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Current Rheological Blood Monitoring Systems: Potential of a Piezo-based Measuring Method as a Haemostasis Monitoring System Compared to a Rotational Rheometer

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Abstract:
In modern intensive care a comprehensive solution for monitoring the coagulation status or blood clotting problems is currently not available, because fast reliable detection of all bleeding-based disorders (coagulation, fibrinolysis, platelet function) cannot be conducted with a single medical device. This situation calls for a comprehensive technical solution, which we think possible to be solved with a rheological piezo-based system. Rheological measurements provide valuable information on the viscoelastic properties of complex fluids. Here, we compared the performance of a commercially available rheological industrial device using shear stress (Kinexus Pro, Malvern) with that of a piezo-based research measuring system (piezoelectric axial vibrator, PAV) applying squeeze flow to sample fluids. Comparative measurements using different xanthan concentrations (0.1 to 5 %) were carried out at 25 and 37 °C. At higher concentrations (1, 2, and 5 %), there was an overlapping frequency range and a consistent range of the viscous and elastic shear viscosity for both systems, allowing direct comparisons. Specifically the lower concentrations of 0.1, 0.2, and 0.5 % xanthan could be used to assess the possibility of both systems to measure blood coagulation, as those concentrations correspond approximately to the viscosity of human blood. Measurement of blood coagulation was then also tested with the PAV. Measurement repeatability was assessed performing blood coagulation measurements over time at different frequencies (10, 100, 300, and 1000 Hz). The middle frequencies of 100 and 300 Hz provided the most repeatable results for blood. Afterwards the activated clotting time (ACT) was performed with PAV at 300 Hz. The piezo-based measuring system was able to differentiate between various heparin blood concentrations (1, 2, and 3 IU/ml). In this study the reliability, repeatability and limitations of the piezo system were examined. Our initial results showed that the piezo system can be used to assess blood coagulation, but further studies are necessary to confirm these promising results. The aim of a fast, small and reliable point-of-care system may be possible with this type of rheological device.

Key words:
Rotational rheometry, piezo-based measuring method, piezoaxial vibrator (PAV), haemorheology, blood coagulation

1 INTRODUCTION

Almost one million heart surgeries involving the use of a heart-lung machine are performed annually worldwide [1]. The contact of blood with foreign surfaces can lead to life-threatening coagulation, which is prevented by using anticoagulants such as heparin [2]. The external blood flow during surgery is named extracorporeal circulation (ECC) and encompasses several methods such as extracorporeal membrane oxygenation (ECMO), extracorporeal life support (ECLS), dialysis and ventricular assist devices (VAD) [3]. The ECMO and ECLS respectively provide cardiac and respiratory support. The ventricular assist device is a medical pump that is used to support weakened hearts [4]. Hence, during and after ECC, which can last several hours, continuous monitoring of the coagulation status is necessary to prevent bleeding or thrombosis [5]. Modern intensive care does not currently provide a comprehensive solution for monitoring of the coagulation status or blood clotting problems, because fast reliable detection of all bleeding-based parameters (coagulation, fibrinolysis, platelet function) cannot be conducted with

a single medical device. The effect of ECC on the haemostatic system and appropriate anticoagulation management remain important research topics [3].

A system that is able to perform fast and complex analyses of the haemostasis system with low or no sample preparation near the patient (point-of-care) is thus required. A possible solution to this problem is rheometry. Rheometric techniques provide important information about the microstructure and dynamics of complex fluids such as polymer solutions and suspensions of colloid particles. Human whole blood can be regarded as a complex suspension of various cell types and proteins that is subject to continuous variations. Human whole blood can be studied as a Newtonian as well as a non-Newtonian fluid, and mathematical models have shown a correlation between haematocrit and haemodynamic factors [6, 7]. There are many different ways to measure rheological properties, each with its own advantages and limitations. In the following section, a concise overview of relevant methods is presented.

2 OVERVIEW OF CURRENT RHEOLOGICAL BLOOD MEASURING SYSTEMS

One microrheological method is fluorescence correlation spectroscopy (FCS). Microrheology can be performed through active manipulation of the sample by applying external forces like magnetic or electric fields or through passive manipulation, for instance by introducing tracers. The passive microrheological FCS uses a confocal microscope where a sample is irradiated by laser light. The microscope monitors the spontaneous fluctuations of the fluorescence signal stemming from the movement of fluorescently labelled particles. The strength and duration of this fluctuation can be quantified by correlation with the recorded signal intensity [8]. The rheological properties of materials can be investigated using this method, but it needs large laboratory equipment and therefore cannot be used next to a patient during extracorporeal circulation. An acoustic wave viscosimeter involves an in-line measurement that integrates the sensor into the measurement process without active interference. Acoustic shear waves are directed through the sample by a quartz crystal resonator and detected on the other side by a quartz crystal sensor. The measuring system is robust against vibration and shock, because there are no moving parts in the in-line system. Furthermore this system can measure the dynamic viscosity under laminar and turbulent flow [9,10]. However, for blood analysis, differentiation between the viscous and elastic modulus is necessary to obtain more information about the viscoelasticity of blood during extracorporeal circulation.

The following rheological methods may be even better suited to detecting blood coagulation. Cavitation rheology investigates the rheological properties of red blood cells. A laser (wavelength of 532 nm) is focused on the sample for 6 ns. Due to the high light intensity at the focal point an expanding bubble is formed (cavitation). These bubbles reach a diameter of 90 – 120 μm before collapsing. The red blood cells are deformed due to the elongation and collapse of the bubbles. The elasticity of the red blood cells can be determined by the time taken to recover the original shape of the blood cells [11,12]. This method can be applied to study the properties of blood cells but it cannot be used for haemostasis monitoring. Sonorheometry generates an acoustic radiation force that is directed onto a 1 ml whole blood sample to induce small localized displacements within the sample. The resulting displacements are detected as shifts in the returning echoes. With the help of an ultrasound motion tracking system the displacements are quantified and indicate the viscoelastic properties [13,14]. Sonorheometry is a research method that is currently being tested in a clinical trial at the University of Virginia. This system will be compared to existing coagulation monitoring technology in heart surgery and will be tested for use in surgical and intensive care settings [15]. In free oscillation rheology (FOR) the time taken to start coagulation can be determined by the clotting onset time (COT). For this purpose, citrated blood or plasma at 37 °C is added to a free oscillating cuvette. The oscillation is initiated every 2 seconds by a magnetic field with a frequency of 11 Hz. An optical detector registers the damping D and frequency F of the container. During the coagulation process, the damping increases and the frequency decreases. Therefore, the endpoint of coagulation can be detected by a change in the elasticity [16, 17]. This system is similar to thromboelastography (TEG and Rotational Thromboelastometry ROTEM), but free oscillation rheology is not a widely used method in investigation of coagulopathy in bleeding patients [18].

