AN AUTOMATED REAL TIME PHYSIOLOGICAL VISCOMETER

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ABSTRACT

Viscosity as hemorheological criteria has become far more important after the clinicians started to blame it for some pathological conditions. There is a bunch of study showing that the blood viscosity is apparently relevant with erythrocyte aggregation, endothelial damage, intimal thickening, coronary artery disease (angina pectoris), and diabetic ischemia. Besides, there is suspicion of strong association with sudden deafness, sudden vertigo, ischemic retinopaty, aseptic bone necrosis and many other diseases of ischemia.

Creating a handy and reliable viscosimeter will be very helpful for both researchers and clinicians that try to investigate the relation between elevated blood viscosity and some disorders. Blood viscosity information is also valuable in monitoring the patient's body reaction to medical treatment.

An Automated Real Time Viscometer with the microcapillary tubes was developed using reusable dome transducers, high gain, low noise, low frequency DC preamplifiers, an AD/DA converter card and a PC with appropriate software that can measure flow rate with small scale precision and compute the viscosity values at high speed.

The automated real time physiological viscometer is currently assembled in a laboratory room in the Department of Anatomy, İstanbul Medical Faculty - University of Istanbul, with the name of Viscosity Laboratory. It serves as a routine laboratory.

Preliminary results obtained indicate that the system can be used for creating reference values and monitoring any kind of diseases with a blood viscosity problem.

Keywords: Hemorhelogy, Blood Viscosity, Capillary Viscometer

OTOMATİK EŞZAMANLI FİZYOLOJİK VİZKOMETRE

ÖZET

Hemoreolojik bir kriter olan vizkozite, bazı patolojik durumlardan sorumlu tutulması ile klinik önem kazanmıştır. Kan viskozitesinin alyuvar agregasyonu, endotel yaralanması, intima kalınlaşması, koroner arter hastalıkları (angina pectoris), diabetik iskemi ile açıkca ilişkili olduğunu gösteren bir çok çalışma yapılmıştır. Ayrıca, ani sağırlık, ani baş dönmesi, iskemik retinopati, aseptik kemik nekrozu ve birçok başka iskemik hastalıkların da yükselmiş viskozite ile ilgili olabileceği konusunda çok güçlü şüpheler vardır.

Kolay kullanılabilir ve güvenilir bir vizkometre yapmak, bu hastalıklar ile yükselmiş kan viskozitesi arasındaki ilşkiyi araştıran klinisyen ve araştırıcılar için son derece yararlı olacaktır. Kan viskozitesi ile ilgili bilgi edinmek ayrıca tedavi gören hastanın tedaviye verdiği cevabın izlenmesi için de kıymetli olacaktır.

Tekrar kullanılabilen transduser, yüksek kazanımlı, düşük gürültülü , düşük frekanslı DA amplifikatör, DA/AD konverter kart, kişisel bilgisayar ve uygun bir yazılım kullanılarak, akış hızını hassas biçimde ölçen ve viskozite hesaplayan bir mikrokapiller otomatik eşzamanlı vizkometre yapıldı.

Bu otomatik eşzamanlı vizkometre halen İstanbul Üniversitesi, İstanbul Tıp Fakültesi, Anatomi Anabilim Dalında bulunan viskozite laboratuarında kurulmuş durumdadır. Bu laboratuar rutin laboratuarı olarak hizmet vermekte ve hasta kabul etmektedir.

Bu sistemle yapılan ön çalışmalar, referans değerlerin oluşturulabileceğini ve viskozite sorunu gösteren her çeşit hastalığın izlenebileceğini göstermektedir.

Anahtar kelimeler: Hemoreoloji, kan vizkozitesi, kapiller vizkometre

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Viscosity as a hemorheological criteria has become far more important after the clinicians started to blame it for some pathological conditions. There is a bunch of study showing that the blood viscosity is apparently relevant with erythrocyte aggregation, endothelial damage, intimal thickening, coronary artery disease (angina pectoris) and diabetic ischemia. Besides, there is suspicion of strong association with sudden deafness, sudden vertigo, ischemic retinopathy, aseptic bone necrosis and many other diseases of ischemia.

Flow dynamics of erythrocytes in microvessels is physiologically important for oxygen transport to tissues. Erythrocyte aggregation, as generally recognized, starts immediately after the implementation of a low-shear condition, and the resultant aggregates completely disperse under high-shear flow [1]. Erythrocyte aggregation in low-shear flow increases the blood viscosity and thus decreases the blood flow [1, 2]. The pathological situation often induces the vicious cycle of acceleration of erythrocyte aggregation in various diseases [1, 3, 4]. Even in the high-shear region of microvessels, the pathologically accelerated aggregation of erythrocyte, as in diabetes mellitus, sometimes forms sludge [5]. In these hemorheological disorders, pathological increment of macromolecules or appearance of pathological macromolecules increases plasma viscosity remarkably (so-called plasma hyperviscosity syndrome) and thus blood viscosity by the resultant acceleration of erythrocyte aggregation [1, 2, 6].

In Chapter 2, some examples were given about how viscosity was concerned in clinics. In Chapter 3, a general view to peripheral circulation was told. The answer to the question of "what is viscosity ?" was placed in Chapter 4. In Chapter 5, some principal Law concerning blood flow and the criteria effected this flow were explained. The effect of hematocrit on viscosity was inquired in Chapter 6. Automated real time viscometer's theory and setup were explained in Chapter 7. Clinical output is explained in Chapter 8. Conclusions were placed in Chapter 9.

2. VISCOSITY AS A CLINICAL CONCERN

2.1 Clinical Researches

2.1.1 Viscosity is a Risk Factor for atherotrombotic vascular disease

Blood and plasma viscosity have emerged as independent risk factors for atherotrombotic vascular disease [7, 8]. Blood viscosity increases correlate with the extent of angiographically determined coronary stenosis [9] and the severity of peripheral arterial narrowing [10] as assessed by ankle brachial systolic pressure index. Clinical manifestations of coronary and cerebrovascular disease are associated with an increased blood viscosity [11, 12, 13]. Increases in plasma viscosity are correlated with cerebrovascular symptoms [12] and claudication [10] after adjusting for the extent of arterial narrowing. Plasma viscosity is predictive of ischemic heart disease events in a free-living population [14] and progression to acute myocardial infarction in patients with unstable angina pectoris [15]. The contribution of an increased blood and plasma viscosity to the atherotrombotic vascular disease may result from reduced microcirculatory flow and increased shear stress against the endothelial surface, which enhances the likelihood of plaque rupture [7, 8, 16].

2.1.2 Viscosity in Optic Vascular Disease

Anterior ischemic optical neuropathy and central retinal artery occlusion are characterized by a sudden loss of visual acuity and field. Prognosis of the disease mainly depends on the time interval between acute occlusion and therapeutic intervention, localization of the occlusion and the residual perfusion of the retina. The most promising therapeutic approach is the intervention by hemorheological means such as isovolemic hemodilution by blood letting and replacement by hydroxyethyl starch or dextrane. The reason to treat these patients by heparin-induced plasma protein precipitation is based on a comparison of its rheological effect to hemodilution. Obviously the rheological effects of heparin-induced extracorporeal LDL/fibrinogen-precipitation (HELP) on plasma viscosity and erythrocyte aggregation are more pronounced than those of hemodilution [17]

2.1.3 Coronary Heart Disease and Increased Viscosity

Coronary heart disease (CHD) remains the leading cause of morbidity and mortality in Western society, accounting for nearly 1.1 million myocardial infarctions and over 450,000 deaths each year in the United States [18]. Currently accepted CHD risk factors of hypertension, cigarette smoking, and dyslipidemia only partly account for the high prevalence of this disease [19]. Recent attention has been directed at numerous unconventional CHD risk factors, including homocysteine concentrations, infectious and inflammatory etiologies, and lipoprotein(a) levels [20]. Evidence continues to mount that abnormalities in blood rheology, including elevations of blood and plasma viscosity, are related to various cardiovascular diseases including CHD [7, 21]. In fact, several prospective, multicultural epidemiological studies have found elevated plasma viscosity to be a powerful independent predictor for CHD morbidity and mortality [14, 22]. Increased blood viscosity has been correlated with the severity of CHD as assessed by cardiac catheterization [9], and it is elevated in subjects with angina pectoris [11, 23]. Relative blood viscosity has been found to be transiently elevated in patients after a myocardial infarction, and both the degree of viscosity elevation and time until resolution of hyperviscosity are correlated with myocardial infarct size [24, 25]. In one large epidemiological study, blood viscosity predicted cardiovascular events as effectively as did low-density lipoprotein (LDL) cholesterol and blood pressure, and was a better predictor than a history of tobacco smoking [10].

Numerous studies have reported the benefits of formal cardiac rehabilitation and exercise training programs on improving exercise capacity, obesity indices, plasma lipid and homocysteine levels, behavioral characteristics, quality of life and post-rehabilitation hospitalization costs. Benefits have been noted in several subgroups of patients, including the elderly, women, the obese, diabetics, individuals with dyslipidemia, those with behavioral disorders (depression and hostility), and patients with both high and low baseline exercise capacity [26-34]. Pooled data from randomized trials of cardiac rehabilitation indicate 20% to 25% reductions in major cardiovascular events, including CHD mortality and total mortality, which can only partly be explained by the modest improvements in conventional CHD risk factor [35-36]. In healthy subjects, those who exercise regularly have been found to have reduced blood and plasma viscosity compared to non-exercisers [37-40]. The available data concerning the effect of exercise on viscosity in unhealthy subjects is limited and conflicting. Whereas Reinhart et al [41] and Levine et al [42] found no sig-

nificant reductions in blood or plasma viscosity following formalized exercise programs in subjects with CHD, Ernst et al [43] reported that eight weeks of exercise training significantly lowered relative blood viscosity in patients with peripheral vascular disease, thus resulting in significant increases in functional capacity.

The purpose of this study was to determine if a standard cardiac rehabilitation and exercise training program improves blood rheology of patients with known stable CHD In reference #25 can be found the details of this study [25].

2.1.4 Viscosity can be Sudden Death for Marathon Running

Several studies have demonstrated an acute increase of blood viscosity following different forms of endurance exercise which went along with hematocrit elevation. In addition, plasma viscosity as well as concentrations of different plasma proteins were found to be elevated in the acute response to long-term exercise [10, 29, 30, 36]. Neuhaus et all investigated acute responses of hematological and hemorheological parameters to marathon running. They measured blood viscosity, aggregation and deformability of red cells [44].

2.1.5 Viscosity in Anatomically Abnormal Pulmonary Arteries

Conventional surgery is not an option for some children or young adults with decreased pulmonary blood flow due to hypoplastic, anatomically abnormal pulmonary arteries, those with pulmonary vascular obstructive disease and an intracardiac communication, or those with "primary pulmonary hypertension" and right-to-left shunting through a foramen ovale or true atrial septal defect [20, 28]. Polycythemia is a physiologic response to cyanosis in these patients [20]; the high hematocrit in turn can increase blood viscosity, reduce cardiac output, and raise pulmonary vascular resistance [9, 10, 24]. The elevated pulmonary vascular resistance can increase right-to-left intracardiac shunting, causing further reductions in arterial oxygen saturation and oxygen tension. The only surgical option for these patients is heart/lung transplantation, currently an experimental procedure, especially in children [14, 26]. Medical management of these patients is difficult and consist primarily of periodic phlebotomy with isovolemic saline or protein solution replacement to reduce blood viscosity in an effort to improve systemic oxygen transport [29-31]. An alternative therapeutic approach would be to improve red blood cell deformability, thus de-

creasing whole blood viscosity at any given hematocrit and possibly increasing both effective pulmonary and systemic blood flow. Berman et all performed a study to assess the effects of pentoxifylline on viscosity, hemodynamic variables, and oxygen physiology in patients with inoperable cyanotic heart disease [45].

2.1.6 Polycythemia,

Polycythemia, defined as a venous hematocrit greater than or equal to 0.65, occurs in 2 to 20% of all newborns infants [18-20]. Clinical manifestations have been observed in approximately 50% of newborn infants with hematocrit values of 0.65 or greater [19, 21]. The rise in blood viscosity accounts for clinical manifestations of Polycythemia [7]. Blood viscosity is an important determinant of the resistance to blood flow, and hyperviscosity can impede blood flow to various organs and compromise their oxygen supply [7, 14]. In neonates, hyperviscosity increases the risk of pulmonary hypertension, renal failure, narcotizing enterocolitis, cerebral ischemia, intracranial hemorrhage, and developmental retardation [19, 21, 22, 46-49].

2.2 Reference Group Studies for Viscosity

Rosenson RS et all and Kirby et all established reference values for blood and plasma viscosity evaluated from a disease-free population that required for clinical utility of viscosity measurements [16, 50]

2.3 Statements of Need

Creating a handy and reliable viscosimeter will be very helpful for both researchers and clinicians who try to investigate the relation between elevated blood viscosity and some disorders. Blood viscosity information is also valuable in monitoring the patient's body reaction to medical treatment [51-59].

3. PHYSIOLOGY OF THE PERIPHERAL CIRCULATION

3.1 History

William Harvey (English physician, 1578-1657) largely dispelled the idea that blood ebbed and flowed through the arteries and veins. After studying in Padua under Fabricius, whose study of the venous valves apparently first generated the idea that blood flowed through the veins toward the heart, Harvey arrived at the conclusion that the blood must circulate around and around. In a series of experiments in animals involving vessel ligations, and measurements of the volume of blood flowing from severed vessels, he concluded that "the movement of the blood is constantly in a circle, and is brought about by the beat of the heart." He also measured the volume of blood contained in the ventricles and calculated that the amount ejected by the ventricles in ½ hour was greatly in excess of the volume of blood contained in the entire body, thereby reinforcing his conclusion that the blood must circulate (despite the fact that microscope was just coming into use and capillaries had not yet been demonstrated). These discoveries were summarized in a classic book published by Harvey in 1628 [60].

