \left( \frac{n\pi l}{l} \cdot z \right), \] (4.3)

where \( l \) is the length of the experimental samples, \( T_0 \) and \( T_1 \) are the initial temperatures at two ends of the cylindrical cell and \( \kappa \) is the thermal diffusivity of the material in a cell. The coordinate \( z = 0 \) corresponds to the interface between the face of the piston from the cold tank and the investigational substance. The other interface between the investigational substance and the face of copper membrane corresponds to \( z = 0.053 \, m \).

The rough estimates of the magnitude of time we are interested in for the different agents such as water, ethanol, castor oil and whole blood are given by the first order extent of the exponent.

The preliminary calibration of the experimental setup included the measurements of the thermal conductivity of ethanol as the investigational substance. The measurements gave \( 0.20 \pm 0.03 \, W/m \cdot K \) for the magnitude of thermal conductivity, which compares favorably with the published value for the thermal conductivity of ethanol, \( 0.19 \, W/m \cdot K \) [35].

In order to perform experiments on thermal conductivity, we prepared a few large clots according to the protocol described previously to fill the entire volume of the sample cell. We used the same vacutainers as before to form the clot samples \( 7 - 10 \, cm^3 \) in volume. The typical dimensions of each clot were about \( 11.5 \times 70 \, mm^2 \). The prepared clots were placed into a cylindrical cell and tightly squeezed between the steel piston and a copper membrane which allowed us to remove all air pockets and to provide a good thermal contact between the faces of piston and the copper membrane on one side and the clot on the other side. Two rubber gaskets were
Figure 4.8: Computer simulation of heat transfer in a cylindrical cell. The plots above represent the evolution of the temperature in a cell with blood sample versus time, \( t \) passed since the beginning of the heating of one end of a cylindrical cell. This mathematical model helps to establish the experimental protocol for taking the data in the thermal conductivity measurements. The length \( l \) of a cell is 53 mm, the temperature difference between two ends is 1.5 °C which is typical in our experiments, the thermal diffusivity of blood, \( \kappa \), is \( 0.127 \cdot 10^{-6} \text{ m}^2/\text{s} \). The program code is shown in Appendix 3.

placed on both sides of a copper membrane. They prevented any leaks of water and the possible penetration of air into the cell’s chambers from outside.

After visual control of the content of the cell, it was placed between two water tanks and fixed in place. When these preparation were finished, we started to heat up the water in the hot tank. It took, approximately, one hour to complete heating of the hot tank to 26°C. The temperature of a cold tank was about 22°C. After that we waited two hours for the thermal stabilization in chambers with water and clot. The data on the temperature drops across the clot were taken in the next 15 minutes and then averaged over this period of time. The coefficient of thermal con-
ductivity of the investigated substance (either clot or ethanol) was calculated from the following formula:

\[
K_{\text{unknown}} = K_{\text{water}} \cdot \frac{\delta T_2}{L_1} \cdot \frac{L_2}{\delta T_1},
\]

(4.4)

where \(K_{\text{unknown}}\) is the thermal conductivity of the investigated substance, \(K_{\text{water}}\) is the thermal conductivity of water, \(\delta T_2\) is the magnitude of the temperature difference between two points inside the substance under investigation, \(\delta T_2\) is the distance between these two points, \(\delta T_2\) is the temperature difference between two points in the water and \(L\) is the distance between these two points in the water-filled chamber. Three sets of measurements with different clot samples were carried out. Every set of measurements was completed in one day. Every set of measurements included measuring the temperature differences in the different points of the clot samples. The results of those measurements for porcine and human clots have been averaged.

### 4.6 Thermal elevation measurements

In order to decrease the contribution of other phenomena such as cavitation and streaming into the thermal elevation in the volume of clot, we conducted the measurements of magnitude of the thermal elevation at low amplitude of ultrasonic signal and, in turn, the ultrasonic pressure.

#### 4.6.1 Analysis of the thermal artifacts during the measurements of ultrasound hyperthermia

The thermocouple probes have been used to measure temperature in both \textit{in vivo} and \textit{in vitro} experiments. There are several materials which are used to manufacture thermocouples: copper, nickel, constantan (an alloy of 55% copper and 45%
nickel, chromel-P (an alloy of 90% nickel and 10% chromium), alumel (an alloy of 95% nickel, 25% of manganese and 2% of aluminum). Sometimes, they are covered by special plastic to make them safe to use in the human body. The last fact drastically increases the size of thermocouple and, in turn, the thermal artifacts.

Thermal artifacts are a significant obstacle for accurate measurements of the real temperature in soft tissues. There are three main causes which can produce an artificial thermal elevation in a tissue [9]. The first is due to presence of shear viscosity in a soft tissue and vibration of the thermocouple end. The second is due to absorption in the material of thermocouple (or isolation). The third is due to ultrasound scattering from the probe. One way to decrease these artifacts is to use very small (relatively to the acoustic wavelength) thermocouples. If the ratio of the diameter of thermocouple $d$ to the acoustic wavelength $\lambda$ is about $1/25$, the thermal artifacts are negligible [10]. Other authors suggest different criteria for avoiding errors in the thermal measurements. For example, in order to avoid significant acoustic field disturbance during the temperature measurements, the ratio $d/\lambda$ was suggested to be smaller than $1/20$ in [49]. In contrary, the value of $1/3$ was suggested in [50] in order to avoid the thermal artifacts. In the following example of the ultrasound hyperthermia, the tip of thermocouple with a diameter of wire equal to 125 $\mu$m immersed in pure water and placed at the focus of the ultrasonic transducer was sonicated by the focused ultrasonic beam with wavelength equal to 1.5 $mm$ [48]. The authors observed a significant temperature elevation of 0.6 $^\circ$C during the 1 $s$ insonification with the acoustic intensity of 188 $W/cm^2$. The ratio $d/\lambda$ was $1/12$, which, apparently, was sufficient for the existence of multiple ultrasound artifacts. Only the existence of thermal artifacts can explain the observed effect because the bulk heating of water in this case was negligible due to a very small coefficient of acoustic absorption in water.

The response time of a thermocouple is a very important characteristic in temperature measurements. If the response time is greater then the time over which
the temperature changes, then the instantaneous dynamic thermal response will not be measured and the thermal elevation will not be followed by the thermocouple measuring it. In order to obtain an expression for the thermocouple response time in the case of very simple spherical geometry of a bare wire beaded junction thermocouple shown in Figure (4.9.), we have to solve the following heat equation and find the time dependence of temperature at the center of the bead:

$$\nabla^2 T = \frac{1}{\kappa} \cdot \frac{\partial T}{\partial t},$$  

(4.5)

where $\kappa = \frac{K}{\rho C_v}$ is a thermal diffusivity in $\text{m}^2/\text{s}$, $K$ is a thermal conductivity in $\text{W/m/K}$, $\rho$ and $C_v$ are the density and the specific heat of the material of thermocouple, respectively. Let us assume that the solution of this equation $T(r, \theta, \varphi, t)$ is given by the following expression in spherical coordinates:

$$T(r, \theta, \varphi, t) = U(r, \theta, \varphi) \cdot \exp\{ - \lambda \cdot t \},$$  

(4.6)

where $U(r, \theta, \varphi)$ determines the spatial dependence of temperature inside the bead. The temporal dependence of temperature is given by the exponent. Separation of variables leads to the following solution for the temperature distribution inside the sphere:

$$T(r, \theta, \varphi, t) = T_1 + \frac{2r_0}{\pi} \cdot \left( T_0 - T_1 \right) \cdot \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n} \cdot \frac{\sin \left( \frac{n \pi r}{r_0} \right)}{r} \cdot e^{-\lambda_n t},$$  

(4.7)

where $\lambda_n = \kappa \left( \frac{n \pi}{r_0} \right)^2$, $r_0 = \frac{3}{2} \cdot d$, $d$ is a diameter of a thermocouple wire. In accordance with Equation (4.7), the response time of this bare wire thermocouple is proportional to $\frac{9d^2}{4\kappa^2} \cdot \frac{1}{\kappa}$.

We planned to carry out the measurements of the temperature elevation in blood clots in vitro by choosing $E$-type (Chromel P-Constantan bare wires) thermocouples with the highest coefficient of thermal diffusivity available (see Table (4.3)).
Figure 4.9: The geometry used to model a spherically beaded thermocouple junction.

and with a wire diameter $d$ ranging from 12.7 $\mu m$ up to 381 $\mu m$. The choice of the size of thermocouple and its type are determined by a working frequency in experiment and the convenience in handling it. Thermocouple must provide a fast response, must be easy to handle and must have a diameter less or equal to the diameter required for the elimination of thermal artifacts. For the experiments on the ultrasound hyperthermia in blood clots at 120 kHz, the wavelength of the acoustic wave in water $\lambda$ is 12.4 $mm$ and the thermocouple diameter less or equal to 500 $\mu m$ is required. It is possible to use a hypodermic thermocouple with the diameter of 203 $\mu m$. It has a stiff measuring tip and is easy to handle. In this case, the condition $(d/\lambda) \leq 25$ is well satisfied. At 3.5 MHz we need the wire diameter less or equal 20 $\mu m$. For the experiments on the ultrasound hyperthermia in temporal bone, we may use the $E$ - type thermocouple with the wire diameter of 381 $\mu m$.