Each presented method has its advantages and disadvantages and none can be used as a point-of-care system without modifications. This calls for a comprehensive technical solution, which we intend to solve with a rheological piezo-based system. For comparison, an oscillatory rheometer was used. Oscillatory rheometers can be used to measure within the linear viscoelastic region, where a fluid can be reversibly deformed without being destroyed. As an example a sample is placed between two plates or a plate and a cone. The geometry is selected based on the properties of the sample. Homogeneous samples or dispersions with a certain particle size ($D_{max} \leq 0.1\ d$ with D_{max} particle size and d measuring gap) are measured using the cone/plate system, otherwise the plate/plate system is used [19]. In the

commercially available rheometer used here, the upper plate or cone rotates in an oscillatory fashion while the sample is sheared. Measurements can be taken in two measurement modes that provide all viscoelastic parameters (complex shear modulus G^* as well as storage G' and loss modulus G''). The first mode is the deformation-controlled measurement, whereby the rheometer applies a sinusoidal angular displacement to the sample. In the second mode (shear stress controlled), the system applies a sinusoidal torque [19]. The rotational rheometer 'Kinexus Pro' (Malvern Instruments GmbH Herrenberg, Germany) was used in this work as a commercially available reference device. Samples can be measured over frequency (up to 100 Hz) or over time. At low frequencies the low torque signal-to-noise level limits the measurement. At higher frequencies inertia effects dominate the measurement [20, 21].

Since samples are often used at different application frequencies (shear rates) it is necessary to characterize the viscoelastic behavior of a wider range of frequencies. Therefore, a piezo device called piezoelectric axial vibrator (PAV) was developed and introduced by Pechhold et al. [22]. The linear viscoelasticity of soft matter fluids such as blood and low viscosity polymeric solutions can be characterized by the PAV at higher frequencies than currently used with other rheometers (1 to 10000 Hz). The PAV is based on a rheometric measurement method that enables detection of the viscoelastic properties using a piezoelectric sensor (Figure 1). The lid of the PAV is fixed on the bottom plate and the system is hermetically closed. The bottom plate is moved by an excitation piezo and a periodically squeeze flow of the sample is created inside of the measuring chamber. The deformation of the sample is measured by the voltage of a detection piezo which is used for further analysis (described subsequently). Before starting a sample measurement, an empty measurement (frequency scan of 1 to 10000 Hz) should be performed to give a reference reading U_o. To gain U_o, the voltage of the detection piezo and the sinus phase displacement between excitation piezo and detection piezo are measured. Afterwards, the same measurement is performed for the sample to obtain U and phase displacement. Only if the ratio U/U_o is smaller than 1, the system can differentiate between unloaded and sample measurement. By calculating the ratio U/U_o and the phase displacement of the sinus curve, both G^* and η^* respectively can be determined. As outlined by Kirschenmann [22] it is written that by the help of a mass spring system the equations of motion of the PAV are described in a point mechanical approximation. This approximation allows the correlation between the ratio of the complex voltage U/U_o and of the complex spring constant K^*. For linear viscoelasticity the complex squeeze stiffness K^* can be calculated (Equation 1):

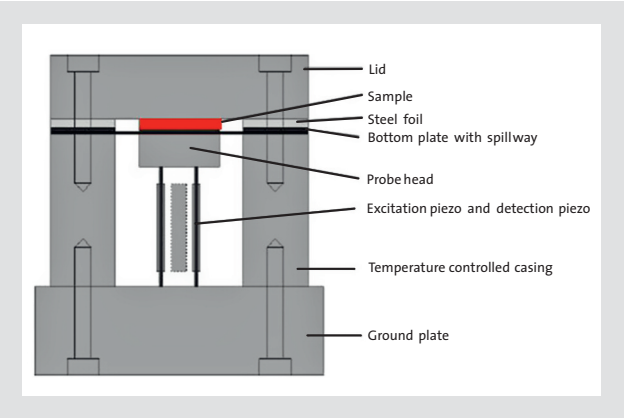

Figure 1: Schematic drawing of the PAV modified after Kirschenmann [22].

$$\frac{1}{G^*} = \frac{3\pi}{2}\frac{R^4}{d^3}\frac{\left(1+\frac{\rho\omega d^2}{10G^*}+\ldots\right)}{K^*} \tag{1}$$

In this equation the radius R and the gap width d of the measuring chamber, complex squeeze stiffness K^*, inertia term $(1 + (\rho\omega^2 d^2)/(10G^*) + \ldots)$ and complex shear modulus G^* are used. The dependence of the geometry is visible in this equation. It explains the need for using calibration oils to adjust the viscosity, before using a new gap width, because a doubling in gap width would change K^* (complex spring constant of the sample) by approximately the eightfold [22]. Pechhold et al. used continuum mechanical calculation to obtain a correlation between the complex spring constant and the complex shear modulus G^*. With these calculations the correlation between the ratio of complex voltage U/U_o and the complex shear modulus were obtained (Equation 2 to 5 shows the conversion from complex shear modulus to complex shear viscosity):

$$G^* = i\omega\eta^* \tag{2}$$

$$G^* = G' + iG'' \tag{3}$$

$$\eta^* = \eta' - i\eta'' \tag{4}$$

$$\omega = 2\pi f \tag{5}$$

with complex shear viscosity η^* and angular frequency ω [22, 24]. The purpose of the current study was to investigate the reliability and repeatability of the PAV and its possible limitations. To enable adaption of the piezo system for medical application a comparison between the PAV and the commercial reference device Kinexus Pro was performed. The PAV should provide convenient, reliable access to the viscoelastic properties of complex fluids like human whole blood. In this experimental work, the PAV was used for measurements at higher frequencies and the rotational rheometer (Kinexus Pro, Malvern)

for those at lower frequencies. The performance of the systems was compared using a viscoelastic sample solution (xanthan). Finally, the ability of the piezo system to detect human whole blood coagulation was investigated using two simple coagulation tests (Quick prothrombin time (PT), activated clotting time (ACT)).