3.2 Some Physical Properties of Blood

Certain physical properties of the blood are important in understanding the pressure and flow characteristics of the circulation. The science of rheology is concerned with investigation of the deformation and flow of matter. A number of different types of cells and formed elements are carried in the blood plasma. With a normal red blood cell (erythrocyte) count of 5 million/mm^3 and a normal white blood cell count of approximately 7000/ mm^3, the red cells constitute about 99.9 % of the circulating cell population. Indeed, the red cells are so numerous that normally they occupy about 40 % of the volume of the blood, a value expressed as a percent termed the hematocrit. Blood plasma is a viscous fluid, and the number of red cells and their characteristics also importantly influence this property [60]

3.2.1 The Hematocrit

The per cent of the blood that is cells is called the hematocrit. Thus, if a person has a hematocrit of 40, 40 per cent of the blood volume is cells and the remainder is plasma. The hematocrit of normal man averages about 42, while that of normal woman averages about 38. These values vary tremendously, depending upon whether or not the person has anemia, the degree of bodily activity, and the altitude at which the person resides. Blood hematocrit is determined by centrifuging blood in a calibrated tube. The calibration allows direct reading of the per cent of cells [61].

3.2.2 Plasma

Plasma is part of the extracellular fluid of the body. It is almost identical to the interstitial fluid found between the tissue cells except for one major difference: plasma contains about 7 per cent protein, while interstitial fluid contains an average of only 2 per cent protein. The reason for this difference is the plasma protein leaks only slightly through the capillary pores into the interstitial spaces. As a result, most of the plasma protein is held in the circulatory system, and that which does leak is eventually returned to the circulation by the lymph vessels. Therefore, the plasma protein concentration is about 3.5 times that of the fluid outside the capillaries [61].

4. PRINCIPLES OF HEMODYNAMICS

The physical principles that apply to the motion of blood through the vascular system are derived from the general laws of hydrodynamics, with certain modifications imposed by the properties of blood and the vascular tree. In the study of hemodynamics, it is natural to begin with the laws that govern the flow of simple liquids through a rigid cylindric tube, since they are the foundation for the more elaborate laws needed to describe the flow of blood through the branching, distensible network of tubes that make up the cardiovascular system [62].

4.1 Viscosity

The flow of liquids through tubes is governed in part by a fundamental property of fluids called viscosity, which is a kind of internal friction between adjacent layers of a fluid. If one portion of a viscous fluid is set in motion by an applied force, this motion is communicated to the adjacent parts of the liquid. Isaac Newton described this phenomenon as a "lack of slipperiness" between the parts of a liquid and derived the earliest theoretical treatment of this general property of fluids from the hypothesis that the resultant internal resistance is proportional to the velocity with which the parts are separated from one another. Fluid moving through a tube may be thought of as a series of thin layers slipping against each other and proceeding at different velocities. Viscosity is defined as the ratio of stress to velocity gradient, where the stress is the force applied per unit area to produce motion of the fluid and the velocity gradient is the change in velocity from one layer to the next. Under ideal conditions the velocity gradient forms a symmetric pattern (Fig. 4.1) [62].

4.1.1 Unit of Viscosity

The standard unit of viscosity is the poise. 1 poise (1 P) being equal to 1 dyne sec/cm^2. Relative viscosity, relating the viscosity of a fluid to that of water (0.01 P at 20°C) is sometimes used instead of these absolute units. Viscosity varies with temperature, increasing as temperature falls [62].

4.1.2 Apparent Viscosity

This definition implies that viscosity is a property of the fluid itself, independent of the actual velocity gradient. Such independence can be demonstrated in water and a number of other liquids, which are therefore termed Newtonian fluids. In blood and certain other substances, however, careful measurements reveal that Newton's fundamental assumption of a velocity gradient proportional to applied stress is not true at all velocities. Nevertheless, the behavior of these Nonnewtonian fluids can be described by their apparent viscosity under specified conditions. The anomalies in the viscous behavior of blood result partly from the presence and orientation of the cells suspended in plasma and partly from the accumulation of red blood cells in the axial portion of the bloodstream. The physical forces involved are not entirely clear, but the result is a decrease in apparent viscosity with increasing velocity of flow and with decreasing vessel radius. The magnitude of these effects is small in vessels more than 0.5 mm in diameter at flow rates in the physiologic range, and for most purposes the viscosity of blood at 37°C in dog and man may be assumed to remain constant at 0.03 to 0.04 P. This value applies to blood with normal red blood cell concentrations. Viscosity rises with increasing hematocrit [62, 63].

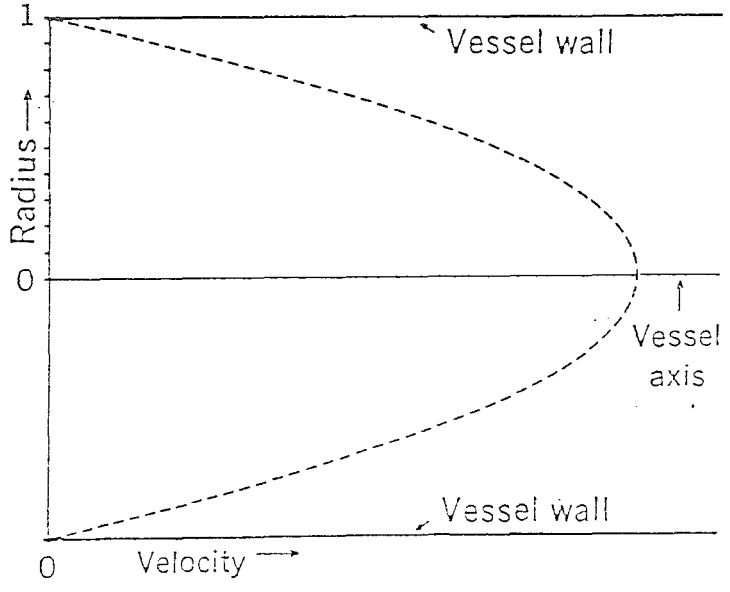


Figure 4.1 Parabolic relationship between blood velocity and distance from vessel axis under condition of laminar flow [62].

5. LAMINAR FLOW IN RIGID TUBES

Newtonian liquids flow through a cylindric tube in a series of concentric cylindric laminae; within each lamina the fluid particles all move with the same velocity, but the velocity of each cylindric shell decreases with its distance from the axis of the stream. The relation between velocity and radial distance from the axis is parabolic (Fig. 1), and flow of this kind is therefore described as parabolic or alternatively as laminar or Newtonian [62].

5.1 Poiseuille's Law

The characteristics of laminar flow can be derived mathematically from the definition of viscosity if we assume that the infinitely thin outermost lamina in contact with the vessels wall is at rest . This derivation demonstrates that the velocity at the axis (r = 0) is twice the average velocity across the stream. The rate of flow (Q), or volume of liquid passing a given cross section Per unit of time, is inversely proportional to viscosity (μ) and directly proportional to the drop in pressure (P_1 - P_2) Per unit length of the vessel (L) and the fourth power of the radius.

$$Q = \frac{\pi R^4 (P_1 - P_2)}{8L\mu} \qquad \text{Eq. (1)}$$

In other words, under these conditions flow depends on four factors: (1) the constant (π /8), in which π relates to the circular cross section of the tube and 8 appears in the process of integration; (2) the reciprocal of the viscosity (1/ μ); (3) the dimensions of the tube, in the form R^4 /L; and (4) the pressure exerted to produce flow (P_1 - P_2), in which P_1 is the pressure at the upstream end of the cylinder and P_2 is the pressure at the distal end. Since the radius of the tube appears to the fourth power, relatively small changes in radius produce large changes in flow. The fall in pressure per unit length, (P_1 - P_2)/L, is occasionally referred to as the pressure gradient. Strictly defined, the latter term means the rate of change of pressure along the longitudinal axis of a vessel, but is sometimes loosely used as a synonym for the pressure head (P_1 - P_2), or difference in pressure between two

intravascular sites. The relationship defined by equation 1 is usually called Poiseuille's law, after the French physician and physiologist Poiseuille (1799-1869), whose interest in flow through small blood vessels led him to measure the flow of water in fine capillary tubes. His results, published in detail in 1846, described the relations between pressure head, flow, and tubing dimensions with a high degree of accuracy, although it was not until some years later that the theoretical analysis of viscosity and laminar flow gave the law its present form.

Poiseuille's law describes the laminar, constant flow of Newtonian liquids through rigid cylindric tubes, and its applicability to the circulation depends on the extent to which blood and the vascular system conform to these theoretical assumptions. Blood behaves as a Newtonian fluid in vessels with an internal diameter greater than 0.5 mm, so that in larger vessels, at least, this requirement is satisfied. Flow in these vessels is also laminar as a rule, but flow is pulsatile rather than constant throughout most of the arterial tree. The acceleration and deceleration of blood in pulsatile flow introduce variations in kinetic energy not taken into account in the Poiseuille equation, which for this reason cannot be applied to oscillations of flow, although it gives a fair approximation of the relation between mean pressure and mean flow. A further departure from Poiseuille's law results from the distensibility of blood vessels, which makes vessel radius vary with the applied pressure.

Despite these discrepancies, Poiseuille's law has served as a useful concept in circulatory physiology, if only because its limitations were for a long time matched by experimental limitations imposed by the lack of a satisfactory theoretical treatment of pulsatile flow in distensible vessels and of reliable ways to measure pulsatile flow [62].

5.2 Vascular resistance

According to Poiseuille's law, the ratio of pressure drop to rate of flow is a function of all the forces that retard blood flow – viscosity, length, and radius, This ratio has been adopted as an expression of vascular resistance by analogy with Ohm's law for electrical circuits (voltage/current = resistance). The resistance (K) of a vascular bed is defined as the difference between the pressure at the inlet of the bed (P_1) and the pressure at the outlet P_2 divided by the blood flow (Q):

$$K = \frac{(P_1 - P_2)}{Q} \qquad \text{Eq. (2)}$$

Since vascular length can be regarded as constant in most beds and blood viscosity is constant under most conditions, it is evident from Poiseuille's law that resistance depends principally on vessel radius. A change in vascular resistance therefore implies a change in radius of some vessels in the bed, although it does not indicate either the site or the mechanism of this change in caliber. Although the inference that a change in pressure: flow ratio in one part of the circulation is due to alteration of local arteriolar resistance is usually valid, it should be remembered that small arteries (diameter = 2 to 0.1 mm), venules, and even capillaries contribute a significant part of the total resistance in some circumstances.

In considering the mechanisms by which vessel radius and thus resistance can be altered, it is essential to distinguish between the fall in pressure along the length of a vessel, which is the factor related to resistance, and transmural pressure across the wall, which affects the radius of distensible vessels. The caliber of a blood vessel can change passively because of a change in transmural pressure, or it can be changed by activation of vascular smooth muscle. A decrease in resistance, for example, may arise from increased arterial pressure, which tends to raise transmural pressure and distend the vessels passively, or from relaxation of the smooth muscle fibers in the arteriolar walls, which are under active neural control. In other words, measurements of vascular resistance reflect changes in vessel caliber but tell nothing of the mechanism by which these changes are bought about. Moreover, because the vascular tree consists of many parallel channels, changes in resistance may be due to complete closure or reopening of some channels rather than to generalized changes in caliber.

The nonspecific nature of resistance measurements has important practical consequences. When resistance and transmural pressure change in the same direction, there has probably been an active change in the caliber of vessels somewhere in the vascular bed, attributable to changes in the tension of vascular smooth muscle. Under any other circumstances the mechanism of a change in resistance is less certain.

In the absence of laminar flow, even this limited applicability of the concept of resistance is altered. When flow becomes turbulent, the pressure: flow ratio is greater than that predicted by Poiseuille's law because energy is expended in random motion across the stream as well as in maintaining forward flow. Laminar flow obtains in the larger vessels of the circulation, but in very small collapsible vessels such as the capillaries a transition from laminar to turbulent flow may, under some conditions, make Poiseuille's law quite inapplicable to that particular segment. For similar reasons, equation 2 is invalid for partially or completely collapsed veins. The flow of liquids at high velocity through a small orifice is a special instance of nonlaminar flow extensively studied by hydraulic engineers. The dynamic conditions at such an orifice are similar those in heart valves constricted by disease, and equations have been developed to estimate the functional area in such valves from measurements of pressure and flow [62].

6. EFFECT OF HEMATOCRIT ON BLOOD VISCOSITY

Blood is several times as viscous as water, and this viscosity increases the difficulty with which the blood flows through the small vessels. The greater the percentage of cells in the blood – that is, the greater the hematocrit- the more friction there is between successive layers of blood, and this friction determines viscosity. Therefore, the viscosity of blood increases drastically as the hematocrit increases, as illustrated in Figure 6.1. If we arbitrarily consider the viscosity of water to be 1, then the viscosity of whole blood at normal hematocrit is about 3; this means that 3 times as much pressure is required to force whole blood through a given tube than to force water through the same tube. Note that when the hematocrit rises to 60 or 70, which it often does in Polycythemia, the blood viscosity can become as great as 10 times that of water, and its flow through blood vessels is greatly retarded.