According to our model, the bigger value of diffusivity corresponds the shorter
Table 4.3: Typical values of the thermal diffusivity $\kappa$ for the known types of materials, $m^2/s$ [13, 21, 31].

<table>
<thead>
<tr>
<th>Material</th>
<th>$\kappa$ $m^2/s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>$11.6 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Nickel</td>
<td>$2.3 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Constantan</td>
<td>$1.6 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Alumel</td>
<td>$0.66 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Chromel-P</td>
<td>$0.05 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Air</td>
<td>$1.99 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Castor oil</td>
<td>$0.01 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Water</td>
<td>$0.014 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Whole blood</td>
<td>$0.13 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Human kidney</td>
<td>$0.13 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Human liver</td>
<td>$0.15 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Human brain</td>
<td>$0.14 \cdot 10^{-6}$</td>
</tr>
<tr>
<td>Human fat</td>
<td>$0.07 \cdot 10^{-6}$</td>
</tr>
</tbody>
</table>

period of the response time. This is also true for the thermocouples with the bigger diameters.

By definition, the time it takes for the temperature at the center of thermocouple bead, $T_0$, to increase by 63.2 % of the initial difference between $T_0$ and $T_1$, the value of temperature outside the thermocouple, is called the response time of thermocouple. One case was considered in the calculations when the initial temperatures $T_0$ and $T_1$ were $37^\circ C$ and $38^\circ C$, respectively.

The numerical value of the response time can be found on the basis of simple graphical analysis of the plot shown in Figures (4.10) and (4.11) in which the calculations of the temperature changes the center of the welded bead of thermocouple are plotted against the time in accordance with Equation (4.7).

This analysis gives the approximate response time of $130 \mu s$ for a bare wire beaded junction $E$ - type thermocouple and about $4 \mu s$ for a $T$ - type thermocouple. As it is seen on the plots, the magnitude of the response time increases with an increase of the diameter of the thermocouple wires. Since, in our case, the
Figure 4.10: *Time dependence of temperature rise at the center of a bead of thermocouple made of three major materials. T₀ is 37°C and T₁ is 38°C. The diameter of bare wires is 12.7 μm.*

Figure 4.11: *Time dependence of temperature rise at the center of a bead of thermocouple made of three major materials. The diameter of bare wires is 203 μm.*
temperature variation of the thermo-physical properties of materials is small, we may assume that our numerical estimates for the thermocouple’s response times are valid for the entire region of temperatures between 20 °C and 40 °C.

The approximate values of the response time for the different types of thermocouples are presented in the table below. These estimates show that all types of thermocouple are expected to give reliable results in monitoring the temperature of a clot, if the duration of acoustic pulse is greater than 130 µs. The last is true for the entire range of duty cycles and pulse repetition periods, we are dealing with in our experiments. For example, the period of ultrasonic wave at 120 kHz is equal to $8.3 \cdot 10^{-3} s$, which is big enough for the thermocouples to keep up with the temperature changes during the first moments of ultrasound insonification.

### 4.6.2 Experimental setup for observation of ultrasound hyperthermia in clotted blood

The experimental setup for the measurements of ultrasound hyperthermia in blood clots is shown in Figure (4.12). Basic installation included a function generator (Agilent 33250, Agilent Technologies, Inc., Palo Alto, CA), an oscilloscope (LeCroy LT372, LeCroy Corp., Chestnut Ridge, NY), a amplifier, a Plaxiglass® water tank with dimensions of $41 \times 21 \times 21 \ cm^3$ filled with deionized, degassed water, a digital thermometer (HH 506-R, Omega Engineering, Inc., Stanford, CT) and a computer with the installed software. For the experiments at 120 kHz, we used the Ultra
series 2021LF/HF linear amplifier (T&C Power Conversion Inc., Rochester, NY). For the experiments at 1 and 3.5 MHz, we were using the amplifier 150LA (Amplifier Research, Souderton, PA). A sheet of acoustic absorbing material (Aptflex F28, Precision Acoustics Ltd., Dorset, UK) was placed at a 45° angle along the far wall of the Plaxiglass® tank to minimize reflections.

A water tank was connected via tubing to the water thermostat EX111 (Neslab Instruments, Inc., Newington, NH) consisting of a bath circulator and a microprocessor controller. The air trap (Terumo Inc., Indianapolis, IN) was fixed on the pipe coming from the thermostat. The trap reduces significantly the flow of air bubbles coming from water circulator. The gas content of the water in the thermal bath was controlled with a dissolved oxygen meter (Model 407510, Extech Instruments Corp., MA).

![Diagram](image)

**Figure 4.12:** Experimental setup for the measurements of ultrasound hyperthermia in blood clots and cranial temporal bone. Temperature in a water tank had two fixed meanings: 20°C and 37°C.

The acrylic sample holder with the round acoustic window had two ports in the
side walls: the first one for the fixation of thermocouple and the second one as an air bleed. Later, the two sides of this window were being sealed with tegaderm films and space between was filled with plasma. The holder with a sample of clot immersed in plasma is shown in Figure(4.13).

For the experiments with porcine clots, we used pig’s frozen plasma in a Na citrate. For the experiments with human clots, we used a frozen human plasma (18201 FF, Hoxworth Center, University of Cincinnati). Human fresh-frozen plasma (hFFP) was procured from a blood bank in 250 – 300 ml units. Each unit was briefly thawed, aliquoted into 50 ml centrifuge tubes (Fisher Scientific Research, Pittsburgh, PA), and stored at –70°C. Aliquots of plasma were allowed to thaw for experiments, and the remaining amounts discarded following completion of a given experiment.

The peak-to-peak pressure of the ultrasonic beam in the focus was evaluated in the preliminary calibration experiments. During these experiments the hydrophone was placed in the focus of the ultrasonic transducer and the intensity was measured. In all our experiments, the peak to peak pressure was 0.25 MPa. The magnitude of the spatial peak intensity in the focal region of ultrasonic transducer used in these experiments \( I_{sp} = 0.5 \ W/cm^2 \), was chosen on the basis of data for the threshold for inertial and stable cavitation given in [96] and the level of intensities used by the other authors and published in literature concerning an ultrasound thermal bioeffect.

The pulse repetition period (PRP) for all experiments was 10 ms. Since the duty cycle in our experiments was 80%, the spatial peak temporal average intensity \( I_{spta} \) in the area of interest was 0.4 \( W/cm^2 \).

Before the experiments on ultrasound hyperthermia, we degassed both plasma and clot. For this purpose we placed the holder with open air bleed into the vacuum chamber connected to the vacuum pump (Model 8803, Welch® Vacuum Technology, Inc., Skokie, IL). The process of degassing took about 70-85 minutes. After
that the air bleed was locked with the nylon screw and the holder was placed in the water tank.

In the experiments at 120 kHz we used a hypodermic \( T \)-type thermocouple with the diameter of a tip 0.203 mm (HYP0-33, Omega Engineering, Inc., CT). In the experiments at 1 MHz we used a bare wire \( E \)-type thermocouple (CHCO-001, Omega Engineering, Inc., CT) with the diameter of bare wire of 0.025 mm. In the experiments at 3.5 MHz we used a bare wire \( E \)-type thermocouple (CHCO-0005, Omega Engineering, Inc., CT) with the diameter of bare wire of 0.012 mm.

4.6.3 Experimental setup for observation of ultrasound hyperthermia in human temporal bone

The experimental setup for the measurement of ultrasound induced hyperthermia in a human temporal cranial bone was similar to that of described in previous section and shown in Figure (4.14). All measurements of the ultrasound hyperthermia
in a human skull were conducted in the water tank with dimensions $42 \times 21 \times 21 \text{cm}^3$ filled with deionized, degassed water. The temperature in the water tank had two fixed meanings 20 and $37^\circ\text{C}$ and was controlled by the thermo-stabilizing system (EX 111, Neslab Instruments, Inc., Newington, NH).

The big segment of a dry human skull was used in the measurements of ultrasound hyperthermia. The mass of the bone was 111.57 g. The thickness of the skull in the area of temporal bone was $3.8 \pm 0.1 \text{mm}$. The sample of the bone was preliminary degassed in a vacuum chamber (Model8803, Welch®, Welch Vacuum technology, Inc., Skokie, IL). We kept this sample immersed in water during all experiments which we were conducting. Three $E$-type thermocouples (5TT-TG30, Omega Engineering, Inc., CT) with the wire diameter of 0.127 mm were glued with the SuperGlue® to the bone in the location of a temporal window. Two of them were fixed on the front wall of bone and the third one was fixed on the back wall. The use of two thermocouples helped to find the location of the maximum effect.

Figure 4.14: A segment of the human skull with the thermocouple attached to the surface of temporal bone.
of hyperthermia in the case of the misalignment of the skull bone with respect to an acoustical axis of transducer. The temporal window with the fixed to it thermo-couples was placed into the focus of the ultrasonic transducer.

The duration of every experiment was about 8 minutes. The duty cycle of the ultrasound pulses was 80%. The spatial peak intensity at focus of transducer was 0.5 $W/cm^2$ in all conducted experiments. Temperature development in a clot was registered by the digital thermometer (HH 506-R, Omega Engineering, Inc., Stanford, CT) and recorded on the computer.