3 MATERIALS AND METHODS

3.1 ROTATIONAL RHEOMETER

Using Kinexus Pro a shear stress-controlled measurement (0.01 – 20 Pa) was performed at different frequencies in order to verify which shear stress is located in the linear viscoelastic region (LVR). A shear stress of 0.04 Pa and the resulting frequencies from 12.6 to 0.1 Hz in ten steps per decade were determined as the optimum compromise between device errors (torque and inertia effects) and measurement time in preliminary experiments. In all measurements, the 1 °/40 mm stainless steel cone geometry was used together with a lower plate made of anodized aluminium. Comparative experiments with a stainless steel plate instead of the aluminium one did not yield diverging results. In order to prevent drying of the sample a solvent trap was used. Air bubbles in the sample were avoided by a carefully application. For each measurement (n = 10, independent measurements) 340 μl of sample was used.

3.2 PIEZOELECTRIC AXIAL VIBRATOR

A frequency scan of 1 to 10000 Hz was performed using PAV. After taking the unloaded measurement, the device was re-opened, a sample (volume range 10 – 200 μl) was placed on the bottom plate and the lid was carefully fixed in place with a defined pressure. Air bubbles in the sample were avoided. The complex shear viscosity of the sample η^* was determined from the difference between the loaded and the unloaded probe. The measuring gap was adapted using different thicknesses of foil and was chosen according to the viscosity of the sample, such that the thickness varied from 10 to 50 μm. A set of ten measurements (n = 10, independent measurements) was performed with each concentration of xanthan solution at 25 and 37 °C.

3.3 SAMPLE FLUIDS

3.3.1 Calibration fluids

Calibration liquids were used to evaluate basic settings of the PAV at 25 °C. The types of liquids and their viscosity are listed in Table 1. The calibration fluid B210 was also used to verify the reliability of the Kinexus Pro.

3.3.2 Xanthan

Xanthan (Sigma-Aldrich, St. Louis, USA) solutions of various concentrations were used to perform comparative measurements between the Kinexus Pro and PAV. The solutions were prepared by dissolving various masses of xanthan in water (Ampuwa, Fresenius Kabi) to yield the following concentrations in weight percent (0.1, 0.2, 0.5, 1, 2, and 5 %). The solutions were left at room temperature to swell with occasional shaking for 24 h. The higher concentrations (≥ 0.5 %) were then centrifuged (Varifuge 3.2 Rs, Thermo Scientific, USA) for 5 minutes at 20 °C and 100 × g to remove air bubbles. The dilute concentrations were applied to the devices with a pipette, the higher concentrated solutions with a spatula or a cut pipette tip respectively. The measurements with the PAV were done with 150 μl of sample and a 10 µm gap for the 0.1 and 0.2 % xanthan solution, and a 25 μm gap for the 0.5 % xanthan solution. For 1 and 2 % xanthan a 35 μm gap was used, whereas a 50 μm measuring gap was used for the 5 % solution.

3.3.3 Blood

Human whole blood was collected by venipuncture in citrate monovettes (S Monovette 3 ml 9NC, Sarstedt AG, Nümbrecht). Citrated blood was chosen because it is often used in haemostasis monitoring. Trisodium citrate (0.106 mol/l) removes the calcium ions in the human whole blood to inhibit the coagulation process. By suitable selection of activators and anticoagulants, coagulation can be selectively activated or inhibited. In the following section an activator is presented that interferes with the coagulation cascade and induces a three-dimensional network of fibrin fibers and activated platelets.

3.3.4 Thromborel S

Thromborel S (Siemens Healthcare Diagnostics Inc., Marburg Germany) consists of lyophilized thromboplastin from the human placenta (≤ 60 g/l), calcium chloride (about 1.5 g/l), stabilizers and preservatives. It is used to determine the prothrombin time *PT* according to Quick, which shows the ability of the extrinsic coagulation sys-

Sample	Viscosity [mPas]
B210 *	202.8
B31 *	29.24
PMX 200 Xiameter	5.0
PDMS 50 Fluorochem	50.0
Glycerol (85 vol.%)	82.95

Table 1: Calibration fluids with dynamic viscosity at 25 °C. Viscosity values were obtained from the certified viscosity standard calibration sheet (Brookfield certified viscosity standard).*

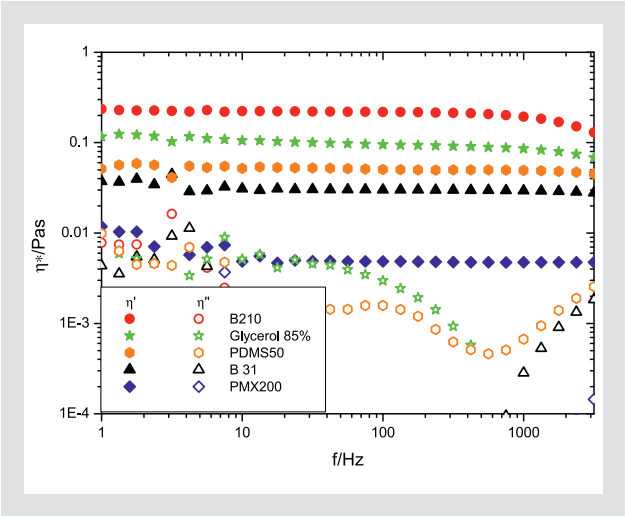

Figure 2: Calibration measurements with the PAV device at 25°C and a 25 µm measuring gap. B210, B31, PMX200, 85 % glycerol and the silicone oil PDMS 50 were used as calibration solutions. The filled symbols are the viscous component and the open symbols represent the elastic component. The drop in viscosity at higher frequencies (> 1000 Hz) for liquids with higher absolute values such as B210 was not within the measurement range when using the specified measuring gap. The lower viscosities (PMX200, PDMS50) at lower frequencies (1–10 Hz) were also out of range (no differentiation between complex U/U_o).

tem to measure the activities of the coagulation factors II, V, VII, and X [25]. When used according to the manufacturer's instructions to determine the *PT* (two parts Thromborel S mixed with one part citrated blood), the blood is strongly diluted and the rheological properties are altered. Therefore, Thromborel was redissolved in a fifth of the recommended volume (2 ml) to achieve a higher concentration and thus a smaller volume of the activator could be added to the blood, keeping the rheological properties closer to the physiological situation.