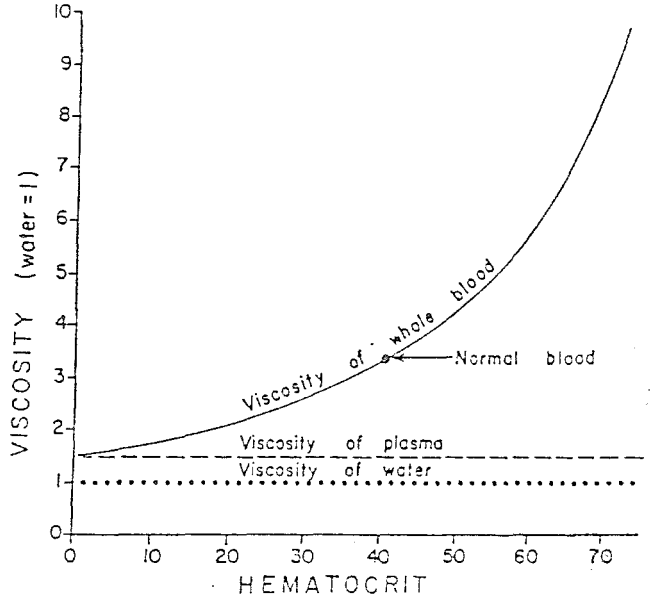


Figure 6.1 Effect of hematocrit on viscosity [61].

Another factor that affects blood viscosity is the concentration and types of proteins in the plasma, but these effects are so much less important than the effect of hematocrit

that they are not significant considerations in most hemodynamic studies. The viscosity of blood plasma is about 1.5 times that of water.

Since most resistance in the circulatory system occurs in the very small blood vessels, it is especially important to know how blood viscosity affects blood flow in these minute vessels:

(1) Blood flow in very minute vessels exhibits far less viscous effect than it does in large vessels. This effect, called the Fahraeus-Lindqvist effect, begins to appear when the vessel diameter falls below approximately 1.5 mm. In vessels as small as capillaries, this effect is so prominent that the viscosity of whole blood is as little as one-half that in large vessels. The Fahraeus-Lindqvist effect is probably caused by alignment of the red cells as they pass through the vessels. That is, the red cells, instead of moving randomly, line up and move through the vessels as a single plug, thus eliminating the viscous resistance that occurs internally in the blood itself. The Fahraeus-Lindqvist effect, however, is probably more than offset by the following two effects under most conditions.

(2) The viscosity of blood increases drastically as its velocity of flow decreases. Since the velocity of blood flow in the small vessels is extremely minute, often less than 1 mm. Per second, blood viscosity can increase as much as 10-fold from this factor alone. This effect is presumably caused by adherence of the red cells to each other (formation of rouleaux and larger aggregates) and to the vessel walls.

(3) Cells also often become stuck at constrictions in small blood vessels; this happens especially in capillaries where the nuclei of endothelial cells protrude into the capillary lumen. When this occurs, blood flow can become totally blocked for a fraction of a second, for several seconds, or for much longer periods of time, thus giving an apparent effect of greatly increased viscosity.

(4) Because of these special effects that occur in the minute vessels of the circulatory system, it has been impossible to determine the exact manner in which hematocrit affects viscosity in the minute vessels, which is the place in the circulatory system where viscosity almost certainly plays its most important role. Nevertheless, because some of these effects tend to decrease viscosity and others tend to increase viscosity, it is perhaps best at present simply to assume that the overall viscous effects in the small vessels are approximately equivalent to those that occur in the larger vessels [61, 63].

7. RAPID RESPONSE MICROVISCOMETER

7.1 Theory

The most common apparatus currently used to measure blood viscosity is the rotary viscometer with either cone-in-cone, cone-on-plate, or concentric cylinder geometry. Although these instruments are of great utility in evaluating blood viscosity in a general sense, the values do not directly translate to microvascular flow because of the difference in measurement flow conditions: sheet flow versus tube flow. The specific flow condition utilized by rotary viscometer approximates sheet flow between parallel plates, one stationary and one moving. This set-up imposes a constant shear rate across the flow cross-section. In tube or vessel flow, however, both shear rate and viscosity have nonlinear distributions across the tube radius. As a result of that variation, viscous resistance to flow in a tube is usually given by a bulk averaged viscosity across the cross-section. The difference between rotary viscometric values and those of tube viscometers is particularly notable in vessels of microcirculation, because effective viscosity in a tube is a strong function of tube diameter in small vessels [64]. Rotary viscometers yield viscosity values significantly larger than those measured in vivo in arterioles [65, 66]. Thus, when effective blood viscosity in arterioles is to be studied by in vitro instrumentation, flow conditions approximating those of the vessels being considered should be established in the viscometer used, if direct correlation to flow physiology is to be made. For arteriolar flow, a tube-type instrument with arteriolar dimensions, geometry, and flow rates will provide relevant viscosity measurements.

For steady laminar flow in single cylindrical vessels, volumetric fluid flow rate (Q) is a function of pressure drop (ΔP), viscosity (μ), vessel length (L), vessel radius, and radial coordinate (r) [67]:

$$Q = \int_0^R \frac{\pi r}{2\mu\mu} \Delta P\left(R^2 - r^2\right) dr \qquad \text{Eq. (3)}$$

Blood is a non-Newtonian fluid and its viscosity dependent not only on tube size and flow rate, but also on the distribution and interactions of the formed elements and chemical constituents in the fluid. Consequently, the integration of Equation (3) for blood requires details of the functional form of viscosity that, in general, has not been obtained. Elegant approximate solutions have been performed which fit experimental data well over limited flow regimes [68-70], but no general solution has yet been achieved. For Newtonian fluids in laminar flow, however, the integration is straightforward [67] and results in the well known Hagen-Poiseuille equation, Equation (4a) below, which can be generalized to non-Newtonian fluids as in Equation (4b) below, by defining μ_e [65] as the effective value required for equality.

$$Q = \frac{\pi R^4}{8L} \Delta P \frac{1}{\mu} \qquad \text{Eq. (4a)}$$

$$Q = \frac{\pi R^4}{8L} \Delta P \frac{1}{\mu_e} \qquad \text{Eq. (4b)}$$

From Eq. (4b), effective viscosity may be obtained by measurements of geometry, pressure difference, and flow rate. Several investigators have done this in tubes of arteriolar dimensions in flow conditions simulating those in arterioles using various methods to measure flow rate [44, 63, 65, 66, 68, 71-73]. For most application, pressure differences may be readily and accurately measured with commercial pressure transducers or manometers and the geometry is easily measured optically with precision.

7.2 Problem

The measurement of flow rate, however, is often problematic for lack of small scale precision flow meters with rapid time response and concerning the protocol and performed man work during test running.

Kirby at al, had developed a rapid response viscometer (Figure 7.1) using two 41 μm tubes connected in series which was very reliable and fast concerning all other either capillary or conventional ones [50]. But still this system needed long procedure to get usable data and very long time and man work to put them in PC. In this microviscometer system a strip chart record system was used, after that data was read on this milimetric chart using a lens. After analyzing this chart, all data were carried to an Excel work sheet for calculation. Additionally, in the capillary part of this system, Kirby had used a disposable transducer (Sorenson Transpac, Abbott Laboratories) which was not very durable and sensitive as midstream transducer. Besides, those transducers had rubber sensitive surface which was not very stable.

7.3 An Automated Real Time Viscometer

In order to overcome this inconvenience, a reusable dome transducer was used instead of disposable one as midstream transducer. For collecting data, only a PC with appropriate software which was more faster, reliable and time saving instead of ink-writing strip chart was used. Additionally, with this system the laboratory was kept tidier, and all data could be stored in digital media that could be reproducible and electronically transferable.

7.3.1 Setup

This automated real time viscometer's setup was established in the laboratory in the Department of Anatomy, Istanbul Medical Faculty, Istanbul University (Figure 7.2).

The microviscosimeter (Figure 7.3) consist of two tubes of polytetrafluoroethylene (PTFE; Zeus Industrial Products) with uniform internal diameters of 41 μm, and lengths of 25 mm (Figure 7.4) connected in series on either side of a small volume pressure transducer. Those hand-made tubes were obtained from Tulane University Hemorheology Laboratory with the cooperation of Dr. Kirby. An attached upstream reservoir contains the test fluid for input to the system. An attached downstream reservoir receives the system effluent. The upstream reservoir is connected to a variable pressure air supply, which drives the test solution through the system. Pressure relative to atmosphere is measured by pressure transducers at two points in the system (Figure 7.5).

7.3.1.1 Transducers

For data harvesting one Ohmeda P23XL reusable dome pressure transducer was mounted between hand made capillary sections (Figure 7.5). For air pressure measurement, another Ohmeda P23XL transducer was used. Those transducers' pressure range was -30 to +300 mm Hg, sensitivity was 5 μV/V/mm Hg ±1% and excitation voltage was 7.5V DC.

The transducers were connected through a 3.7 meters shielded cable to front panel of a preamplifier (Figure 7.6).

7.3.1.2 Preamplifiers

The signals from the transducers were separately amplified using low level DC preamplifiers because the pressure values that we measured were in a range of 20mV to 2V.

Preamplifiers that were used were Grass Instrument Co. Low-Level D.C. Pre-amplifier Model 7P1. Those preamplifiers were high gain, low noise, low frequency, plug in DC preamplifier, with the specifications of CMRR: greater than 1000:1 and unity gain between 0.995 and 1. Those preamplifiers could give 6.2V excitation voltage that had to be needed for transducers.

Preamplifiers were actually designed to be used with a driver amplifier (in this case, Grass Driver Amplifier model 7DA), a part of Grass Polygraph Table. There was no need for this driver amplifiers in our setup, because the I/O board could operate between the range of -5-+5V or 0-10V which was enough for setup's amplificators. They had custom sockets/plugs to fit on the driver amplifier for outputs and power inputs. In order to overcome this incompatibility, then sockets were changed with a general purpose PC connection socket.

A PC power supply which had both +12V and –12V outputs was used to supply the preamplifiers.

The cables that carry the output data were connected to the connector block (National Instruments CB-50 I/O Connector Block with DIN Rail Mounting) as shown in figure 7.7 (Figure 7.8).

This fifty pins connector block was connected to I/O board with 1 meter connecting cable (National Instrument NB1 Cable) (Figure 7.9).

7.3.1.3 I/O Board

The output signals were carried to a PC through a AD/DA converter card (National Instruments, PCI-1200).

PCI-1200 Multifunctional I/O Board for PCI Bus Computers were used as A/D converter. This board was completely software configurable, 12 bit resolution and 100Ks/s sampling rate. PCI-1200 were installed an PCI expansion slot in the computer.

7.3.2 Analog I/O Configuration

After NI-DAQ driver software for PCI 1200 set up, "measurements & automation explorer" and "devices and interfaces" files were opened respectively. PCI's "properties" in the last file was chosen and this device was tested. After testing the device, input signal range was set to ±5V, and "differential" mode was chosen as connection type.

As the last step, "CB-50 connector block" was chosen as accessory item.

7.3.3 Software

LabView 5.01 (National Instruments) was used as software. An appropriate diagram and user interface (Figure 7.10, 7.11) was established which solved the problem shown in flow chart (Figure 7.12, 7.13)

7.3.4 Theory for Setup

When fluid is flowing steadily through the entire system, the Hagen-Pouiseuille flow relationship [Equation (4)] holds throughout the flow system such that the effective viscosity in the test section is a linearly related to the flow resistance in the reference section. Applied to the microviscometer, Hagen-Pouiseuille relation yields both μ_e of a sample placed in the upstream reservoir and Q throughout the system as a function only of measured pressure drops, system geometry (C), and the reference viscosity μ_R of the fluid in the reference section:

$$\mu_e = \left(\frac{R_X}{R_R}\right)^4 \left(\frac{L_R}{L_X}\right) \mu_R \frac{(P_0 - P_1)}{(P_1 - P_2)} = C\mu_R \frac{(P_0 - P_1)}{(P_1 - P_2)} \qquad \text{Eq. (5)}$$

$$Q = \frac{\pi R_R^4}{8 L_R \mu_R} (P_1 - P_2) \qquad \text{Eq. (6)}$$

By this means, effective viscosity and flow rate are continuous on-line output variables which can be directly obtained from PC records of only P_0 and P_1, both being gauge pressures relative to atmosphere pressure (P_2).

Both reservoirs are constructed from 1-ml polyethylene syringe barrels. Both the reference section/reservoir and test section/reservoir are constructed by gluing a 25-mm length of 41-μm tubing into a reservoir which is glued onto a needle hub for Luer-Lok® attachment to mid-stream transducer. These provide reservoirs of 0.5 ml in volume. In practice, using either type of test section/reservoir, about 0.2 ml of blood are loaded into the test reservoir for a data run. The system is cleaned and stored in distilled water.

7.3.5 Performance

Nominal physiologic flow rate in a 41- μm arteriole is about 12 nl/s [50]. The microviscosimeter, as configured, will measure effective blood viscosity at flow rates which bracket nominal values, by varying Q from 0.1 to 25 nl/s using upstream driving pressures of no more than 200 mm Hg. The upstream pressure transducer P_0 is periodically calibrated against a measured mercury column manometer, and the midstream pressure transducer P_1 is calibrated relative to P_0 before every data run. In use, the system parameters are first calibrated with saline filling the entire system downstream of pressure transducer P_0. Saline is then removed from the upstream test reservoir, replaced with the test fluid, and positive upstream pressure applied for at least 5 s to completely flush residual saline from the test section. Positive upstream air pressure causes the test fluid to flow through the test section into the pressure transducer, P_1, where it mixes with saline and drives downstream fluid through the reference section at the same flow rate as in the test section. All measurements are made at steady flow.