The previous studies showed that 80% duty cycle is the most efficient in the ultrasound assisted thrombolysis at least at two frequencies, 120 kHz and 1 MHz [2]. All experiments were conducted with 80% duty cycles at 100 Hz of PRF.
Chapter 5

Obtained results and discussion

5.1 Acousto-mechanical parameters of human and porcine blood clots ($\rho$, $v$ and $\mu$)

The experiments showed that the initial density of the newly prepared blood clots was $(1.08 \pm 0.02) \times 10^3$ kg/m$^3$ for human clots and $(1.06 \pm 0.01) \times 10^3$ kg/m$^3$ for porcine clots, respectively. We also measured the change in the value of specific heat and density due to degassing of clots. The degassed clots had slightly bigger both specific heat and density in comparison with the intact clots. The density of the degassed human and porcine clots was $(1.08 \pm 0.01) \times 10^3$ kg/m$^3$ and $(1.07 \pm 0.03) \times 10^3$ kg/m$^3$, respectively.

The measurements of the speed of sound in porcine clotted blood gave a magnitude of $1547 \pm 1$ m/s at $20^\circ$C and $1577 \pm 2$ m/s at $37^\circ$C. The speed of sound in human clots was $1597 \pm 9$ m/s at $20^\circ$C and $1633 \pm 4$ m/s at $37^\circ$C, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density $\rho$, g/cm$^3$</td>
<td>$1.076 \pm 0.019$</td>
</tr>
<tr>
<td>Sound speed $v$, m/s</td>
<td>$1597 \pm 9$ at $20^\circ$C</td>
</tr>
<tr>
<td></td>
<td>$1633 \pm 4$ at $37^\circ$C</td>
</tr>
</tbody>
</table>

The comparison between the densities and specific heat coefficients for the newly prepared and degassed porcine and human clots is presented in Figures (5.13) and (5.14).
Table 5.2: Acousto-mechanical parameters of the porcine blood clots.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density $\rho$, $g/cm^3$</td>
<td>1.058 ± 0.014</td>
</tr>
<tr>
<td>Sound speed $v$, m/s</td>
<td>1547 ± 1 at 20°C</td>
</tr>
<tr>
<td></td>
<td>1577 ± 2 at 37°C</td>
</tr>
</tbody>
</table>

The pressure amplitude attenuation coefficient for each frequency was determined in three sets of experiments using three different blood clot samples for every type of clotted blood. In every experiment, we also did the transverse scans of acoustic pressure in both directions: $x$ and $y$. The example of the acoustic field profiles at 120 kHz along with the error bars representing the standard deviation for the experiments with porcine clots is presented in Figure (5.1). All curves presented in Figure (5.1) are the results of procedure of averaging mentioned below. The nice smooth symmetric shape of the two upper curves justifies the assumption that no distortion of the acoustic field was brought by introducing the plastic frame of a

![Figure 5.1](image-url)
sample holder filled with only saline between the transducer and the hydrophone. The relative reduction of acoustic pressure in the third curve was caused by the attenuation of ultrasound in the material of clot which was calculated in accordance with Equation (2.9) and (2.14).

The results of the transverse pressure profile measurements at 3.5 MHz are shown in Figure (5.2). In this case, the coefficient of attenuation was determined by averaging the results over the region limited by the width of acoustic beam.

![Graph showing the lateral scans of acoustic pressure in the focal plane of a 3.5 MHz transducer](image)

**Figure 5.2:** The lateral scans of acoustic pressure in the focal plane of a 3.5 MHz transducer. Representation of the experimental results for the evaluation of the amplitude attenuation coefficient in porcine clotted blood.

Standard deviation was computed after averaging the results obtained in all nine experiments for every frequency and over the scans performed in two mutually perpendicular directions in order to assess the variability. The final results with the corresponding standard deviations are shown in Tables (5.3).
Table 5.3: The averaged values of the amplitude coefficients of ultrasonic attenuation in porcine and human clots at the different frequencies with the corresponding standard deviations.

<table>
<thead>
<tr>
<th>Frequency, MHz</th>
<th>Amplitude attenuation in porcine clots, Np/cm</th>
<th>Amplitude attenuation in human clots, Np/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>0.10 ± 0.01</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>1.00</td>
<td>0.17 ± 0.02</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>3.50</td>
<td>0.3 ± 0.1</td>
<td>0.23 ± 0.04</td>
</tr>
</tbody>
</table>

5.1.1 Frequency dependence of the amplitude coefficient of attenuation

In general, the ultrasonic attenuation in biological tissues displays the power dependence versus frequency, which can be generalized in the following expression:

\[ \mu = \mu_0 \cdot f^m, \]  

where \( \mu \) is a coefficient of attenuation, \( \mu_0 \) is a normalization constant, \( f \) is a frequency, \( m \) is a power coefficient. Most soft tissues and body liquids exhibit a value of \( m \) close to unity [83]. It is true for the frequency range from 1 to 50 MHz or even higher [82]. It is different from the frequency dependence of ultrasonic attenuation in the homogeneous media such as air or water, in which the attenuation coefficient is proportional to the square of frequency.

It should be also admitted that the magnitude of \( m \) may be frequency dependent. Although such frequency dependence is characteristic of the measurements artifacts due to the presence of gas bubbles, there are some evidences that this effect may be the real property of the tissue which is being investigated [83].

As it was pointed out in [19], the frequency dependence of the attenuation coefficient in soft mammalian tissue is almost linear for the entire range of ultrasound frequencies utilized in medicine i.e. 0.5 -15 MHz. Attenuation in soft tissue decreases with the increasing water content and rises up with the increasing content
of proteins in it. The frequency dependence of absorption in general is similar to that of attenuation [19]. Knowledge of the coefficient $m$ may help in understanding of the likely relative contributions of different attenuation mechanisms taking place in the particular type of biological tissue.

![Figure 5.3](image-url)

**Figure 5.3: Frequency dependence of the pressure amplitude attenuation coefficient in blood clots.** The solid and dashed lines refers to a least square fit of the data to the form $\mu = \mu_0 \cdot f^m$, where $\mu$ is the pressure amplitude attenuation coefficient, $f$ is the center frequency, and the $R$ values listed in the legend are the correlation coefficients and represent a goodness of fit.

The straight lines on the plot presented in Figure (5.3) are the least square fit to the obtained data. The slope of the lines is very close to one third, which means that the experimentally measured coefficient $m$ is equal to 0.3 for both types of blood clots.

The information on the temperature dependence of the ultrasound attenuation in biological tissue is very scarce [98]. The results of *in vivo* experiments in the spinal cord of a mouse, conducted by Dunn at 1 MHz, showed that the amplitude
absorption coefficient $b$ in the temperature range between 0 and $35^\circ C$ is given by the following analytical relation:

$$ b(T) = \frac{1}{10} \cdot \left[ 2 - e^{0.016 \cdot (35 - T)} \right], \quad (5.2) $$

where $T$ is the temperature of the tissue in degrees Centigrade, and $b$ is measured in $Np/cm$ [97].

The results on the thermal elevations at the different frequencies obtained in our study and presented in Section (5.6), show that there is a slight temperature dependence of the absorption in clotted blood. The temperature elevation rates, which are proportional to the magnitude of attenuation, increase with the increase of the temperature of both type clot samples. The results on the thermal elevations obtained in our study show that there is a slight temperature dependence of the absorption in a clotted blood. The temperature elevation rates, which are proportional to the magnitude of attenuation, increase, in general, with the increase of the temperature of both type clot samples. The obtained results on the maximal thermal elevations also justify this assumption. This is valid for all frequencies we use in our work. It does not concern the attenuation in cranial bone: at both temperatures which were used in our experiments, the rates of temperature rise were the same within the limits of the uncertainty in temperature measurements.

### 5.2 Ultrasound transcranial attenuation in human skull

The experimental results on the attenuation of ultrasound through the temporal window at 1 MHz of a human skull specimen were gathered. Experiments showed that there is significant attenuation of ultrasound through the temporal bone of skull at 1 MHz. The attenuation measurement yielded 90 % loss in the value of peak-to-peak pressure at the location of MCA (see Figure (5.4)). The magnitude of
peak-to-peak pressure was:

\[ P_{field\ at\ MCA} = 1.3 \cdot U_{MCA} = 0.02 \cdot 1.3 \text{ MPa} = 0.026 \text{ MPa}, \]  

(5.3)

where \( U_{MCA} \) is the value of voltage registered by hydrophone and 1.3 MPa/V is the conversion factor for a given type of hydrophone at 1 MHz. The purpose of these investigations was to determine whether misalignment of the acoustic beam with the temporal bone would yield the increased attenuation. The evaluation of the coefficient of attenuation was done on the basis of the following formula:

\[ \alpha_{sk} = 20 \cdot \log \frac{P_{free\ field}}{P_{field\ at\ MCA}}, \]  

(5.4)

where \( \alpha_{sk} \) is the value of the transcranial attenuation and \( P_{free\ field} \) is the acoustic pressure created by the ultrasound transducer and registered by the hydrophone without the skull in a tank.

Figure 5.4: Axial acoustic pressure profile along the way of propagation of ultrasonic beam through the skull. The pressure, proportional to the voltage, is presented on the graph in accordance with Equation (5.3).
The resulting attenuation varied between $-17.5\, dB$ to $-21\, dB$ with the angle variation between $0\, ^\circ$ (when the sound beam strikes the skull wall normally) and $12\, ^\circ$ (the oblique incidence). The experiment showed that we have the minimum attenuation under the normal incidence of the ultrasonic beam (see Figure (5.5)). At 120 KHz, the coefficient of attenuation across the same skull specimen was $4.5\, dB$ [9, 7, 8]. All measurements were made in the room temperature range: $20\pm 1\, ^\circ C$.