3.3.5 ACTIVATED CLOTTING TIME (ACT) – KAOLIN

During Extracorporeal circulation (ECC) human whole blood is heparinised and routinely monitored with the activated clotting time (ACT). Heparin is a polydisperse, negative loaded sulfated mucopolysaccharid, which is used to inhibit the coagulation process. Heparin is dosed in "international units" to realise a reproducible pharmacological effect which was defined by the world health organisation (WHO). In combination with the platelet count, statements about the intrinsic coagulation system and about disseminated intravascular coagulopathy (pathological use of coagulation factors) can be made. Kaolin is the surface activating substance and was used as coagulation activator for this study [26].

3.4 STATISTICAL ANALYSIS

Data are presented as means with standard deviation (SD). Normally distributed data were analysed using One Way ANOVA with Bonferroni's multiple comparison test to analyse differences between groups. Non-normally distributed data were analysed using a non-parametric test (Kruskal-Wallis test with Dunn's multiple comparison test). Statistical significance of each concentration of the respective viscous and elastic component was defined as $p < 0.05$. All statistical analyses were performed using GraphPad Prism (Version 5, GraphPad Software, La Jolla, USA). Further diagrams were drawn with the help of Origin Pro 8 (Origin Lab Corporation).

4 RESULTS AND DISCUSSION

4.1 CALIBRATION OF PIEZOELECTRIC AXIAL VIBRATOR

In order to guarantee the correct performance of PAV an initial calibration was carried out before performing further measurements. Kinexus Pro measurement accuracy was also tested with a calibration oil (B210). To demonstrate that the PAV measures correctly, the 25 °C measurements were presented first, because there are much more viscosity data available for calibration fluids at 25 °C. Afterwards the measurements at 37 °C (body temperature, needed later for blood measurements) were performed and compared to literature values. For the PAV different calibration oils and liquids of known viscosity at 25 °C were used: B210, B31 (both carboxylic oils), glycerol 85 vol. %, silicone oil PMX 200, and silicone oil PDMS 50 (polydimethylsiloxane). Figure 2 shows the calibration performed with a measuring gap of 25 μm according to Kirschenmann [22]. Several calibration oils were measured by varying individual parameters (e.g. gap thickness) to adjust the viscosity. The PAV applies very small deformations (< 5 nm) and therefore all subsequent measurements with xanthan and blood were in the linear viscoelastic region. The same calibrated parameters (according to Kirschenmann) for 25 °C were used to measure samples at 37 °C (e.g. Glycerol 85 % η^* = 40 mPas [27] and 10BW η^* = 5.86 mPas, viscosity value from calibration sheet). The results showed (data not shown) that the calibration parameters for that temperature could also be used at 37 °C. At low frequencies (1 to 10 Hz), the low viscosity oils (PMX200, PDMS50) that were measured with a measuring gap of 25 μm at both temperatures (25 and 37 °C) were out of range, i.e. complex U/U_o was close to 1. The PAV could therefore not differentiate between loaded and unloaded measurements, which created a strong measurement uncertainty and unusually high statistical spread. Therefore, as seen in preliminary experiments, lower measuring gaps such as 10 μm provided more reliable results. The small measuring gap however could not be used for blood without affecting the tender fibrin network built during clotting process. The higher viscosity samples (B210 and Glycerol 85 %) showed a

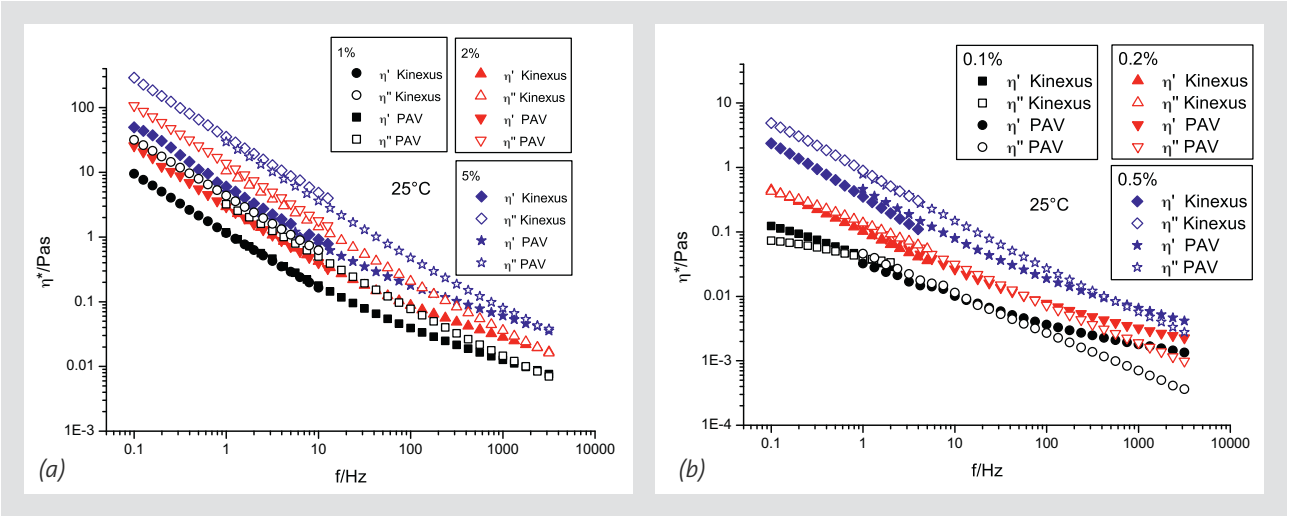

Figure 3: The frequency dependence of different xanthan concentrations (a) 1, 2, 5 % xanthan and b) 0.1, 0.2, 0.5 % xanthan measured using Kinexus Pro and PAV at 25°C. The mean of viscous and elastic component (n = 10, independent measurements) of each concentration is shown in this diagram. Measurements at lower frequencies were made by the Kinexus, and at higher frequencies by PAV. All measurements were in the linear viscoelastic region (LVER) (Filled symbols = viscous component, unfilled symbols = elastic component).

decline in viscosity readings at higher frequencies (> 1000 Hz) that was not within the measurement range at 25 μm, because the sample is too stiff at higher frequencies at that measuring gap (Figure 2). To obtain higher frequency ranges (> 1000 Hz) for B210, higher measuring gaps such as 35 or 50 μm should be used.