Because each viscosity run requires only about 1 μl of fluid, the saline in the 0.2 ml mid-stream transducer is diluted with blood to no more than a 0.5 % solution. As verified by post-test saline calibrations done after every test run, such a dilution does not measurably affect the viscosity of the reference fluid. All measurements for which post-test saline calibrations did not match pre-test calibrations are discarded. All data runs for which the initial and final calculated values of μ_e are not identical are discarded and the run repeated after clearing the test section. The test and reference sections may be oriented either vertically or horizontally, as the present system is configured. Cells do not readily adhere to the PTFE test section. Only after long periods without flow do occasional cells stick to the tube wall. These are displaced by flow rates of less than 1 nl/s. Blood is never allowed to remain at rest in the test section for duration greater than a few seconds during a test run.

For the present apparatus configuration and flow domain, the Reynolds number is always less than 0.5, with laminar flow entrance lengths [67] less than 0.002 %, so that end effects due to flow development may be considered negligible. Using an acceleration analysis of Hagen-Poiseuille flow [74], the noncompliant flow response to step increases in applied pressure gradient is calculated to be within 0.5 % complete in 0.2 ms. For the present system using blood, with an instrument response time of 3 s, acceleration effects are negligible.

7.3.6 Instrument precision

A standard oil, Cannon Certified Viscosity Standard S3 (Cannon Instrument Company, State College, PA) and five concentrations of sodium chloride solution (1, 2, 5, 10, and 20 % by weight, viscosity values from Weast, 1978) were used to test the accuracy of the microviscosimeter over the flow rate range of 0.7-35 nl /s. The standard deviations (std dev) of the distribution of the differences of μ_e measurements from Cannon Standard values ranged from 2.3 % at 0.7 nl/s to 1.6 % at 35 nl/s, and those of the NaCl solution tests, at a flow rate of 7.5 nl/s, were $\leq$ 1.3 %. Full scale instrument response time was 3 s after a step function input. Each complete test run of 15 different flow rates required less than 3 minutes.

Because of the short duration required for a complete test, non-anticoagulated blood could be used. To test the reproducibility of the instrument, repeated measurements were made on such samples drawn from cubital veins. At nominal flow rates (5-20 nl/s) the standard deviation of the μ_e distribution of repeated measurements of the same sample is less than 2 % of the mean value. Thus the instrument is precise to about 2 % in both accuracy and

reproducibility. Three anticoagulants (15 U.S.P. units/ml of sodium heparin, 1.5 mg/ml EDTA, and 10 % dilution with buffered sodium citrate) were used and compared to non-anticoagulated blood from 10 subjects over a flow rate range of 0.5-20 nl/s. All anticoagulants, with time, distorted μ_e to some degree. Sodium heparin and EDTA always caused less than 2.5 % increase in μ_e, whereas sodium citrate decreased it variably by as much as 10 %. Sodium heparin proved to cause the least deviation and provided for values more stable with time than did the others. The average μ_e difference between non-anticoagulated blood (viscosity measured immediately post-draw) and sodium heparin anticoagulated blood, drawn immediately afterward and measured within 2 hr of blood draw, ranged from 0.8 % at 0.5 nl/s to 1.8 % at 20 nl/s, but the mean values were not statistically different for up to 4 hr. Using repeated measurements of the same sample, the reproducibility of sodium heparin anticoagulated blood was determined to be less than 2 % deviation at flow rates above 2.5 nl/s. All measurements reported herein used sodium heparin anticoagulated blood.

7.3.7 Experimental protocol

The test and reference sections were cleaned with distilled water and the entire system was filled with 0.9 % NaCl and purged of bubbles A calibration was done at both high and low flow rates to determine the calibration constant C (Eq. (5)). If the value of C differed between high and flow rates, a system defect was indicated and the entire system was cleaned, reassembled, and re-calibrated. Using 21-gauge needles, blood was drawn from veins of the cubital fossa into 10-ml Vacutainers® (Figure 7.14) with 15 U.S.P. units Na heparin per milliliter. Whole blood effective viscosity was measured within 1 hr. Fifteen different driving pressures from 2 to 200 mm Hg were used to vary the flow rate in the test section. Hematocrit was measured after microcentrifugation at 13,000 x g for 6 minutes. Plasma was obtained by centrifugation of anticoagulated blood (Figure 7.15). Plasma viscosity is stable for several hours but was measured within 2 hr of draw. All measurements were done at temperatures between 20 and 25°C. Some results were shown in figures 8.1, 8.2, 8.3 and 8.4.

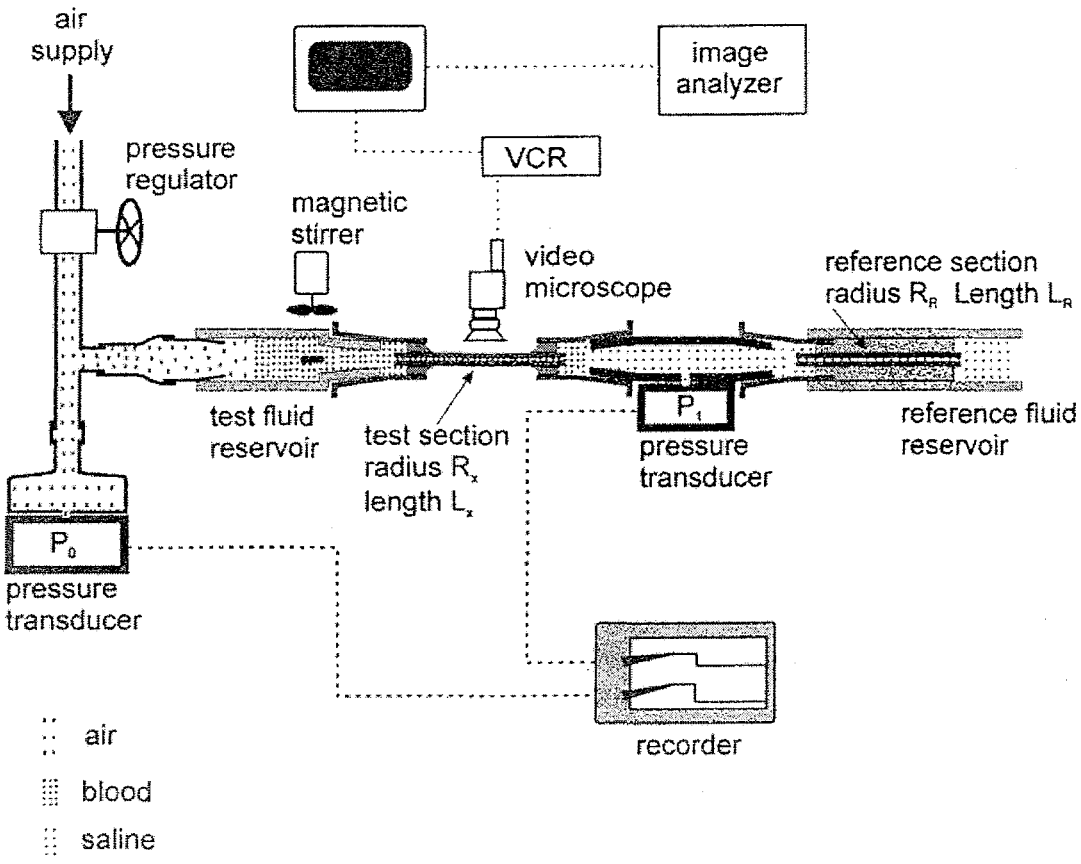


Figure 7.1 Schematic diagram of microviscometer (Kirby, G [50])

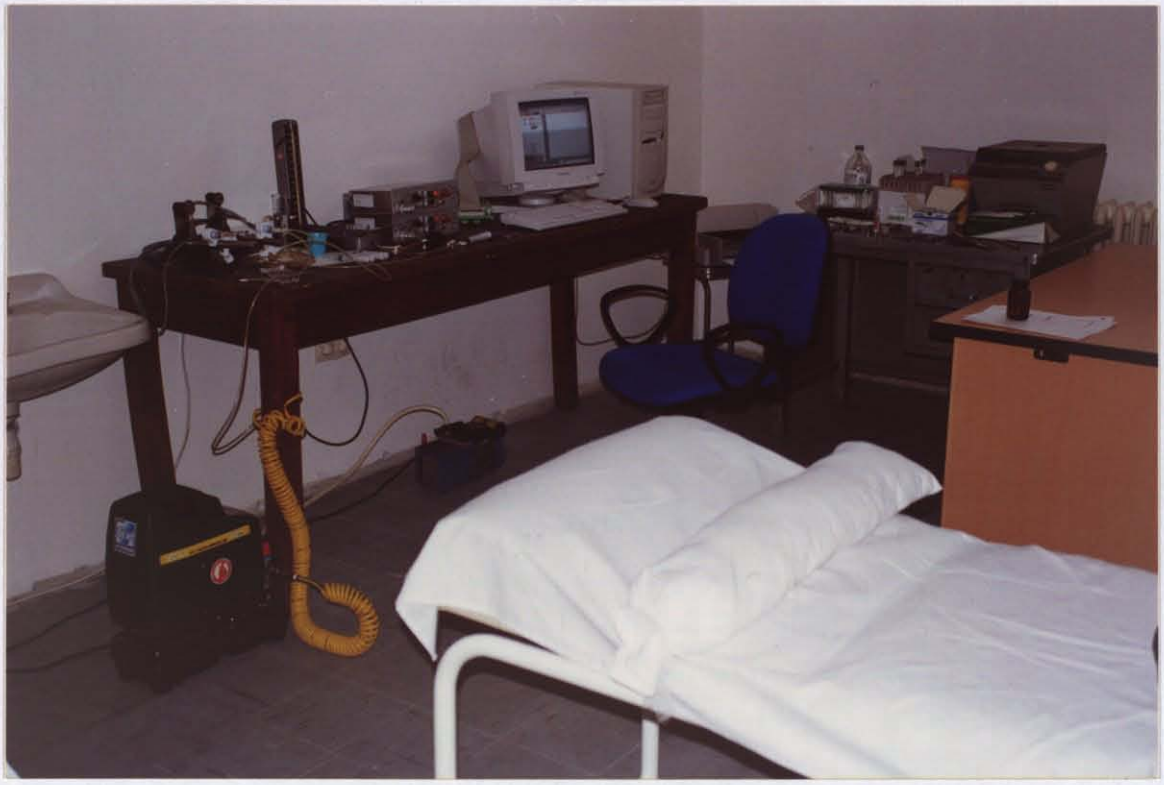


Figure 7.2 Viscosity Laboratory in Department of Anatomy

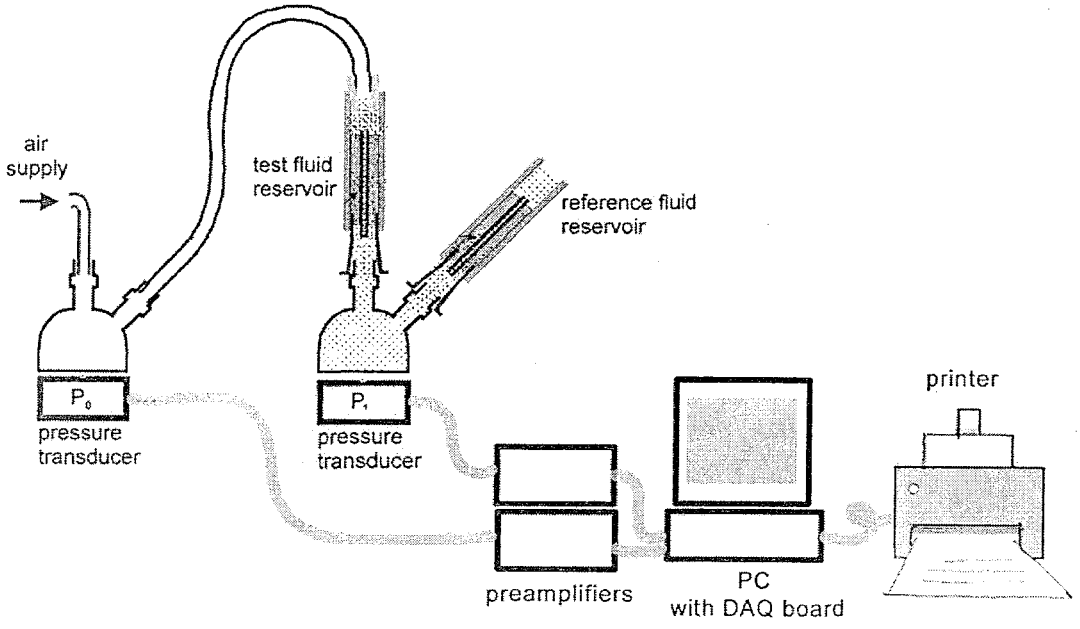


Figure 7.3 Schematic diagram of real time viscometer.