Our results on transcranial ultrasound attenuation at 1 MHz and 120 kHz are in good agreement with those obtained in [44].

In the addition to the data on the ultrasound attenuation in the cranial bone, we need for the further discussion the data on the other thermal and acoustomechanical properties of the skull bone. These physical properties of the human skull compiled from literature are presented in the Table (5.4).
Table 5.4: Acoustic and thermal properties of human skull bone.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuation $\mu$, Np/cm</td>
<td>1.5</td>
<td>[25], at 1 MHz</td>
</tr>
<tr>
<td>Density $\rho$, g/cm$^3$</td>
<td>1.61-1.85</td>
<td>[13, 61]</td>
</tr>
<tr>
<td>Specific heat $C_V$, J/g · K</td>
<td>1.59</td>
<td>[31]</td>
</tr>
<tr>
<td>Heat conductivity $K$, W/m · K</td>
<td>1.16</td>
<td>[31]</td>
</tr>
<tr>
<td>Thermal diffusivity $\kappa$, m$^2$/s</td>
<td>0.49 · 10$^{-6}$</td>
<td>[31]</td>
</tr>
<tr>
<td>Sound speed $c$, m/s</td>
<td>3.36 · 10$^4$</td>
<td>[18, 129]</td>
</tr>
</tbody>
</table>

5.3 Thermal properties of human and porcine blood clots ($C_V$, $K$ and $\kappa$)

The results of the experimental measurements of specific heat, thermal conductivity, and the calculation of thermal diffusivity are presented in the following Tables (5.5) and (5.6). The magnitude of the physical constants was determined after a series of measurements. The accuracy is given by the value of the standard deviation in the experimental measurements. The total number of clots used in 21 experiments on the measurement of the coefficient of specific heat was 202 for human clots. In the case with porcine clots, we used 336 clots in 30 experiments. A batch of 7-12 clots was used in an individual experiment.

The average magnitudes for the coefficients of specific heat of the newly prepared human and porcine clots were $(3.48 \pm 0.76) \times 10^3$ J/kg · K and $(3.23 \pm 0.46) \times 10^3$ J/kg · K, respectively. After degassing, the densities and the coefficients of the specific heat changed. The densities of degassed human and porcine clots were $(1.08 \pm 0.01) \times 10^3$ kg/m$^3$ and $(1.07 \pm 0.03) \times 10^3$ kg/m$^3$. The coefficients of specific heat were $3700 \pm 800$ J/kg · K and $3900 \pm 800$ J/kg · K, respectively. The bar plots corresponding to these results along with a standard deviations are presented in Figures (5.6) and (5.7).

The results of measurements of coefficient of thermal conductivity were being averaged over three specimens in which the experiments were carried out. They
gave the following value for the thermal conductivity of porcine clots: 0.55 ± 0.13 W/m · K. The average magnitude of the coefficient of thermal conductivity of human clot was 0.59 ± 0.11 W/m · K.

**Table 5.5: Thermal parameters of the human blood clots**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific heat $C_V$, J/g · K</td>
<td>3.48 ± 0.76</td>
<td>Measured</td>
</tr>
<tr>
<td>Heat conductivity $K$, W/m · K</td>
<td>0.59 ± 0.11</td>
<td>Measured</td>
</tr>
<tr>
<td>Thermal diffusivity $\kappa$, m²/s</td>
<td>$(0.16 ± 0.05) \cdot 10^{-6}$</td>
<td>Calculated</td>
</tr>
</tbody>
</table>

**Table 5.6: Thermal parameters of the porcine blood clots**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific heat $C_V$, J/g · K</td>
<td>3.23 ± 0.46</td>
<td>Measured</td>
</tr>
<tr>
<td>Heat conductivity $K$, W/m · K</td>
<td>0.55 ± 0.13</td>
<td>Measured</td>
</tr>
<tr>
<td>Thermal diffusivity $\kappa$, m²/s</td>
<td>$(0.16 ± 0.05) \cdot 10^{-6}$</td>
<td>Calculated</td>
</tr>
</tbody>
</table>

The data on the specific heat and density of clotted blood are consistent with the data of the specific heat and density for blood, plasma and air, as it is seen in Chapter 2.

The presence of air, which has the heat capacity of 1.01 J/g · K, may significantly influence of the thermal and acoustic properties of clotted blood. The evidence of such an influence was obtained after the degassing of the specimens of human and porcine clots. The bubbling during degassing and the the measurements of density and specific heat obtained after degassing witness about a significant part of a gas presented in a volume of clot. The specific heat of whole blood, on average, is less then that of plasma, i.e. 3.93 J/g · K.

The results obtained in our experiments help to evaluate the share of air in the content of blood clots, since the total value for the specific heat, and, in a less extent, the density of clots is given by the heat capacity and the density of two major components of the thrombus: formed elements and air. Degassing of clots in a vacuum chamber significantly reduces the amount of air in thrombi. This, in turn,
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Figure 5.6: Density and specific heat of two types of porcine clots: intact and degassed ones.

Figure 5.7: Density and specific heat of two types of human clots: intact and degassed ones.
changes the total magnitudes of density and specific heat of the degassed blood clot. Simple calculations give the relative concentration of air and structured elements in porcine and human blood clots. For porcine clot, 77% of total mass of clot is given by structured elements and the other 23% is given by air. In the case with human clot the percent of the structured elements and air was 89% and 11% respectively. On the other hand, as we shall see later, there is no evidence of the presence of a gas after acoustic scanning of the clots. Probably, this means that the size of the air bubbles is significantly less than the wavelength in acoustic beam at the highest frequency of 3.5 MHz used in our experiments, i.e. 140 μm.

The data obtained by means of electron microscopy show that the distribution of fibers inside a clot becomes denser after insonification. Ultrasound irradiation also changes the diameter of the fibrin fibers in clots approximately by a factor of 1.2 [70].
5.4 Thermal elevation in human and porcine blood clots due to ultrasound exposure

5.4.1 Timely limits for adiabatic model

In order to determine experimentally the timely limits for the validity of our adiabatic model, the preliminary experimentation was needed. For this purpose, we recorded the development of temperatures at two points in a clot during the ultrasound insonification. The first point was at the center of a clot. The second point was at the interface between the clot and the surrounding plasma. We compared those temperatures with the temperature in a water tank. The change in the temperature differences against the time is shown in Figure (5.8).

We preliminary degassed the plasma, either human or porcine, which we used in the experiments with blood clots. This was done to eliminate the additional artifactual temperature elevation due to presence of the gas in plasma and the consequent

![Figure 5.8: The temperature elevation in a porcine clot due to exposure to a 1 MHz ultrasound at 20 °C. Two temperature differences are shown. The first is the temperature difference between the center of the insonated clot and the temperature in a water tank. The second is the temperature difference between the temperature of the outer boundary of a clot and the temperature in a water tank.](image)
heating of the gas by ultrasound. The plot in Figure (5.9) demonstrates the presence of such artifacts in the porcine plasma. After degassing, the artifactual temperature rise drops to the level of uncertainty in the measurements of temperature.

The temporal dependence of the temperature increase in blood clots exposed to pulsed ultrasound in the frequency range between 0.12 and 3.5 MHz with an $I_{SPTA}$ of 0.4 W/cm² is shown in Figure (5.10)-(5.14). The solid lines represent theoretical prediction of the temperature increase built in accordance with Equation (2.24) and the data points represent experimentally measured ultrasound hyperthermia. Experimental data points represent the average values for the temperature rise obtained in two in vitro experiments. The error bars in the theoretical curves were assessed by combining the effects of the range of acousto-mechanical and thermal properties measured in the clotted blood, as well as the size range of the heat source, which depends on the physical dimensions of the clots.

The experiments showed that the temperature elevation rates do not depend on
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Figure 5.10: Thermal elevation in porcine clots exposed to 120 kHz ultrasound at 37 °C. The intensity $I_{spta} = 0.4 \text{ W/cm}^2$, pulse repetition period 10 ms.

Figure 5.11: Thermal elevation in porcine clots exposed to 1 MHz ultrasound at 37 °C. The intensity $I_{spta} = 0.4 \text{ W/cm}^2$, pulse repetition period 10 ms.
Figure 5.12: *Thermal elevation in porcine clots exposed to 3.5 MHz ultrasound at 37°C. The intensity $I_{sptu} = 0.4 \text{ W/cm}^2$, pulse repetition period 10 ms.*

Figure 5.13: *Thermal elevation in human clots exposed to 120 kHz ultrasound at 37°C. The intensity $I_{sptu} = 0.4 \text{ W/cm}^2$, pulse repetition period 10 ms.*
Figure 5.14: Thermal elevation in human clots exposed to 1 MHz ultrasound at 37°C. The intensity $I_{spta} = 0.4\ W/cm^2$, pulse repetition period 10 ms.

Figure 5.15: Thermal elevation in human clots exposed to 3.5 MHz ultrasound at 37°C. The intensity $I_{spta} = 0.4\ W/cm^2$, pulse repetition period 10 ms.
the magnitude of the duty cycle used in the experiment. In turn, the final magnitude of the temperature rise depends on the duty cycle, as it was expected. It shows good consistency with a duty cycle of ultrasound pulse: the greater duty cycle we apply, the bigger maximal temperature rise is.