4.2 FREQUENCY SPECTRUM OF KINEXUS AND PAV

After initial calibration of the PAV according to Kirschenmann, the performance of the commercial rheological device (Kinexus, Malvern) was compared with the piezo-based research measuring system (PAV). First, some xanthan solutions were measured over time to prove the stability of the xanthan solutions at a certain frequency. The higher concentrations of 2 and 5 % xanthan had slight changes in viscosity after being transferred to the measurement chamber. Therefore, these concentrations were held in the measuring chamber for 30 min to allow material relaxation before starting measurement. Figure 3 shows the frequency spectrum of the different xanthan concentrations at 25 °C and Figure 4 shows the frequency spectrum at 37 °C. For comparative purposes the results of both devices are depicted in the same figure. The Kinexus Pro measured low frequencies whereas the PAV measured the high frequencies. In both figures the mean result (n = 10 independent measurements) for the respective concentrations is shown. The higher concentrations of 1, 2, and 5 % xanthan (Figure 3a) were tested first. At these concentrations, the data points of the viscous component of both Kinexus and PAV were overlapping within a certain frequency range. The same was true for the elastic component. Then the tenfold less concentrated xanthan solutions (0.1, 0.2, and 0.5 %) were examined (Figure 3b). The measurements recorded by both devices at the three concentrations also had an

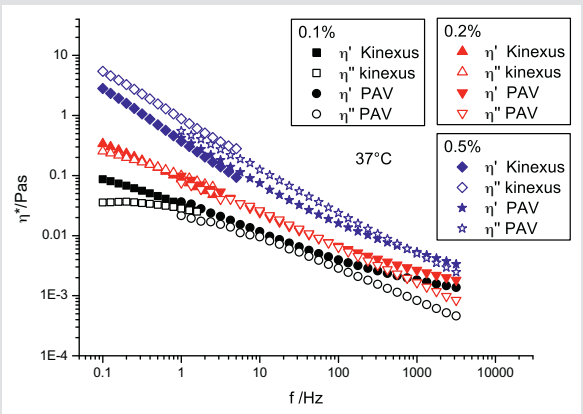

Figure 4: The frequency dependence of different xanthan concentrations: 0.1, 0.2, and 0.5 % xanthan at 37°C measured using Kinexus Pro and PAV. The mean of viscous and elastic component (n = 10, independent measurements) of each concentration is shown in this diagram.

overlapping range. Since the blood measurements were carried out at 37 °C, measurements with xanthan were performed at this temperature in addition to the experiments at 25 °C to demonstrate repeatability (Figure 4). At 37 °C only 0.1, 0.2, and 0.5 % xanthan were examined. These three xanthan solutions were comparable with the viscosities of freshly drawn blood (50 Hz, η^* = 0.003 Pas [28]) or blood during and after the coagulation process. This suggests that the xanthan solutions are appropriate to simulate the viscosity of blood at its diverse clotting stages. Furthermore, these concentrations provided comparable results using the Kinexus Pro and PAV within their overlapping frequency ranges, i.e. the viscous component of the Kinexus Pro and the viscous component of the PAV had a consistent range. For the elastic components of both devices the

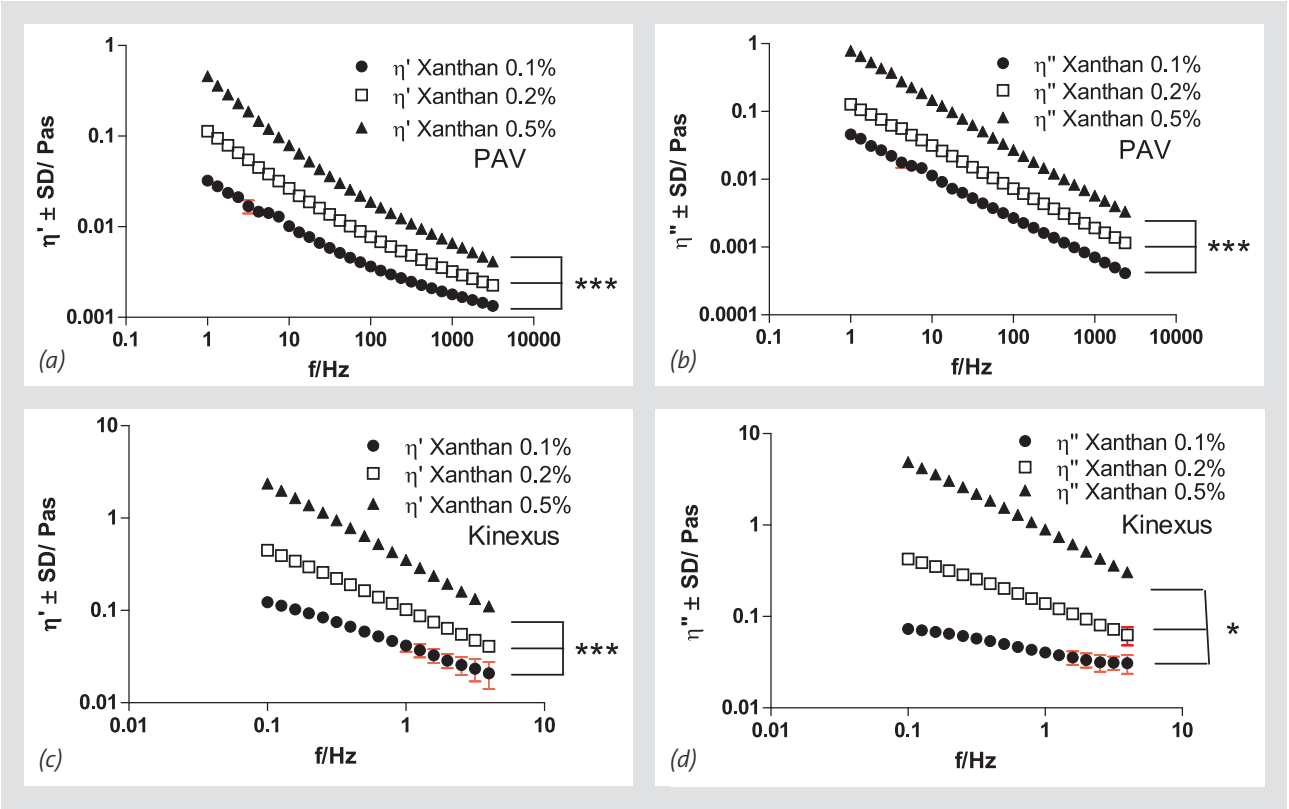

*Figure 5: Measurements of various xanthan solutions at 25 °C. The viscous and elastic component were analysed using both Kinexus Pro and PAV. The mean of viscous and elastic component (n = 10, independent measurements) of each concentration is shown. Significance of each concentration of the respective viscous or elastic component was set at * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

same phenomenon was observed. In summary, the viscous components of the measurements obtained by PAV and Kinexus and also their respective elastic components had overlapping data points within a certain frequency range. After calibration and the comparison of both devices at 25 and 37 °C, it can thus be concluded that PAV can measure reliably in that viscosity range.