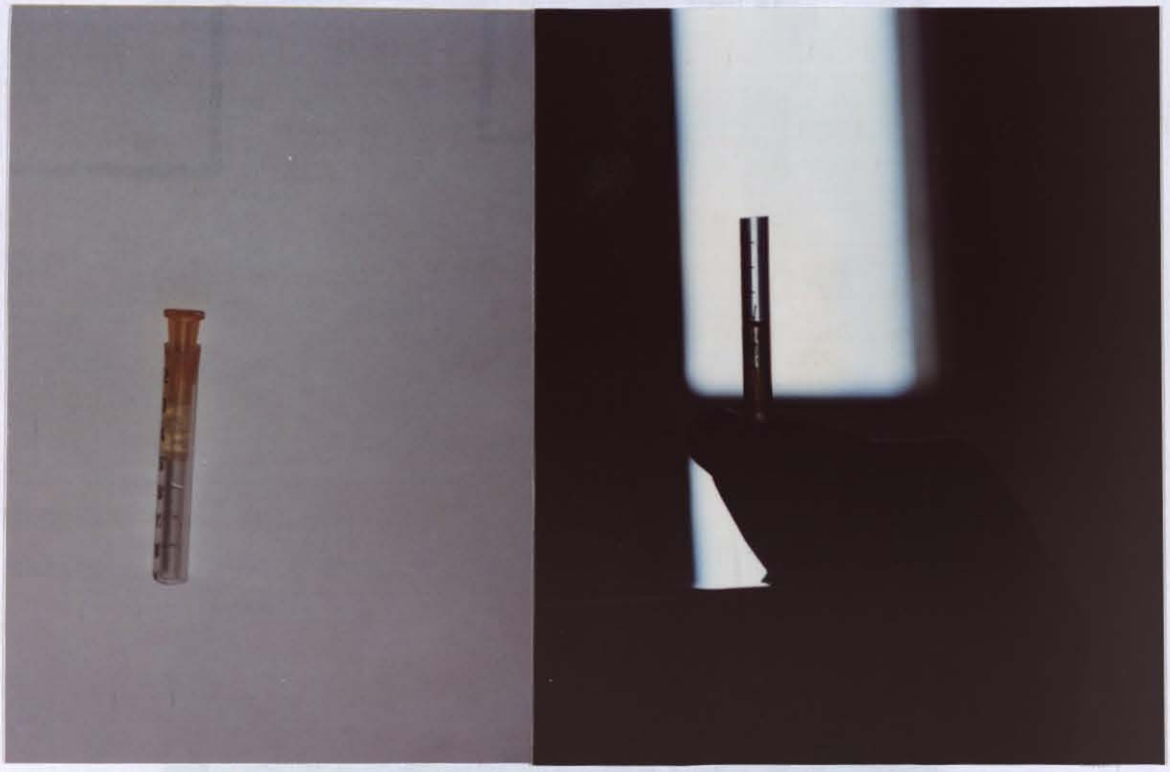


Figure 7.4 Capillary tube in hand-made reservoir.

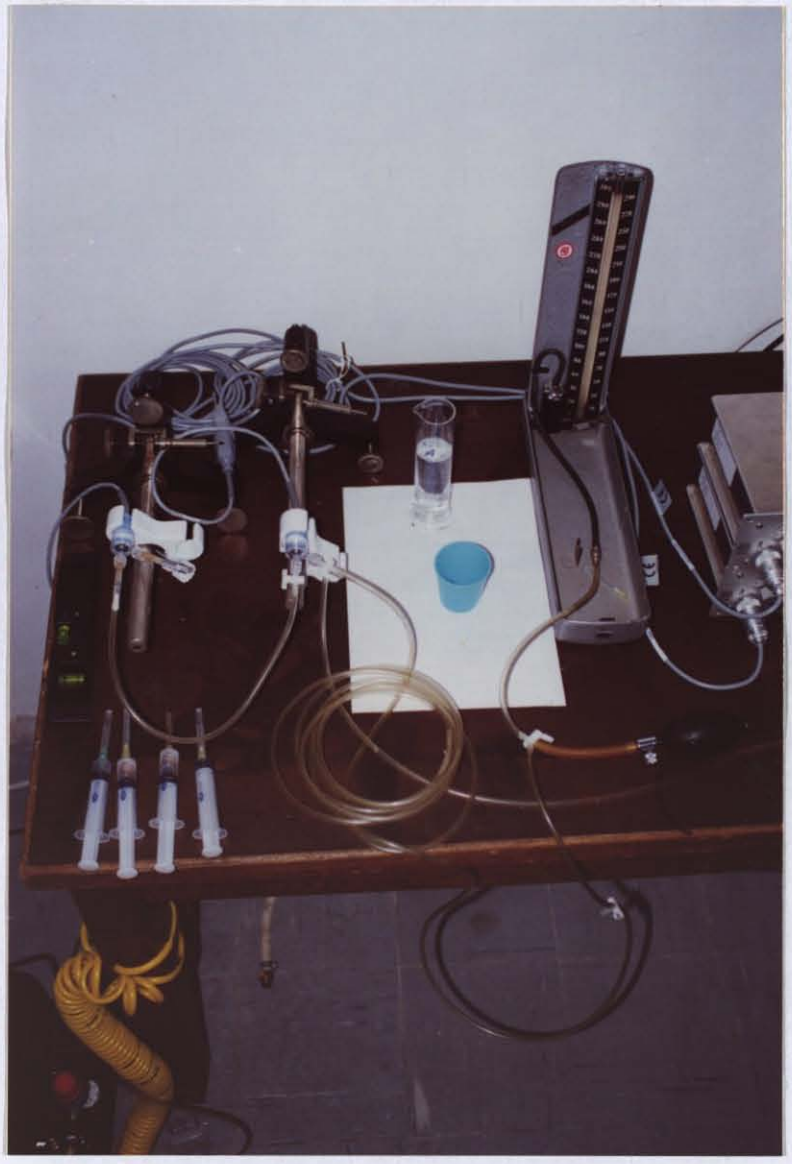


Figure 7.5 Capillary tubes and transducers set up.

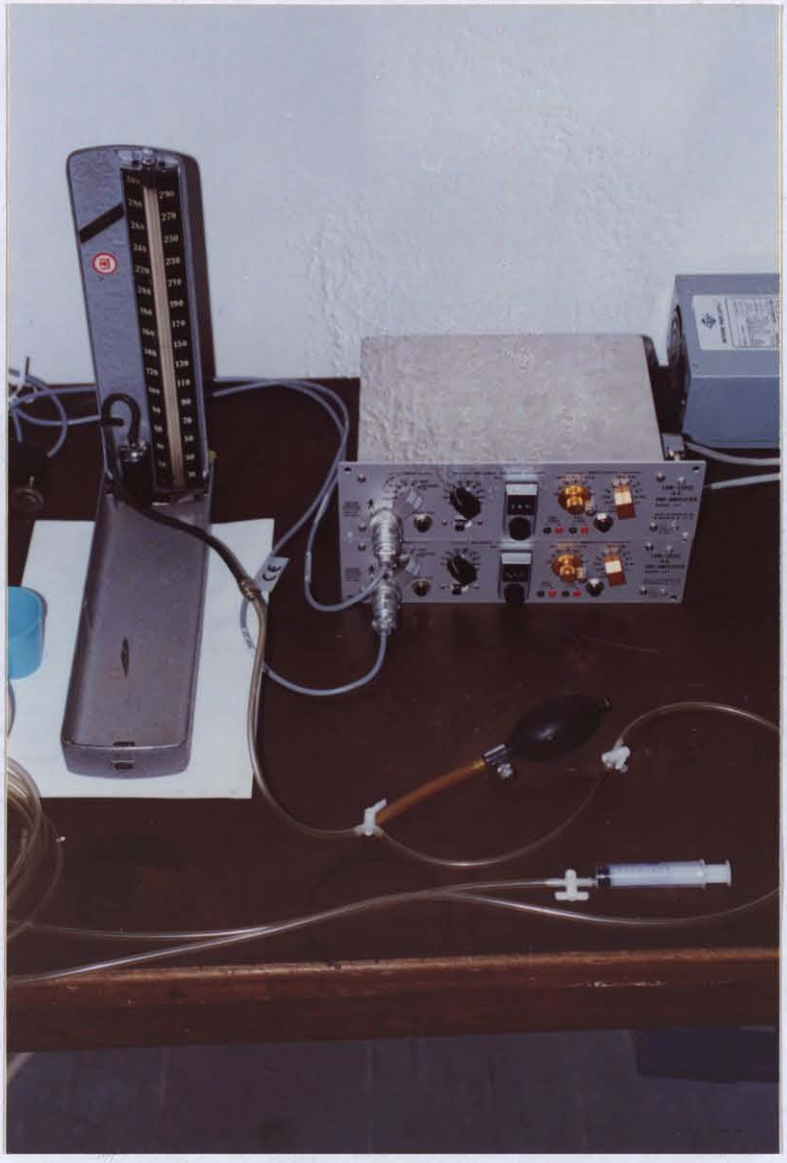


Figure 7.6 Preamplifiers and mercury column manometer set up.

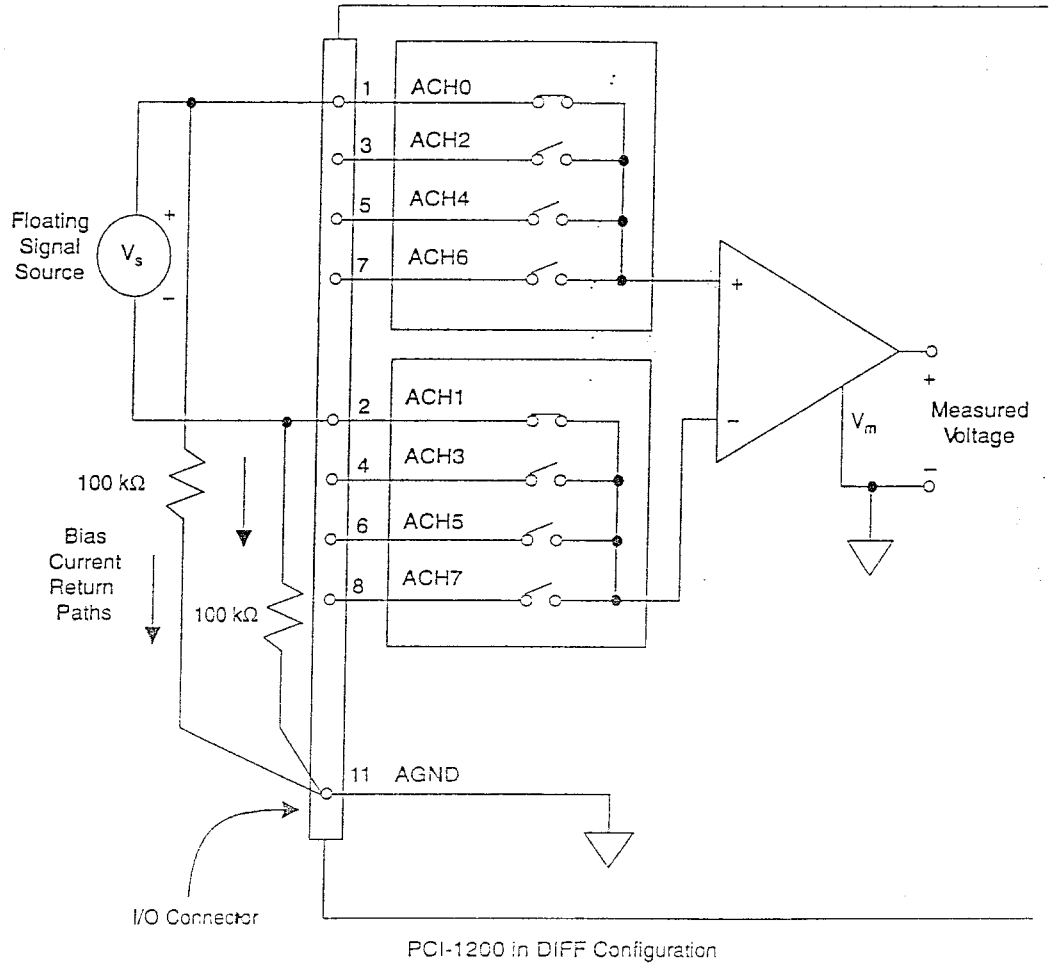


Figure 7.7 Differential input connections for floating sources (from, NI, PCI-1200 User Manual)

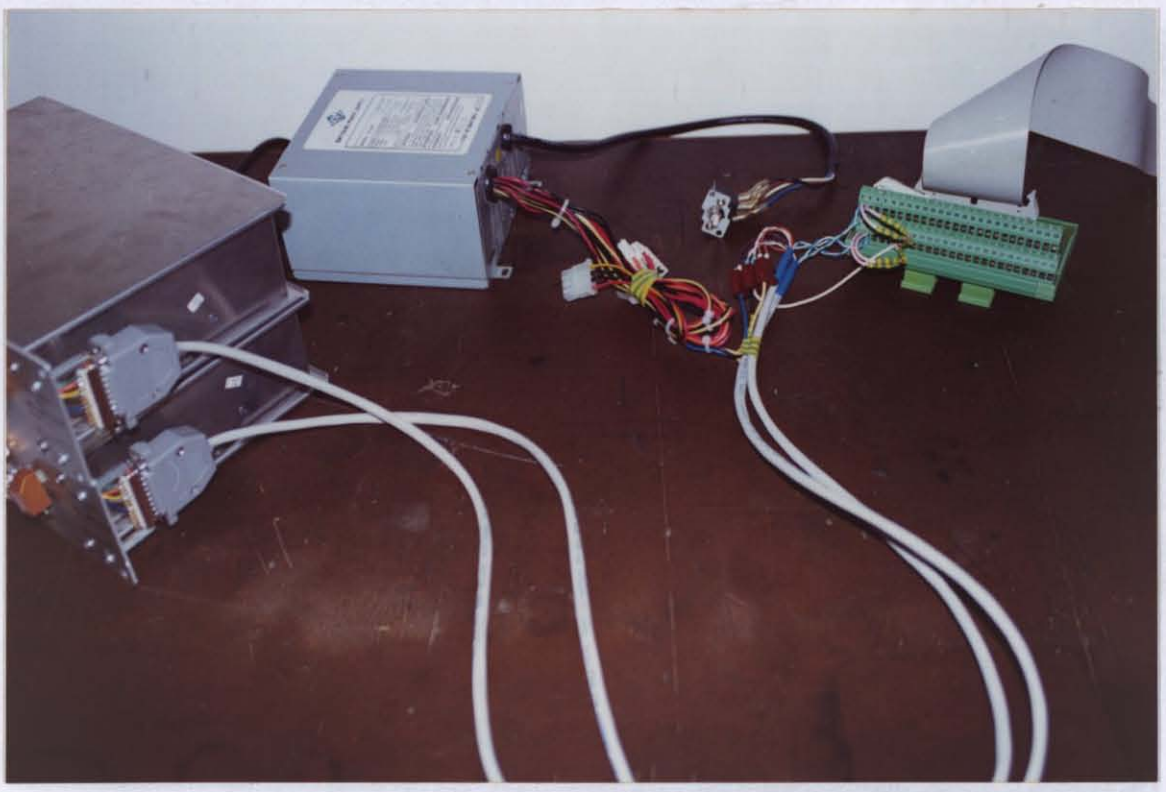


Figure 7.8 Connection of preamplifiers to connector block and power supply.