5.5 Thermal elevation in human temporal bone due to ultrasound exposure

The curves representing a typical process of heating of the temporal bone of human skull exposed to the pulsed ultrasound are shown in Figures (5.16)-(5.21). The duration of all experiments did not exceed 300 s. The initial stage of the heating is characterized by the sharp elevation of the temperature at the area insonified by ultrasound beam. The time, it takes to reach the maximum value of hyperthermia, is different for different frequencies. It decreases with the increase of frequency. It is approximately $80 - 100$ s at 120 kHz. At 1 MHz and 3.5 MHz this time is shorter: $50 - 70$ s and $35 - 40$ s, respectively. After that, all curves flatten and the temperature reaches a maximum. The flattened part of the curves has more oscillatory character at $37^\circ C$ than at $20^\circ C$. This effect may be explained by the circulation of water in the water tank at $37^\circ C$. At $20^\circ C$, the water in experimental tank was still.

There is a significant difference between the temperature elevation at the front walls of the skull and that of at the back wall at 3.5 MHz. This may indicate the attenuation of ultrasound wave on the way from the front wall to the back one.
Figure 5.16: Ultrasound hyperthermia in the human temporal bone exposed to 120 kHz ultrasound at the room temperature.

Figure 5.17: Ultrasound hyperthermia in the human temporal bone exposed to 120 kHz ultrasound at 37°C.
Figure 5.18: Ultrasound hyperthermia in the human temporal bone exposed to 1 MHz ultrasound at the room temperature.

Figure 5.19: Ultrasound hyperthermia in the human temporal bone exposed to 1 MHz ultrasound at 37°C.
Figure 5.20: Ultrasound hyperthermia in the human temporal bone exposed to 3.5 MHz ultrasound at the room temperature.

Figure 5.21: Ultrasound hyperthermia in the human temporal bone exposed to 3.5 MHz ultrasound at 37 °C.
Table 5.7: Experimental results of the maximum temperature rise measured in tissue exposed to pulsed ultrasound \textit{in vitro}, the corresponding temperature rise rates, and the corresponding standard deviations (PRP=10 ms, $P_{ptp} = 0.25$ MPa, $I_{SPTA} = 0.4 \text{ W/cm}^2$, $T_{tank} = 20^\circ\text{C or } 37^\circ\text{C}$, $t = 300$ s).

<table>
<thead>
<tr>
<th>Maximum thermal elevation</th>
<th>Temporal bone</th>
<th>Porcine clots</th>
<th>Human clots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$20^\circ\text{C}$</td>
<td>$37^\circ\text{C}$</td>
<td>$20^\circ\text{C}$</td>
</tr>
<tr>
<td>$T$ ($^\circ\text{C}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 kHz</td>
<td>0.49</td>
<td>0.61</td>
<td>0.11</td>
</tr>
<tr>
<td>1 MHz</td>
<td>1.05</td>
<td>1.00</td>
<td>0.22</td>
</tr>
<tr>
<td>3.5 MHz</td>
<td>0.94</td>
<td>0.85</td>
<td>0.25</td>
</tr>
<tr>
<td>$\Delta T/\Delta t$ ($^\circ\text{C}/s$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 kHz</td>
<td>0.01</td>
<td>0.01</td>
<td>0.0005</td>
</tr>
<tr>
<td>1 MHz</td>
<td>0.02</td>
<td>0.02</td>
<td>0.005</td>
</tr>
<tr>
<td>3.5 MHz</td>
<td>0.03</td>
<td>0.03</td>
<td>0.005</td>
</tr>
</tbody>
</table>

The results shown in Table (5.7) were obtained in two sets of experiments. We had two experimental runs with the same segment of a human skull and two experiments with two different clots. Later, the maximum values of the thermal elevation in each experiment were recorded and the average values for them were determined and presented in the table above. The temperature elevation rate was assessed on the basis of the recorded temperature change over the initial 30 s of ultrasound insonification.

5.6 Comparison the results of analytical thermal model with measured values of temperature rise in clotted blood and cranial bone

The magnitude of the ultrasound induced hyperthermia increase with an increase of ultrasound frequency. As we expected, its magnitude is greater for the temporal bone and less for both types of clotted blood. Our experiments showed that the registered thermal elevations are well consistent with the duty cycles used in experiments at all frequencies: if we increase the duty cycle by a factor of $\frac{3}{4}$, the
hyperthermia also increases by this factor. The information on the physical properties of human cranial bone and clotted blood used in our numerical calculation are presented in Tables (5.2)-(5.5) and (5.8).

Table 5.8: Coefficient of attenuation in human cranial bone. Compilation of data from [8, 18, 25, 73-76].

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>0.12</th>
<th>1.0</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of absorption (Np/cm)</td>
<td>0.12</td>
<td>1.5</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Measured values of the maximum temperature increase in porcine and human blood clots and in human temporal bone exposed to pulsed ultrasound with \( I_{SPTA} = 0.4 \, W/cm^2 \) are presented in Table (5.7). The maximum temperature increase in clotted blood (0.33 ± 0.04°C for both types of clots) was noted at 3.5 MHz. The temperature rise in cranial bone exposed to a pulsed ultrasound was greater than the temperature increase in blood clots at each frequency. Note, however, that for all three frequencies, the temperature increase on the surface of human cranial bone does not exceed 1°C. The maximum thermal elevation of 1.00 ± 0.06°C was achieved on the proximal surface of the cranial bone exposed to 1 MHz pulsed ultrasound. No significant difference in the temperature elevations between the proximal and distal surfaces of the bone were observed for 0.12 MHz and 1 MHz. However, at 3.5 MHz, the temperature elevation on the proximal wall is somewhat higher (0.4 to 0.6°C), probably due to significant attenuation of the ultrasound in the bone. The temperature on the proximal and distal bone surfaces for all frequencies is plotted as a function of time in Figures (5.16)-(5.21). For all ultrasound hyperthermia measurements in both bone and clotted blood, a steady state temperature increase was achieved after an initial 30 to 120 s period of insonification.

Using Equation (2.24) and Equation (2.29), the predicted values of the ultrasound hyperthermia in porcine or human clots and bone after 300 s of ultrasound insonification in the frequency range between 0.12 MHz and 3.5 MHz are presented
in Table (5.9). Note that the theoretical estimates for the thermal increase in clots due to ultrasound exposure to 0.4 $W/cm^2$ pulsed ultrasound ($I_{SPTA} = 0.4 W/cm^2$) do not exceed 0.88°C for all three frequencies. Implicit in the derivation of the predicted thermal increase is a calculation of the reflected and absorbed ultrasonic energy. Also included in the calculation is the beam aperture, which was not identical for the three frequencies. The 3.5 MHz transducer was focused and the beam diameter is smaller than the 0.12 and 1.0 MHz unfocused beams. Thus the predicted temperature increase at 3.5 MHz in clotted blood is less than the temperature increase calculated at either 0.12 or 1.0 MHz. The upper and lower limits of

Table 5.9: Numerical estimates for the magnitudes of temperature rise and the temperature rise rates at the center of the human cranial bone, porcine, and human blood clots exposed to pulsed ultrasound ($I_{SPTA} = 0.4 W/cm^2$) for 300 s.

<table>
<thead>
<tr>
<th>Temperature rise and temperature rise rate</th>
<th>Temporal bone</th>
<th>Porcine clots</th>
<th>Human clots</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$ (°C)</td>
<td>0.29</td>
<td>0.58(+0.20,−0.13)</td>
<td>0.38(+0.32,−0.16)</td>
</tr>
<tr>
<td>120 kHz</td>
<td>0.82</td>
<td>0.71(+0.22,−0.15)</td>
<td>0.55(+0.37,−0.19)</td>
</tr>
<tr>
<td>1 MHz</td>
<td>0.88</td>
<td>0.49(+0.48,−0.26)</td>
<td>0.33(+0.25,−0.13)</td>
</tr>
<tr>
<td>3.5 MHz</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\Delta T/\Delta t$ (°C/s)</th>
<th>0.04</th>
<th>0.024 ± 0.004</th>
<th>0.017 ± 0.009</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 kHz</td>
<td>0.47</td>
<td>0.040 ± 0.008</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>1 MHz</td>
<td>2.44</td>
<td>0.07 ± 0.03</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

the variation of the temperature elevation presented in this table correspond to the error bars shown in Figures (5.10)-(5.15). Propagated errors for the temperature rise rates were calculated for the initial, adiabatic stage of heating in accordance with Equation (2.25) by the standard procedure.
Chapter 6

Conclusions

The acousto-mechanical and thermal properties of the human and porcine clotted blood were determined experimentally and add to the knowledge base of the physical properties of clotted blood. The results obtained allowed us to make numerical estimates of a magnitude of the thermal elevation during ultrasound insonification of blood clots for enhanced thrombolysis and may be helpful in the development of high intensity focused ultrasound and other therapeutic ultrasound applications.