4.3 STATISTICAL ANALYSIS OF THE VISCOUS AND ELASTIC COMPONENT OF DIFFERENT XANTHAN CONCENTRATIONS AT 25 AND 37 °C

The concentrations of 0.1, 0.2, and 0.5 % xanthan were chosen because the three concentrations had similar viscosity values as human whole blood during the coagulation process. First frequency sweeps were performed (n = 10 independent measurements) with different xanthan solutions (0.1, 0.2, and 0.5 %) on both devices. Afterwards, the elastic and viscous components of the performed sweeps were plotted separately at 25 °C (Figure 5) and the viscous component η' (η'' as elastic component, respectively) of those xanthan concentrations was compared at the various frequencies. For each individual frequency, the 10 data points from the different experiments were analysed for normal distribution. Having a normal distribution within one frequency, then the data points of the different concentrations were further analysed with a ONE-Way ANOVA and Bonferroini's multiple comparison post-hoc test for significance in elastic or viscous component. If data points of a frequency scan were not normally distributed the non-parametric Kruskalis-Wallis test with Dunn's multiple comparison post-hoc test was used to test for significantly different η' or η''. The significance of each concentration of the respective viscous or elastic component was defined as $p < 0.05$. The statistical significance therefore relates to the $\eta' \pm SD$ (η'', respectively) between concentrations at single frequencies. None of the analysed concentrations had overlapping data points within a frequency. With these analyses, we could show that both systems were able to measure significantly different elastic or viscous components over the used frequencies with the used xanthan concentrations. This was also true for measurements at 37 °C (Figure 6). For both devices, all concentrations yielded significantly different data for viscous or elastic components. In summary, the frequency scan showed that differentiation of the various xanthan concentrations was possible (at least at $p < 0.05$). Thus, all concentrations could be distinguished from each other.

4.4 REPEATABILITY MEASUREMENTS USING CITRATED BLOOD AND THROMBOREL WITH PAV OVER TIME

In order to investigate whether or not the PAV device is suitable as a haemostasis monitoring system, measurements were performed with citrated blood and Thromborel. Figure 7 presents an example of the coagulation

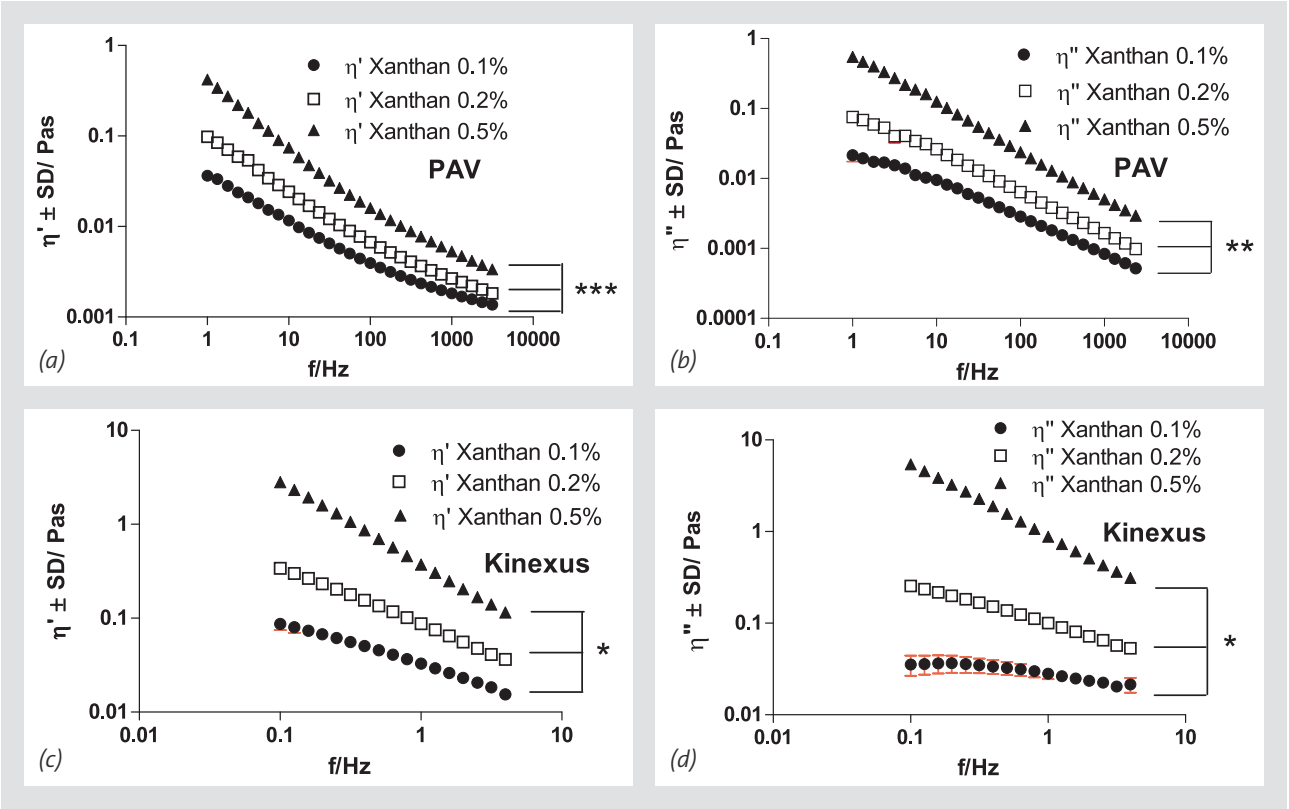

*Figure 6: Measurements with various xanthan solutions at 37 °C. The viscous and elastic component were analysed using Kinexus Pro and PAV. Significance of each concentration of the respective viscous or elastic component was set at * = p < 0.05, ** = p < 0.01, *** = p < 0.001.*

process at 300 Hz over time. The viscous and elastic components can be seen. Uncoagulated blood (dashed lines) showed a constant viscous (red) and elastic (green) component. Coagulation was initiated by adding Thromborel and η' and η'' increased over time. At approximately 100 s the curves of the viscous and elastic component still increase at a constant rate, but the slope is less as in the first seconds. The clotting time (*CT* = 18.5 s) was determined as the intersection between uncoagulated blood (red) and the slope of the viscous component. The determined clotting time in whole blood is in the normal range of 15 – 21 s (prothrombin time, *PT*) [29]. From that time point onwards, the calculated tan δ of elastic and viscous component was chosen as the measurement parameter instead of using the viscous and elastic components separately. Parameter tan δ provides information about the ratio of the viscous and elastic component (tan $\delta = \eta'/\eta''$), thus information about the relationship of liquid and solid proportions and which of those states predominates. If tan $\delta < 1$, the elastic component of the material is dominant. If tan $\delta > 1$, the viscous component is dominant. During blood coagulation, the state of the sample changes over time and with it the tan δ. It can thus be a sensitive parameter to detect blood coagulation.