Figure 7.9 Connection of connector block to PC.

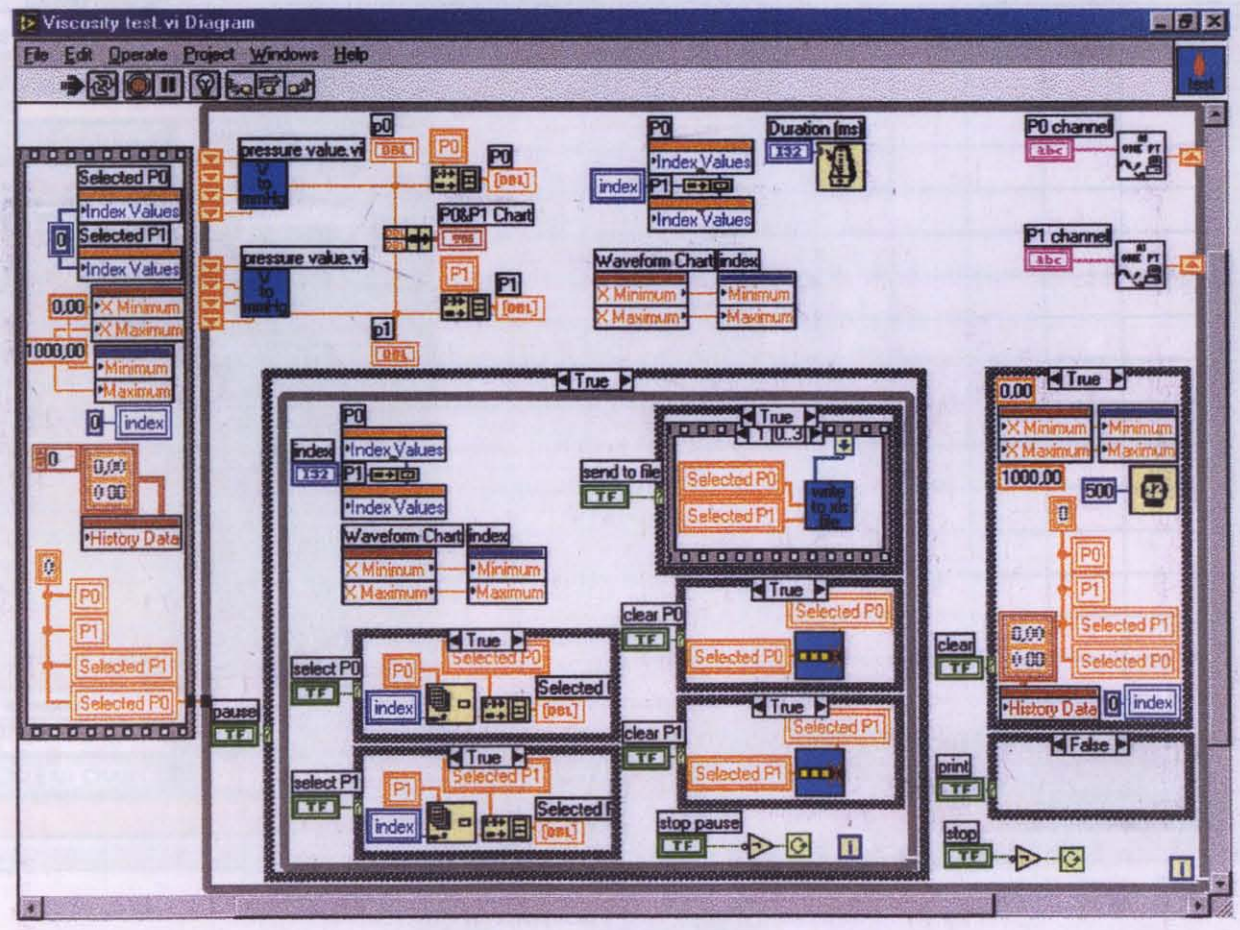


Figure 7.10 Block diagram of user interface (LabView 5.01)

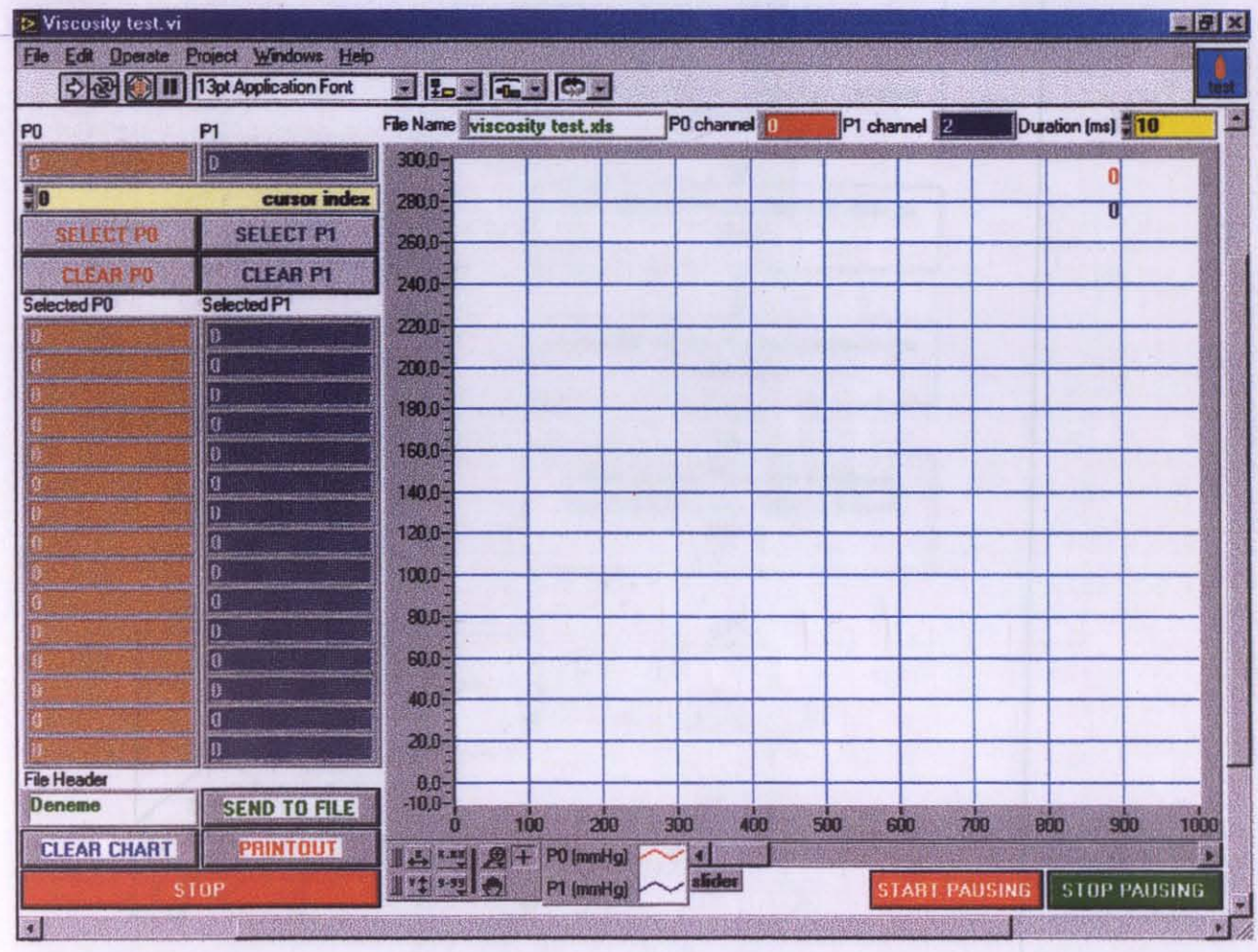


Figure 7.11 User interface

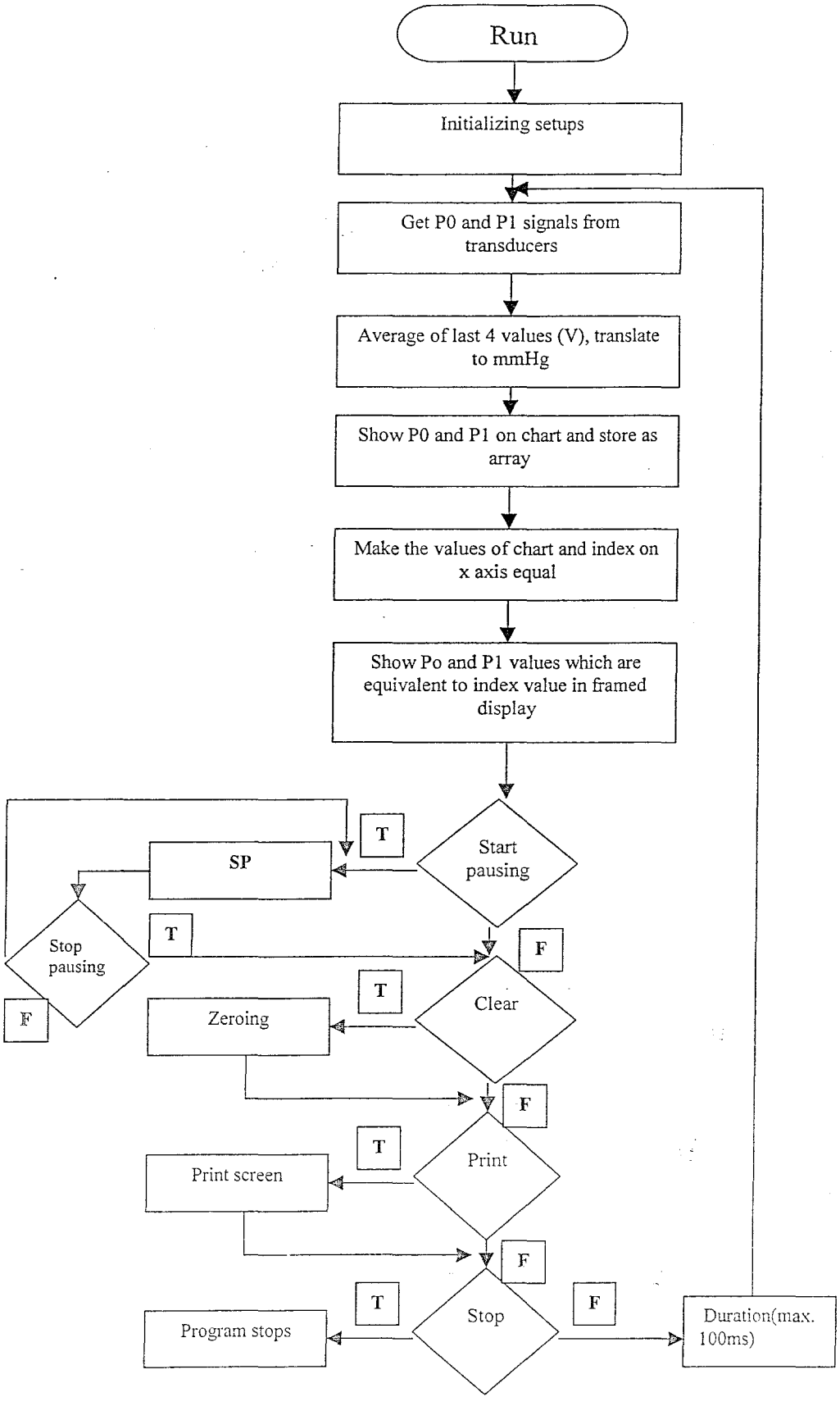


Figure 7.12 Real time viscometer flow-chart (a)