The temperature rises in the human cranial bone and the human and porcine clotted blood at 20 and 37 °C were determined experimentally in vitro. Ultrasound insonification of human temporal cranial bone with a spatial peak, temporal average intensity of 0.4 W/cm² at 3.5 MHz produces a maximum temperature rise of 1.0 °C on either side of the skull bone. Nor was there significant thermal elevation in blood clots either at this frequency. The theoretical estimates of the magnitude of thermal elevation during ultrasound insonification of the blood clots are higher than those measured experimentally. Thus temperature increases predicted by the theory can be used as an upper limit for ultrasound hyperthermia. In the presence of perfusion, the mild thermal elevation would be even smaller.

The average diameter of the middle cerebral arteries is about 3 – 4 mm, which may correspond to the clot size two times less than we used in this study. The numerical results on the temperature rise in blood clots obtained in our in vitro
experiments may be used for the calculation of the change of kinetic rates of the ultrasound enhanced thrombolysis or fibrinolysis. These changes are proportional to the temperature changes in tissue according to Formula (1.5). The comparison of the change in the effectiveness of the ultrasound assisted thrombolysis in the presence of rt-PA with the magnitude of the temperature elevations for different frequencies (i.e. 0.12 MHz, 1.0 MHz, 3.5 MHz) or at different temperatures of the clot (i.e. 20 °C or 37 °C), provides the information necessary for the estimation of the numerical contribution of thermal effects in the process of a clot disruption.

Indirect proof of the slight temperature dependence of the coefficient of attenuation was obtained in the thermal elevation measurements. Experiments showed that the temperature elevation rates in the insonified blood clots have different magnitudes at 20°C and 37°C. The slight increase in temperature elevation rates with the increase of the temperature of clots indicates the increase of ultrasound attenuation in the material of clots.

Understanding the potential for thermal mechanisms involved in the interaction of ultrasound with blood thrombi during ultrasound-assisted thrombolysis is an important first step in improving thrombolytic efficacy while minimizing unwanted thermal bioeffects. The obtained results on the magnitudes of ultrasound hyperthermia in cranial bone and blood clots may be helpful in the development of high intensity focused ultrasound and other therapeutic ultrasound applications.

6.1 Analysis of the obtained results on the thermal elevation due to pulsed ultrasound exposure

The analysis of the obtained results should be started with the analysis of the structure of the clotted blood. The formed blood clot has the same main constituents as a whole blood: red cells, platelets, fibrin fibers. Density, specific heat and thermal conductivity for the whole human blood according to the compilation of
the literature data are: $(1.05 - 1.60) \times 10^3 \text{ kg/m}^3$, $(3.61 - 3.89) \times 10^3 \text{ J/kg \cdot K}$, $0.49 - 0.55 \text{ W/m \cdot K}$ [13, 31, 32]. Human red blood cells have the density of $1.093 \times 10^3 \text{ kg/m}^3$ [13]. Their specific heat is $3.21 \times 10^3 \text{ J/kg \cdot K}$ and thermal conductivity is $0.45 - 0.482 \text{ W/m \cdot K}$ [13, 53]. The isolated fibrin fibers have the density of $1.3 \times 10^3 \text{ kg/m}^3$ [32]. The magnitudes of all these physical properties in the formed clots are determined by the concentration of the mentioned above components. This suggestion is consistent with the experimental results obtained in this work: the density, the heat capacity, the thermal conductivity are higher for the human clots because they have a higher hematocrit than the porcine clots. It is interesting to compare the same properties for the other types of soft tissues. The typical values for the density of the different human organs such as kidney, liver, brain lie in the range between $1040 - 1060 \text{ kg/m}^3$ [31]. The specific heat of brain is $3.68 \times 10^3 \text{ J/kg \cdot K}$, liver is $3.60 \times 10^3 \text{ J/kg \cdot K}$, kidney is $3.89 \times 10^3 \text{ J/kg \cdot K}$ [31]. The thermal conductivity of brain is $0.528 \text{ W/m \cdot K}$, liver is $0.565 \text{ W/m \cdot K}$ and kidney is $0.544 \text{ W/m \cdot K}$ [31]. The attenuation of ultrasound at 1 MHz in the whole human blood according to the published data is $0.017 \text{ Np/cm}$ [36]. In our experiments, the attenuation of ultrasound in porcine clotted blood at 1 MHz was $0.17 \text{ Np/cm}$. It is closer to the magnitude of ultrasonic attenuation in liver ($0.17 \text{ Np/cm}$), muscles ($0.2 - 0.25 \text{ Np/cm}$) and brain ($0.12 \text{ Np/cm}$) and much bigger than the attenuation of ultrasonic wave in a whole blood [61]. This fact may be explained by the significantly inhomogeneous structure of the material of clot which may cause the scattering of acoustic wave. The inhomogeneity of blood clot includes air pockets, the fibrin network region with different densities of fibrin, red cells, platelets, and cavities filled with serum. The histology of clotted blood justifies these assumptions (see Figure (6.1)).

The attenuation of the ultrasound in the clotted blood is determined by the absorption of acoustic wave in the macromolecules of different proteins forming the material of clot [17]. The magnitude of the coefficient of attenuation is close to the
Figure 6.1: Photo of the cross section of a porcine clot with a 20-× magnification. The porcine clot was stained with an antifibrinogen antibody (Dako Corp., Carpinteria, CA) which stains fibrin fibers brown [96].

coefficient of attenuation in the other types of soft biological tissues such as liver, kidney, brain containing the same type of proteins. In contrary to the linear dependence between the attenuation and frequency which is typical in soft tissues, our results showed more shallow power dependence of attenuation in the frequency range between 0.12-3.5 MHz: the amplitude coefficient of attenuation is proportional to the frequency in the $0.3^{rd}$ power as shown in Figure (5.3). This type of the frequency dependence of attenuation is similar to the frequency dependence of ultrasound attenuation in lung ($0.2^{nd}$ power), skeletal muscles ($0.8^{th}$ power), skin ($0.6^{th}$ power) [83]. The theoretical calculations of the frequency dependence of the absorption in a whole blood clot reported by the Applied Physics Laboratory of the University of Washington showed the $0.9^{th}$ power in the dependence between the coefficient of absorption and frequency [32]. The absorption in this case is de-
terminated by the relaxation mechanism in protein macromolecules. The obtained frequency dependence of the amplitude coefficient of attenuation probably reflects the fact of the dependence of the attenuation on the relative concentration of the proteins in clots. The standard deviation in the thermal conductivity and specific heat data reflects the natural variability of the physical properties of blood clots. The presence of air pockets and an inhomogeneous fibrin structure in the clots might also contribute to the standard deviation of the thermal conductivity and specific heat experimental results.

The techniques which were used in this work due to the direct approach, simplicity and affordability may be applied for the investigation of the other types of soft biological tissues such as brain tissue, liver tissue etc.

The analysis of the data on clotted blood obtained in this work showed a good consistency with the same properties of other similar type of biological materials such as blood, plasma, red cells, serum, although the ultrasonic attenuation is higher than we expected.

The theoretical prediction of the temperature elevation in human and porcine clots presented in Table(5.9) tends to overestimate the actual temperatures measured, sometimes by a factor of two. Several factors could contribute to this discrepancy, including errors in the assumed size and shape of the clot, the absorption coefficient used, or the position of the thermocouple within the clot. The temperature increase depends on the square of the radius of the clot and any error in the measurement of the clot size is squared. Note that scattering was ignored and the coefficient of absorption was taken directly from the coefficient of attenuation. Note, also, that the absorption coefficient for human and porcine clots was measured at 20°C, but our experiments were conducted at 37°C. The positioning of the thermocouple tip inside the clot samples could have been slightly off center and we did not monitor the thermocouple placement during the experiments. Since the temperature decreases for any position away from the center of the clot, the
thermocouple location might contribute to the measurements of the temperature increase appearing lower than the theoretical predictions. At 3.5 MHz, the agreement between theory and experiment is improved. Our theoretical model and our experimental set-up did not incorporate blood perfusion. Thus actual in vivo temperature increases will likely be lower. The initial heating rate of human clots exposed to 3.5 MHz pulsed ultrasound ($I_{SPTA} = 0.4 \text{ W/cm}^2$) was 0.01°C/s. This initial heating regime is considered to be adiabatic. A rough estimate of the heating rate in soft tissue exposed to pulsed ultrasound was presented by Nyborg and Ziskin and is given by a simple empirical formula which depends on the attenuation coefficient and the spatial average, temporal average intensity [33]. Using the attenuation coefficient for clotted blood and a spatial average, temporal average intensity of 0.4 W/cm², their formula yields an estimate of the heating rate of 0.01 °C/s, which agrees with our data. Temperature rise was quantified in human muscles exposed to 3.0-MHz pulsed ultrasound with a spatial average, temporal average intensity of 0.5 W/cm² for 10 min [64]. A temperature increase of 2.8°C was noted in these in vivo experiments. Using Equation(2.26), and utilizing the exposure parameters as in [64], the predicted temperature increase is 2.5°C, which is in close agreement with their measured value of 2.8°C. Note that the size of the thermocouple used (0.4 mm) by these investigators experimentally could possibly have produced an artifactually higher temperature increase at 3.0 MHz. Similarly, Carstensen et al. measured the temperature elevation in rat liver exposed to 2.0-MHz continuous wave (CW) ultrasound with an $I_{SAT}A$ of 0.57 W/cm² [57]. The maximum temperature increase measured by these investigators in rat liver was 1.8°C. Using the same ultrasound exposure parameters as input to Equation(2.26) in human liver with absorption coefficient of 0.132 $Np/cm$ [129], a 1.3°C temperature increase is predicted, which is also consistent with the experimental data. The temperature elevations measured in blood clots exposed to low intensity (< 0.5 W/cm²) pulsed ultrasound were extremely small (~ 0.2°C) for all three frequencies (0.12, 1.0, 3.5
MHz). Enhanced rt-PA thrombolysis has been demonstrated \textit{in vitro} in both human and porcine clots exposed to low intensity ultrasound [2]. Therefore, it is unlikely that a thermal mechanism contributed significantly to this thrombolytic enhancement.