To obtain more information about the coagulation process, four different frequencies (10, 100, 300, and 1000 Hz) were measured in a continuous cycle at 37 °C. A measuring gap of 25 μm was chosen because under such conditions the blood should have more space to

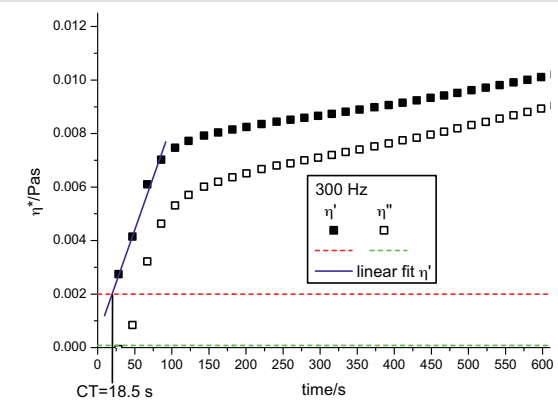

Figure 7: Example of measurement of citrated blood and Thromborel using a measuring gap of 25 µm at 300 Hz and at 37°C. The dashed lines represent the viscous (red) and elastic component (green) of uncoagulated citrated blood (obtained by own measurement at the same system). The addition of Thromborel led to an increase of the viscous and elastic component.

form a three-dimensional clot. The repeatability of the viscoelastic measurements of coagulated human whole blood was elucidated (Figure 8, *n* = 7, sample preparations from the same donor even on different days). This figure shows the mean values with their respective standard deviations. The figure shows that the largest deviations were observed at 10 Hz (*SD* = 115.68), because at the frequency with a measuring gap of 25 μm the

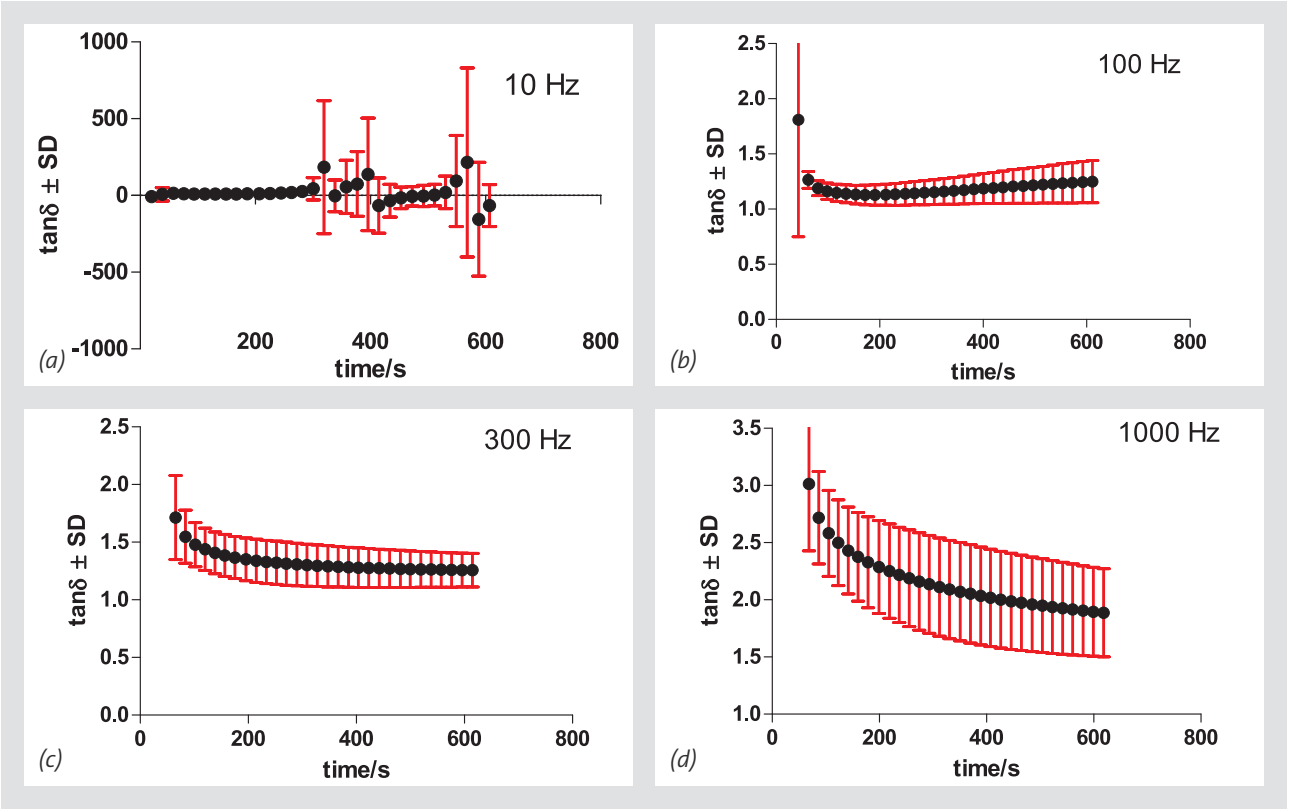

Figure 8: Repeatability measurements using citrated blood and Thromborel. A measuring gap of 25 µm was used for the measurements at 10, 100, 300, and 1000 Hz and at 37°C. The mean of tan δ (n = 7, number of sample preparations from the same donor even on different days) of each measuring point and the standard deviation are shown. The high standard deviation at 10 Hz indicates the lack of repeatability. The best results were obtained at 100 and 300 Hz, which gave a low standard deviation. A larger standard deviation was observed at 1000 Hz.

measurement was out of range (no differentiation between complex U/U_o). The most reliable results were obtained by using 100 and 300 Hz, because at these frequencies the standard deviations of the measurements were low ($SD_{(100Hz)}$ = 0.96, $SD_{(300Hz)}$ = 0.3), and thus had a better repeatability compared the other frequencies ($SD_{(10Hz)}$ = 115.68, $SD_{(1000Hz)}$ = 2.85). The highest frequency (1000 Hz) needs to be investigated further, because repeatability appeared to increase with higher frequency.