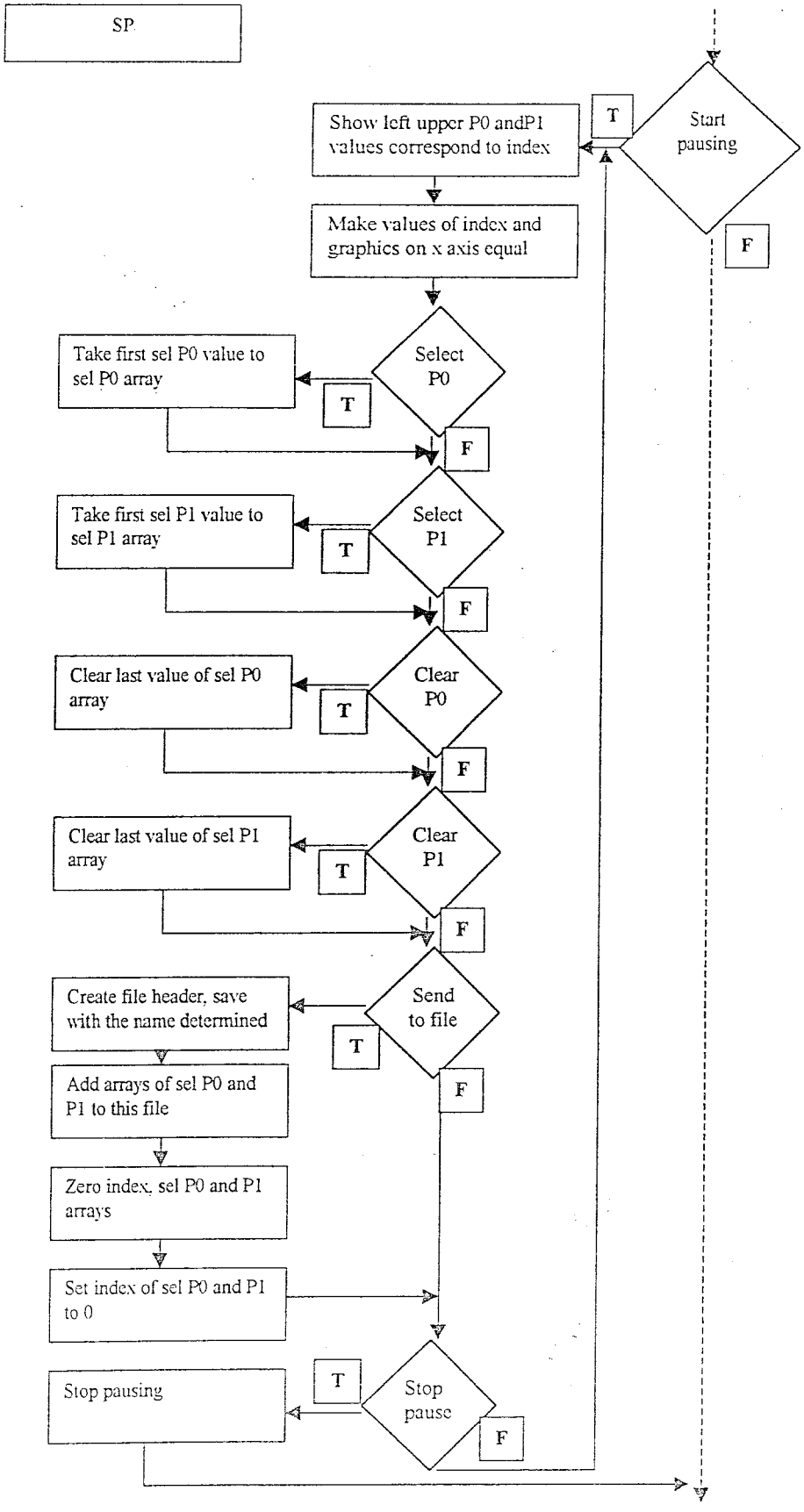


Figure 7.13 Real time viscometer flow-chart (b)

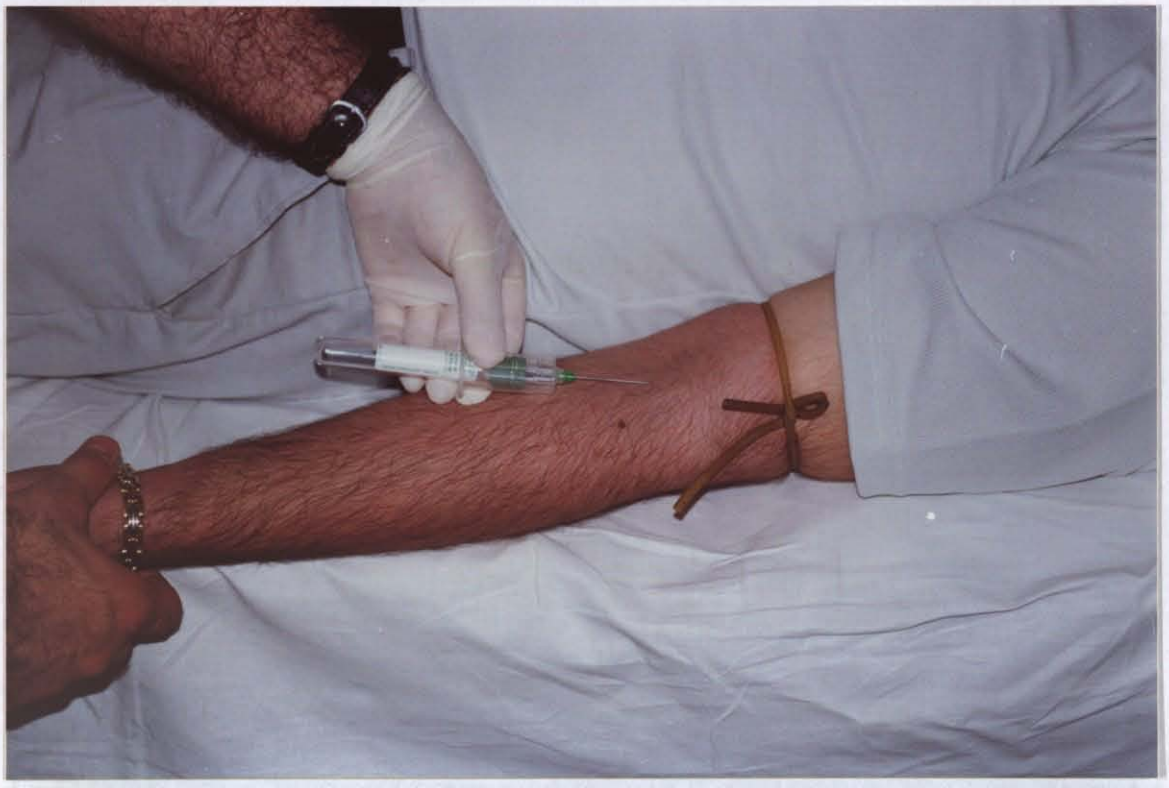


Figure 7.14 Drawing blood from a patient using Vacutainers.

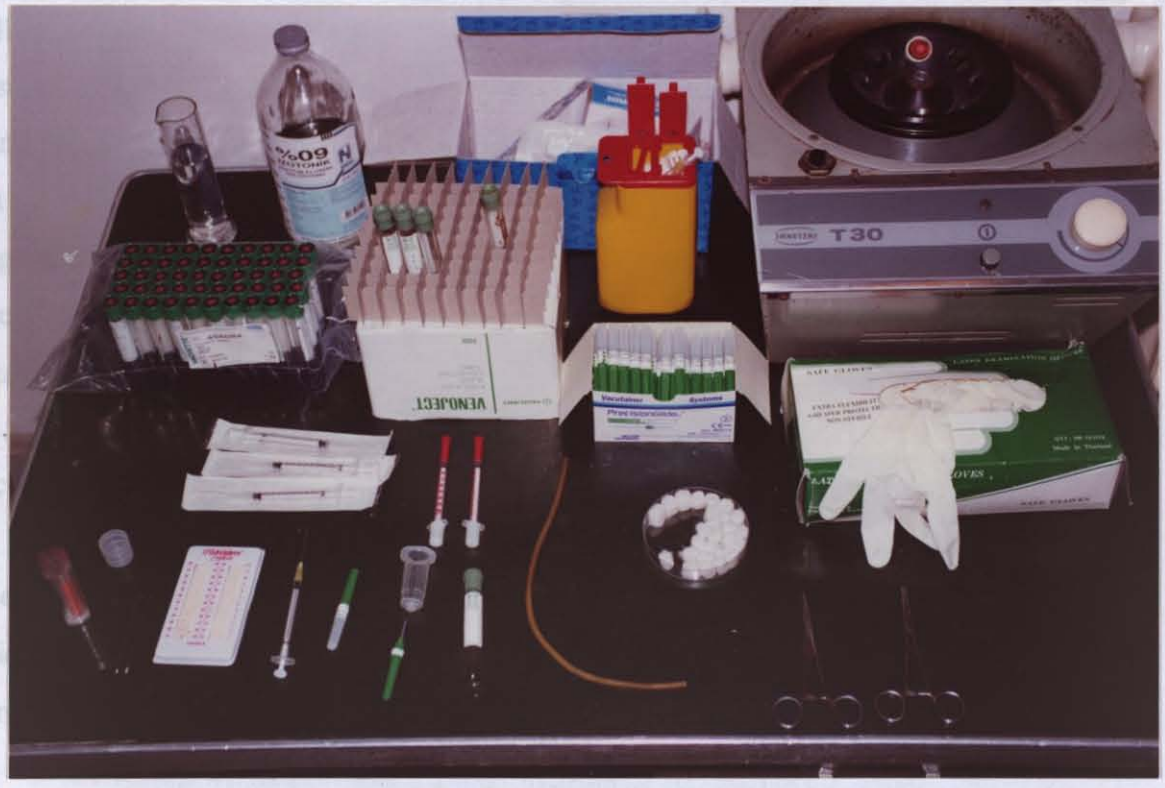


Figure 7.15 Blood handling table.

8. CLINICAL OUTPUT

In this Chapter we aimed to demonstrate the output of the viscosity measurements and describe the details of the worksheet used as patient report.

Figure 8.1 is an example for blood plasma viscosity report, correspond to Case 1 measured by Dr Kirby, in Hemorheology Laboratory, Department of Anatomy, Tulane Medical School, New Orleans, USA, shows a normal blood viscosity curve. Data from transducers, N0 and N1, was multiplied by sensitivity values (S0 and S1) then divided by 10 to find pressure values, P0 and P1, in mm Hg. Different sensitivity setting were used to cope with the resolution requirements of the setup. Q (flow rate) and μ (viscosity) were computed using the Eq.(5) and Eq.(6) respectively. On the right; the chart shows the relation between those Q and μ values. "Characteristic Curves" values (pink dots on the chart) correspond to the table on the top center. These values are the set reference points that are always 0.5, 0.1, 2.5, 5.0, 10.0, 20.0. These related N values at these reference Q points are always computed for comparison purpose. On the top left, saline calibration measurement values (as N0 and N1) both for blood and plasma, and additionally, the temperature of environment and Hct values of the case are shown. On the top right, geometrical values of the test and reference sections, blood and plasma K (vascular resistance), μ (viscosity) values of blood and plasma reference saline are also shown.

In Figure 8.2, Case 2 shows normal blood and plasma viscosity. The computations and method used to produce table in worksheet 2 created in Viscosity Laboratory, Department of Anatomy, Istanbul Medical Faculty, University of Istanbul, are exactly same with Case 1 which were created at Tulane University. Except for N0 and N1 values were in mV in Figure 8.1 while corresponding pressure values are presented as P0 an P1 in Figure 8.2. Dividing those values by ten gives us the P0 and P1 values. In this case the sensitivity values were 1.

In Figure 8.3, Case 3 shows abnormal blood viscosity values at the flow rates of 10nl/s and 20 nl/s. In this case N0 and N1 values were also in mV, and the sensitivity values were 1.

In Figure 8.4, Case 4 shows abnormal blood viscosity values at the flow rates of 0.5nl/s and 1.0 nl/s. In this case N0 and N1 values were actually in mm Hg (P0 and P1), and the sensitivity values were actually 1 on preamplifier. For the sake of using same worksheet, data was first multiplied by 10, then they were written on worksheet, and S0 and S1 values were made 10 for getting same values on P0 and P1 columns.