\section*{6.2 Relevance to the clinical application of transcra-nial ultrasound thrombolysis for the stroke therapy}

After successful treatment, the blood perfusion in brain feeding blood vessels is restored. Tissue plasminogen activator (t-PA) currently is the only medicine which was approved to treat new strokes [117]. It is called the clot-busting medication. The safe concentration of rt-Pa injected into the human blood stream lies between 0.35 and 0.85 $mg/ml$. This medicine works with the body’s own chemicals, and helps dissolve the blockage in the blood vessel in the brain that may be causing the stroke restore a blood vessel perfusion. It is the same drug that is often used to treat heart attacks. The studies of t-PA have shown that it can restore the motor functions of brain and reduce disability from stroke by 30%. At the same time, it has potentially serious side effects that include bleeding within the brain. Not all people with stroke can receive the clot-busting drug t-PA. For example, people with a repetitive case of stroke, recent major surgery or suffering from diabetes are at the high risk of blood intracerebral bleeding.

For t-PA to work, it must be given within three hours of the early stage of is-chemic stroke symptoms. The earlier the drug is given within those three hours, the better it works. Symptom onset is defined as the time the patient was last known to be all right. If a patient awakens with symptoms, the symptom onset time is set back to the hour when a patient went to sleep. This criterion alone may exclude many people from receiving this drug. This is also why it is so important to get to
a stroke team for preliminary evaluation. A patient must not have any evidence of bleeding on the CT scan of the head. The clot-busting medication is not used for anyone having a hemorrhagic stroke. That is why it is critical to know what kind of stroke the patient is having. After evaluation, the doctor decides whether a patient should receive treatment with this drug and discuss the risks and benefits of giving it. Some doctors may believe that the drug is less effective. If given, strict guidelines must be met for the administration of this drug to prevent bad side effects. Other treatments for acute stroke are being tested. At some hospitals, clot-busting drugs are given through a small catheter that, through an IV, is threaded up into the neck and into the artery where there is a blockage. This treatment can potentially be used up to 6 hours after onset of stroke symptoms. Many other new treatments for stroke are being developed. It may be possible to participate in a study of a new stroke drug or other acute treatment.
Chapter 6. Conclusions

Application of ultrasound for the medical treatment of ischemic stroke allows reducing the concentration of rt-PA used for this purpose. The successful application of ultrasound requires choosing the appropriate parameters of ultrasound: amplitude, frequency, duty cycle [69]. Lower frequencies and duty cycles reduce the undesired heating and the lower amplitudes eliminate the risk of a blood hemolysis and hemorrhage [76].

Alexandrov et al. reported recently the results of the successful application of Doppler ultrasonography for the treatment of patients with acute ischemic stroke [79]. His group was using the standard head frame to position the transducer on the patients’ head against the left transtemporal cranial window. The use of transcranial 2 MHz Doppler ultrasonography after rt-Pa was administered to the patients increased the number of those who had the complete recanalization in the proximal middle cerebral artery after 2 hours of treatment. The application of the continuous Doppler ultrasound also slightly increases the final recovery from the ischemic stroke.

The analysis of all possible mechanisms involved in the ultrasound assisted thrombolytic effect is presented in [80]. After the qualitative analysis of every biological effect of ultrasound, the author of this study did not give preference to any of them as a main cause of ultrasound thrombolysis. Only the synergistic effect of all bioeffects may result in the effective ultrasound-assisted thrombolysis, the author concludes.

6.3 Suggestions for further research

One of the possible logical continuation of this project is the investigation of the ultrasound hyperthermia and in turn, the ultrasound assisted thrombolysis in the presence of contrast agents. It is a known fact, that the increased temperature in-
creases the enzymatic activity of rt-Pa, gives the basis for the assumption that the contrast agent may significantly increase the thrombolitic effect of ultrasound. The future research may include the investigations of physical mechanisms and different factors (such as a size, a type of a contrast agent, its concentration) influencing ultrasound-assisted thrombolysis in the presence of a contrast agent.

The further research may include the development a theoretical model for the ultrasound hyperthermia in a clotted blood in the presence of a blood perfusion. This may bring some corrections to the existing theory. In this case, the theoretical calculations may be closer to those observed in experiments.

Further experimental work may include the measurements of ultrasound hyperthermia in blood clots in the presence of plasma, rt-PA and liposomes. These experiments are closer to the in vivo experiments.

The further research may include the development of theory of dissipation of ultrasonic wave in the netlike medium of the clotted blood. The experimental results on the frequency dependence of attenuation obtained in this work may be the verification of validity of the developed theory.

The further research may include more detailed investigation of the ultrasound absorption in wider rage of frequencies. The measurements of the ultrasound absorption in soft tissues may help to identify the abnormalities in their structure and the diseased tissues. Since a temperature is a very influencing factor for the metabolic rates, the heat transfer problems considered in this work may be very useful in the analysis of the temperature distribution in tissue during insonification. Particularly, they may be applied to the problem of ultrasound tissue ablation, cancer treatment by high-intensity focused ultrasound (HIFU).
Appendix A

MatLab applications

A.1 Temperature distribution inside a thermocouple bead

% Calculation of temperature distribution inside
% the spherical thermocouple bead (Constantan) (Tmp1.m);
% February 15, 2004

function z=Tmp1(r,t)

global D %D - Diameter of the bare wires of thermocouple, meters;

k=1.6*10^(-5);% Constantan diffusivity, m2/s;

ro=1.5*D;% Radius of the sphere(bead of thermocouple), meters

T1=38;% Temperature on the surface
T0=37;% Temperature at the center
T11=0; n=1; while n <600000
    Lambda=k*((n*pi)/ro)^2;
    T11=T11+((-1)^(n+1))/n*exp(-Lambda*t)*sin((pi*n*r)/ro)/r;
    n=n+1;
end

z=T1+T11*2*(ro/pi)*(T0-T1);

% Matlab 6.5 Release 13
% File - Tmp1.m
A.2 Determination of a response time for the different types of thermocouples

% Response time plots( Script203.m );
% February 21,2004
% The diameter of bare wires 203 m;
clear all;

global D %D - Diameter of the bare wires of thermocouple, meters;

% Time scales for different materials :
zet=0:5*10^(-5):5*10^(-2); ket=0:5*10^(-5):10^(-1);
tet=0:5*10^(-6):10^(-3);

D=203*10^(-6);% Diameter of bare wires, m
r = 10^(-8);% The mathematical location
    % of the center of the thermocouple bead, m

Temperature1 = Tmp1(r,zet);%Data on Constantan
Temperature2 = Tmp2(r,ket);%Data on Chromel P
Temperature3 = Tmp3(r,tet);%Data on Copper

save('Temperature1.txt', 'Temperature1', '-ascii');
save('Temperature2.txt', 'Temperature2', '-ascii');
save('Temperature3.txt', 'Temperature3', '-ascii');

semilogx(zet,Temperature1,'r--'),grid
axis([1.5*10^(-5) 10^(-1) 37 38]),%Sets up the range of variables to display

axis([1.5*10^(-5) 10^(-1) 37 38]),%Sets up the range of variables to display
title('Temperature at the center of a 203 m bare wire thermocouple bead'), xlabel ('Time, s'), ylabel('Temperature, deg.(C)');

hold on; semilogx(ket,Temperature2,'b-.');
semilogx(tet,Temperature3,'g');

legend('Constantan','Chromel P','Copper',2);
set(gca,'FontName','Times New Roman','FontSize',14);

hold off;

% Matlab 6.5,Release 13
A.3 Temperature distribution in a cylindrical cell

Response time plots (ScriptCyl.m);
November 10, 2004
The length of experimental cell is 53 mm;
clear all; global z t1 t2 t3 t4

Time scales for different materials
t1 = 0; t2 = 1200; t3 = 3600; t4 = 7200;
l = 0.055; % Length of a cell, m
z = 0:0.0001:0.053; % Limits of a cell, m