To compare the deviations between xanthan and citrated blood the coefficient of variation *v* was used. We defined biological measurements with a coefficient of variation smaller than 20 % as repeatable [30]. The coefficients of variation of xanthan (exemplary xanthan 0.5 %, 25 µm gap, $v_{(10Hz)}$ = 5.55 %, $v_{(100Hz)}$ = 1.02 %, $v_{(300Hz)}$ = 1.05 %, $v_{(1000Hz)}$ = 1.17 %) were lower than the coefficients of variation of human blood with thromborel ($v_{(10Hz)}$ = 480.39 %, $v_{(100Hz)}$ = 12.01 %, $v_{(300Hz)}$ = 13.45 %, $v_{(1000Hz)}$ = 20.45 %). In comparison to xanthan, measurements with citrated human whole blood had larger deviations. This is a typical observation with biological material, as in contrast to uniform xanthan solutions, blood has natural biological variability. Even when blood from the same donor is used repeatedly, there is day-to-day variability. Furthermore, the viscoelastic properties of human whole blood changed over time after the addition of the coagulation activator Thromborel. Altogether, these changes generally lead to larger variations when measuring blood samples. Important here is the observation that even with a higher variability of biological samples, the frequencies 100 and 300 Hz had a lower coefficients of variation than 10 or 1000 Hz and seem to be more suitable to measure blood coagulation.

4.5 VARIOUS HEPARIN CONCENTRATIONS WITH KAOLIN WITH PAV OVER TIME

Human whole blood is heparinised systemically during extracorporeal circulation and routinely controlled with the activated clotting time (ACT) to ensure patient safety. Figure 9 shows an ACT experiment with PAV at 37 °C. The calculated tan from elastic and viscous components is shown over time at 300 Hz. It can be seen that higher heparin concentrations led to later clotting time points. For example 3 IU/ml of heparin blood had the highest clotting time value of 306.5 s and reached a higher steady state tan δ value of 4 than heparin blood with 1 and 2 IU/ml. Freshly drawn blood from the neutral monovette without the use of a coagulation activator provided the highest clotting time with 552.4 s. Before starting an ECC, the ACT should have a value at least of 400 s (normal range about 80–120 s) [31]. In conclusion, the PAV is capable to differentiate between various heparin concentrations. The first results with the PAV are promising, but further experiments are

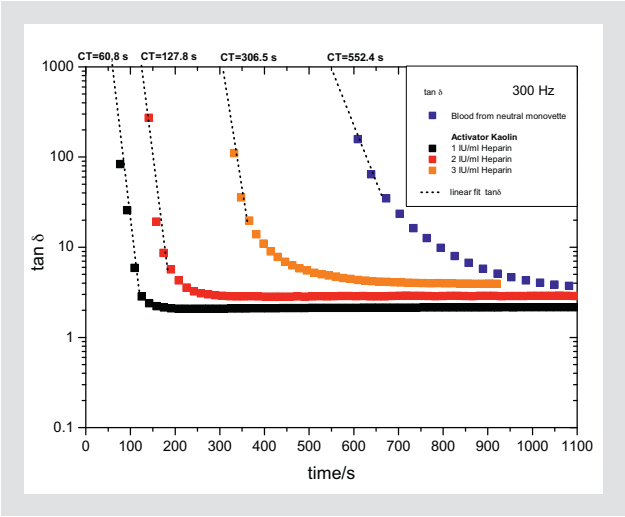

Figure 9: Comparison of tan δ and clotting time CT of freshly drawn blood with and without anticoagulation (heparin) and coagulation activator (kaolin). Freshly drawn human whole blood without coagulation inhibitor showed the natural coagulation process in absence of a coagulation activator (blue, with the longest coagulation time of 552.4 s). In comparison to that, blood was anticoagulated with various heparin concentrations and activated with kaolin. All measurements were performed at 37°C and 300 Hz. It can be seen that with increasing anticoagulation (1 - 3 IU/ml heparin) tan δ decreased at later clotting time points. At 3 IU/ml of heparin the clotting time was at 306.5 s (orange) and had a higher tan δ value of 4 than 2 IU/ml (tan δ = 3, red) and 1 IU/ml (tan δ = 2; black).

necessary to conclude if the piezo based measuring system has a potential as a future haemostasis monitoring system.

In clinical practice the thromboelastography (TEG) is used for sophisticated haemostasis monitoring. That system is able to show elasticity over time and provide many parameters such as clotting time and clot firmness [32]. The first experiments showed that PAV can provide the viscous and elastic component and the clotting time. Further studies are necessary to investigate if the PAV has a potential for haemostasis monitoring system. Overall, this study showed that the PAV was reliable, repeatable and comparable to a commercially available rheometer. The blood experiments showed that the coagulation of blood can be detected and various heparin blood concentrations can be differentiated by the piezo-based system.

5 CONCLUSIONS

After calibrating the PAV using conventional calibration fluids, its performance was compared with an industrial reference device (Kinexus Pro) and both systems provided comparable results. Different xanthan concentrations (0.1 – 5 %) were examined to investigate the comparability with 0.1, 0.2, and 0.5 % being chosen due to their similarity to various states of human whole blood. For instance, the viscosities of freshly removed blood or blood during and after the coagulation process were simulated by the xanthan solutions. Following the coagulation of citrated human whole blood with Thromborel, four frequencies (10, 100, 300, and 1000 Hz) were selected to prove the repeatability of the viscoelastic measurement. The lowest standard deviation was observed at 100 and 300 Hz. In future experiments only one frequency should be selected instead of measuring several frequencies in a continuous cycle (discrete frequency scan) to obtain faster results. The experiments showed that the activation of blood with Thromborel or with kaolin could be detected by the PAV device. It showed the potential of that measuring system, but these results stay preliminary and further experiments are necessary to confirm the promising results.

Advantages of the PAV is that small sample volumes (20 μl) can be used, the measuring chamber is hermetically closed to prevent an evaporation of the sample and results can be obtained faster by higher frequencies (> 100 Hz) than other rheometers. Further studies will be performed with blood activators or inhibitors to confirm the ability of PAV to detect blood coagulation. The ultimate goal is to provide a rheological system for the medical field that has the ability to perform a fast and complex analysis of the haemostasis system with low or no sample preparation near the patient (point-of-care).

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