Temperature1 = Cyl1(z, t1); % Data on Copper for t1
Temperature2 = Cyl2(z, t2); % Data on Copper for t2
Temperature3 = Cyl3(z, t3); % Data on Copper for t3
Temperature4 = Cyl4(z, t4); % Data on Copper for t4

save ('Temperature1.txt', 'Temperature1', '-ascii'); save
('Temperature2.txt', 'Temperature2', '-ascii'); save
('Temperature3.txt', 'Temperature3', '-ascii'); save
('Temperature3.txt', 'Temperature3', '-ascii');

subplot(2,2,1); plot(z, Temperature1, '-rs'); grid
set(gca,'FontName','Times New Roman','FontSize',12);
axis([0 53*10^(-3) 22 23.5]), % Sets up the range of variables to display
title ('Initial temperature distribution across the cell'), xlabel
('Distance z, m'), ylabel ('Temperature, deg.(C)'), legend('t =0
s',2),
hold on; subplot(2,2,2); plot(z, Temperature2, '-bd'); grid
axis([0 53*10^(-3) 22 23.5])
title ('Temperature evolution across the cell');
xlabel ('Distance z, m');
ylabel ('Temperature, deg.(C)'); legend('t =1200 s',2);

subplot(2,2,3); plot(z,Temperature3,'-go'); grid
axis([0 53*10^(-3) 22 23.5])
title ('Temperature evolution across the cell');

xlabel ('Distance z, m'); ylabel ('Temperature, deg.(C)'); legend('t =3600 s',2);

subplot(2,2,4); grid plot(z,Temperature4,'-mx'); grid
axis([0 53*10^(-3) 22 23.5]);
title ('Final temperature distribution across the cell');

xlabel ('Distance z, m');
ylabel ('Temperature, deg.(C)'); legend('t = 7200 s',2);

hold off;

Matlab 7.1,
End of file - ScriptCyl.m

File - Cyl.m
function y=Cyl1(z,t)


t1=0.0;% Time passed, s
z=0:0.0001:0.053;%z-coordinate range, m
l=53*10^(-3);%Length of cylinder,m
k=0.127*10^(-6);%Thermal diffusivity for water, m2/s
T11=0; T0=22; T1=23.5;

n=1;

while n <100000
    Lambda=k*(((n*pi)/l)^2);
    T11=T11+((-1)^(n+1)/n)*sin((pi*n*z)/l)*exp(-Lambda*t1);
    n=n+1;
end

y=T0+((T1-T0)/l)*z+2*T11*(T0-T1)/pi;

%End of file - Cyl.m
%File - Cyl2.m
function w=Cyl2(z,t)

%clear all;
t2=1200;% time passed, s
z=0:0.001:0.053;%z-coordinate range, m
l=0.053;%the length of cylinder,m
k=0.127*10^(-6);%Thermal diffusivity for water, m2/s
T11=0; T0=22; T1=23.5;

n=1;
    while n <100000
        Lambda=k*((n*pi)/l)^2;
        T11=T11+((-1)^(n+1)/n)*sin((pi*n*z)/l)*exp(-Lambda*t2);
        %+((-1)^(n+1)-1)/(n*pi)*2*cos((pi*n*z)/l)*exp(-Lambda*t2);
    end
    n=n+1;
end
w=T0+((T1-T0)/l)*z+2*T11*(T0-T1)/pi;

%End of file - Cyl2.m
Appendix B

Mathematica applications

B.1 Determination of the focal zone

Axial dependence of the transducers’ radiation fields (November, 2003)

Clear All;

a1 = 6.14/2; (*Radius of transducer, cm-> 120 kHz*)
a2 = 2.54/2; (*Radius of transducer, cm-> 1 MHz*)
a3 = 1.9/2; (*Radius of transducer, cm-> 3.5 MHz*)

(Print["Sound speed in water c = ", 1483, " m/s"); (*Print["Sound speed in the clotted blood c = ", 1590, "m/s"]*)

lambda1 = 1.23; (*Wavelength of 120 kHz acoustic wave in cm*)
k1 = 2*Pi/lambda1; Print["Wavevector 1 (120 kHz) = ", k1, " cm\(^{-1}\)");

lambda2 = 0.149; (*Wavelength of 1 MHz acoustic wave in cm*)
k2 = 2*Pi/lambda2; // N Print["Wavevector 2 (1 MHz) = ", k2, " cm\(^{-1}\)");

lambda3 = 0.042; (*Wavelength of 3.5 MHz acoustic wave in cm*)
k3 = 2*Pi/lambda3; Print["Wavevector 3 (3.5 MHz) = ", k3, " cm\(^{-1}\)");

d1 = Plot[Sqrt[2 - 2*Cos[k1*((Sqrt[((a1^2 + x^2)]) - x))]], {x, 0, 80},
      PlotStyle -> {Hue[.35], Thickness[0.0045]}, GridLines -> Automatic,
      AxesLabel -> {"Distance, mm", "Pressure"}];
d2 = Plot[Sqrt[2 - 2*Cos[k2*((Sqrt[((a2^2 + x^2)]) - x))]], {x, 0, 150},...
`PlotStyle -> {Hue[.85], Thickness[0.0035]}, GridLines -> Automatic, AxesLabel -> {"Distance, mm", "Pressure"}] ;
d3 = Plot[Sqrt[2 - 2*Cos[k3*((Sqrt[(a3^2 + x^2)]) - x))], {x, 0, 80},
PlotStyle -> {Hue[.6], Thickness[0.0003]}, GridLines -> Automatic,
AxesLabel -> {"Distance, mm", "Pressure, a.u."}] ;
Show[d1, d2, d3, PlotRange -> {0, 2.3}];

B.2 Calculation of the thermal elevation in human clot

<< Graphics'Legend'

Clear All;
L = 6.7*10^-3; (* Diameter of a clot, m *)
a = L/2;

b21 = 0.09*10^-2; (* Attenuation in a human clot at 0.12 MHz, 1/m *)
b22 = 0.17*10^-2; (* Attenuation in a human clot at 1 MHz, 1/m *)
b23 = 0.23*10^-2; (* Attenuation in a human clot at 3.5 MHz, 1/m *)

gam12 = ((1 - Exp[(-2)*L*b21]))/((2*L*b21)); (* Spatial average intensity due to decline of US in a clot *)

gam22 = ((1 - Exp[(-2)*L*b22]))/((2*L*b22)); (* Average intensity due to decline of US in a clot *)

gam32 = ((1 - Exp[(-2)*L*b23]))/((2*L*b23)); (* Average intensity due to decline of US in a clot *)

Intens21 = 0.4*10^-4*gam12; (* Spatial peak temporal average ultrasonic intensity at 80% DC, W/m^2 *)

Intens22 = 0.4*10^-4*gam22; (* Spatial peak temporal average ultrasonic intensity at 80% DC, W/m^2 *)

Intens23 = 0.4*10^-4*gam32; (* Spatial peak temporal average ultrasonic intensity at 80% DC, W/m^2 *)

ro = 1.08*10^-3; (* Density of human clots *)
\[ C_v = 3.48 \times 10^{-3}; \]

\[ \kappa = 0.16 \times 10^{-6}; \quad (\text{Thermal diffusivity of a blood clot, m}^2/\text{s}) \]

\[ K = 0.59; \quad (\text{Thermal conductivity of human clots, W/m K}) \]

\[ \text{Temp91}_t := \frac{(b21 \cdot \text{Intens21} \cdot a^2)}{K} \left(1 - \text{Erf}\left[\frac{a}{2 \sqrt{t \cdot \kappa}}\right]\right) - \frac{(2 \cdot \sqrt{\kappa})}{a \sqrt{\pi}} \cdot Sqrt[t] \cdot \text{Exp}\left[-\frac{a^2}{4 \cdot t \cdot \kappa}\right]; \]

\[ \text{Temp92}_t := \frac{(b22 \cdot \text{Intens22} \cdot a^2)}{K} \left(1 - \text{Erf}\left[\frac{a}{2 \sqrt{t \cdot \kappa}}\right]\right) - \frac{(2 \cdot \sqrt{\kappa})}{a \sqrt{\pi}} \cdot Sqrt[t] \cdot \text{Exp}\left[-\frac{a^2}{4 \cdot t \cdot \kappa}\right]; \]

\[ \text{Temp93}_t := \frac{(b23 \cdot \text{Intens23} \cdot a^2)}{K} \left(1 - \text{Erf}\left[\frac{a}{2 \sqrt{t \cdot \kappa}}\right]\right) - \frac{(2 \cdot \sqrt{\kappa})}{a \sqrt{\pi}} \cdot Sqrt[t] \cdot \text{Exp}\left[-\frac{a^2}{4 \cdot t \cdot \kappa}\right]; \]

\text{Plot}\left[\{\text{Temp91}_t, \text{Temp92}_t, \text{Temp93}_t\}, \{t, 0, 300\}\right];

\text{PlotLegend} \rightarrow \{"120 \text{ kHz}", "1 \text{ MHz}", "3.5 \text{ MHz}"\};

\text{PlotStyle} \rightarrow \{\text{Hue}[0.012], \text{Hue}[0.05], \text{Hue}[0.41]\};

\text{LegendPosition} \rightarrow \{.9, (-.45)\};

\text{LegendLabel} \rightarrow \text{"Frequency"};

\text{LegendOrientation} \rightarrow \text{Vertical};

\text{Frame} \rightarrow \text{True};

\text{FrameLabel} \rightarrow \{"Time (s)", "Temperature elevation, C", "Heat conduction losses in human clots (sphere)"\};

\text{TextStyle} \rightarrow \{\text{FontFamily} \rightarrow \text{"Times"}, \text{FontSize} \rightarrow 14\};

\text{GridLines} \rightarrow \text{Automatic};

\text{Print["\\n"]};

\text{Print["Temperature rise at the center of human clots at 120 kHz = ", \text{Temp91}[300] // N, " C"]};

\text{Print["Temperature rise at the center of human clots at 1 MHz = ", \text{Temp92}[300] // N, " C"]};

\text{Print["Temperature rise at the center of human clots at 3.5 MHz = ", \text{Temp93}[300] // N, " C"]};

\text{Print["\\n"]};
Bibliography