Name

date	exp #	age
04.12.90	90003	20

MACROS
control C to generate characteristic curves

	BLOOD	PLASMA
Calibration N_0	40,0	40,0
Calibration N_1	19,8	20,0
Temperature (C)	22,8	23,4
Hct, %	39,1	
plasma viscosity, cp	1,56	
Shape	16	

CHARACTERISTIC CURVES

Q (nl/s)	Mu (cp)	T (%/cp)	Deviation from ref group mean viscosity @ Hct	VR, #std dev
0,5	3,22	**12,14**	**normal**	**-0,27**
1,0	3,01	**13,01**	**normal**	**-0,38**
2,5	2,88	**13,60**	**normal**	**-0,32**
5,0	2,81	**13,90**	**normal**	**-0,23**
10,0	2,79	**14,01**	**normal**	**-0,06**
20,0	2,77	**14,13**	**normal**	**0,11**

		Q(nl/s)	Mu(cp)	N_0	S_0	N_1	S_1	P_0 mmHg	P_1 mmHg
blood	1	0,20	2,87	20,8	1	5,1	1	2,08	0,51
	2	0,28	3,05	30,3	1	7,1	1	3,03	0,71
	3	0,36	3,18	41,0	1	9,3	1	4,10	0,93
	4	0,52	3,26	30,3	2	13,5	1	6,06	1,35
	5	0,70	3,19	40,0	2	18,1	1	8,00	1,81
	6	0,92	3,06	20,3	5	23,7	1	10,15	2,37
	7	1,38	2,97	29,7	5	35,5	1	14,85	3,55
	8	1,90	2,92	40,3	5	24,4	2	20,15	4,88
	9	2,84	2,86	29,7	10	36,5	2	29,70	7,30
	10	3,83	2,84	39,8	10	19,7	5	39,80	9,85
	11	5,89	2,84	30,6	20	30,3	5	61,20	15,15
	12	7,73	2,79	39,7	20	39,8	5	79,40	19,90
	13	9,79	2,75	19,9	50	25,2	10	99,50	25,20
	14	14,57	2,77	29,8	50	37,5	10	149,00	37,50
	15	19,66	2,78	40,3	50	25,3	20	201,50	50,60
plasma	1	1,56		40,0	50	15,0	50	200,00	75,00
	2	1,55		40,1	50	15,1	50	200,50	75,50

Ref sect L =	25,0	mm
Test sect L =	25,0	mm
Ref radius =	20,5	um
Test radius =	20,5	um
blood K =	0,980198	
blood ref saline mu =	0,951960	cp
plasma K =	1,000000	
plasma ref saline mu =	0,938691	cp

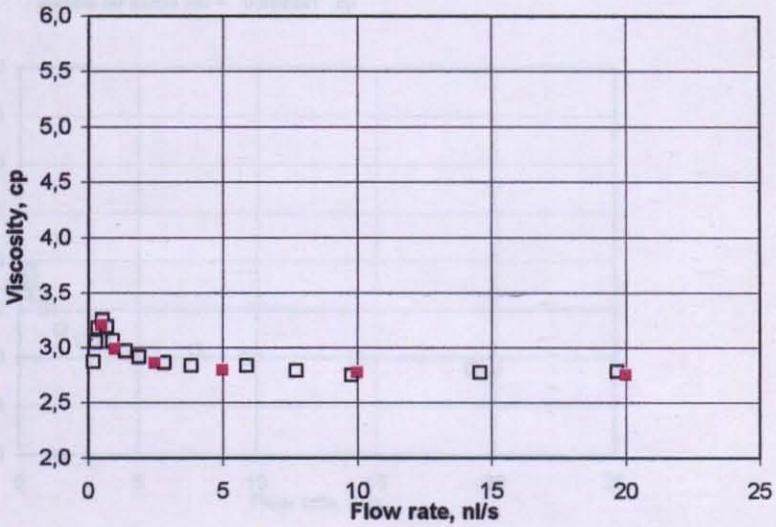


Figure 8.1 Case 1

Figure 8.2 Case 2

Name

date	exp #	age
5.18.00	200022	29

MACROS
control C to generate characteristic curves

	BLOOD	PLASMA
Calibration N_0	200,0	198,0
Calibration N_1	100,0	99,0
Temperature (C)	24,0	23,4
Hct, %	44,0	

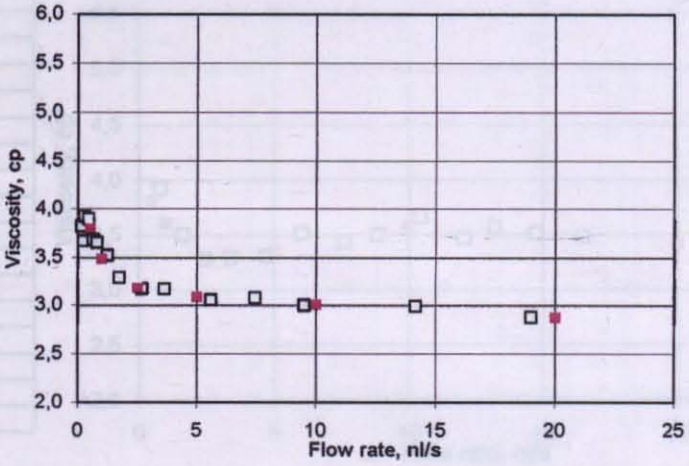
plasma viscosity, cp	1,57
Shape	36

CHARACTERISTIC CURVES

Q (nl/s)	μ (cp)	T (%/cp)	Deviation from ref group mean viscosity @ Hct	VR, #std dev
0,5	3,80	11,58	normal	0,25
1,0	3,49	12,60	normal	0,04
2,5	3,18	13,82	normal	-0,22
5,0	3,09	14,24	normal	-0,27
10,0	3,01	14,60	normal	-0,32
20,0	2,88	15,28	normal	-0,65

		Q(nl/s)	μ (cp)	N_0	S_0	N_1	S_1	P_0 mmHg	P_1 mmHg
blood	1	0,16	3,82	20,0	1	3,9	1	2,00	0,39
	2	0,24	3,67	29,8	1	6,0	1	2,98	0,60
	3	0,30	3,92	39,8	1	7,6	1	3,98	0,76
	4	0,46	3,89	60,4	1	11,6	1	6,04	1,16
	5	0,65	3,68	80,6	1	16,2	1	8,06	1,62
	6	0,82	3,66	101,0	1	20,4	1	10,10	2,04
	7	1,23	3,52	148,5	1	30,9	1	14,85	3,09
	8	1,76	3,28	200,0	1	44,0	1	20,00	4,40
	9	2,71	3,17	300,0	1	67,8	1	30,00	6,78
	10	3,64	3,16	402,0	1	91,0	1	40,20	9,10
	11	5,59	3,05	602,0	1	140,0	1	60,20	14,00
	12	7,45	3,07	806,0	1	186,5	1	80,60	18,65
	13	9,51	3,00	1010,0	1	238,0	1	101,00	23,80
	14	14,18	2,99	1500,0	1	355,0	1	150,00	35,50
	15	19,02	2,88	1955,0	1	476,0	1	195,50	47,60
plasma	1	1,56		200,0	1	75,0	1	20,00	7,50
	2	1,57		201,0	1	75,2	1	20,10	7,52

Ref sect L =	25,0	mm
Test sect L =	25,0	mm
Ref radius =	20,5	um
Test radius =	20,5	um
blood K =	1,000000	
blood ref saline mu =	0,925715	cp
plasma K =	1,000000	
plasma ref saline mu =	0,938691	cp



Name

date	exp #	age
04.28.00	102	50

MACROS
control C to generate characteristic curves

	BLOOD	PLASMA
Calibration N_0	1997,0	1998,0
Calibration N_1	992,0	994,0
Temperature (C)	22,0	22,0
Hct, %	42,0	
plasma viscosity, cp	1,81	
Shape	8	

CHARACTERISTIC CURVES

Q (nl/s)	Mu (cp)	T (%/cp)	Deviation from ref group mean viscosity @ Hct	VR, #std dev
0,5	3,83	**10,96**	**normal**	**0,68**
1,0	3,62	**11,60**	**normal**	**0,66**
2,5	3,27	**12,83**	**normal**	**0,34**
5,0	3,37	**12,45**	**normal**	**1,13**
10,0	3,59	**11,70**	**abnormal**	**2,53**
20,0	3,43	**12,24**	**abnormal**	**2,46**

Ref sect L =	25,0	mm
Test sect L =	25,0	mm
Ref radius =	20,5	um
Test radius =	20,5	um
blood K =	0,987065	
blood ref saline mu =	0,970125	cp
plasma K =	0,990040	
plasma ref saline mu =	0,970125	cp

		Q(nl/s)	Mu(cp)	N_0	S_0	N_1	S_1	P_0 mmHg	P_1 mmHg
blood	1	0,80	3,92	107,0	1	21,0	1	10,70	2,10
	2	1,64	3,50	200,0	1	43,0	1	20,00	4,30
	3	2,48	3,29	288,0	1	65,0	1	28,80	6,50
	4	3,39	3,29	395,0	1	89,0	1	39,50	8,90
	5	4,73	3,31	552,0	1	124,0	1	55,20	12,40
	6	6,02	3,51	737,0	1	158,0	1	73,70	15,80
	7	7,63	3,43	917,0	1	200,0	1	91,70	20,00
	8	8,85	3,50	1079,0	1	232,0	1	107,90	23,20
	9	10,45	3,66	1322,0	1	274,0	1	132,20	27,40
	10	12,05	3,46	1459,0	1	316,0	1	145,90	31,60
	11	13,19	3,57	1636,0	1	346,0	1	163,60	34,60
	12	14,72	3,51	1801,0	1	386,0	1	180,10	38,60
	13	16,47	3,48	2002,0	1	432,0	1	200,20	43,20
	14	0,80	3,92	107,0	1	21,0	1	10,70	2,10
	15	1,64	3,50	200,0	1	43,0	1	20,00	4,30
plasma	1	1,79	999,0	1	349,0	1	99,90	34,90	
	2	1,84	2001,0	1	686,0	1	200,10	68,60	

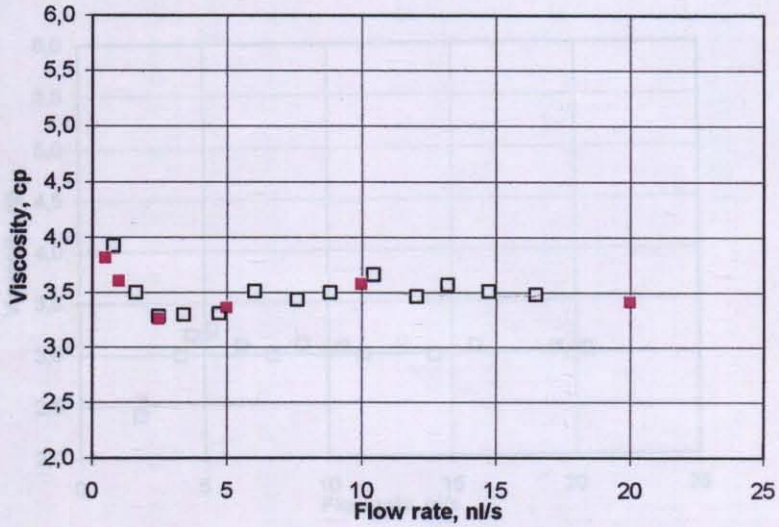


Figure 8.3 Case 3

Figure 8.4 Case 4

Name		date	exp #	age
		3.16.00	200019	38

	BLOOD	PLASMA
Calibration N_0	201,0	198,0
Calibration N_1	107,0	107,0
Temperature (C)	25,0	25,0
Hct, %	42,0	
plasma viscosity, cp	1,62	
Shape	-97	

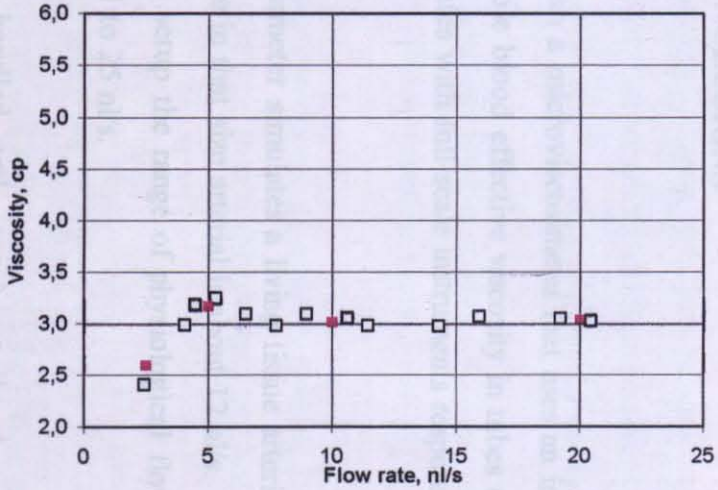
CHARACTERISTIC CURVES

Q (nl/s)	Mu (cp)	T (%/cp)	Deviation from ref group mean viscosity @ Hct	VR, #std dev
0,5	0,63	66,58	abnormal	-6,76
1,0	1,05	39,95	abnormal	-5,39
2,5	2,60	16,13	normal	-1,49
5,0	3,18	13,21	normal	0,46
10,0	3,02	13,89	normal	0,22
20,0	3,04	13,80	normal	0,67

MACROS
control C to generate characteristic curves

Ref sect L =	25,0	mm	
Test sect L =	25,0	mm	
Ref radius =	20,5	um	
Test radius =	20,5	um	
blood K =	1,138298		
blood ref saline mu =	0,904717	cp	
plasma K =	1,175824		
plasma ref saline mu =	0,904717	cp	

		Q(nl/s)	Mu(cp)	N_0	S_0	N_1	S_1	P_0 mmHg	P_1 mmHg
blood	1	1,23	1,03	6	10	3	10	6,00	3,00
	2	2,04	1,65	13	10	5	10	13,00	5,00
	3	2,45	2,40	20	10	6	10	20,00	6,00
	4	4,09	2,99	39	10	10	10	39,00	10,00
	5	4,50	3,18	45	10	11	10	45,00	11,00
	6	5,32	3,25	54	10	13	10	54,00	13,00
	7	6,54	3,09	64	10	16	10	64,00	16,00
	8	7,77	2,98	74	10	19	10	74,00	19,00
	9	8,99	3,09	88	10	22	10	88,00	22,00
	10	10,63	3,05	103	10	26	10	103,00	26,00
	11	11,45	2,98	109	10	28	10	109,00	28,00
	12	14,31	2,97	136	10	35	10	136,00	35,00
	13	15,95	3,06	155	10	39	10	155,00	39,00
	14	19,22	3,05	186	10	47	10	186,00	47,00
	15	20,44	3,03	197	10	50	10	197,00	50,00
plasma	1	1,60		200,0	10	80,0	10	200,00	80,00
	2	1,64		198,0	10	78,0	10	198,00	78,00



9. CONCLUSIONS

This study describes how to establish a microviscosimeter that uses an inline micro-flowmeter that can accurately measure whole blood effective viscosity in tubes of arteriolar diameter at physiologically nominal flow rates with full-scale instruments response time of 3 seconds.

Using microtubes with the 41 μm diameter simulates a living tissue arteriolar vessel. In the body, physiological nominal flow rate in that size arterial is about 12 nl/s. Although, this flow rate is not always steady, in this setup the range of physiological flow rate was represented more than necessary that was 0.1 to 25 nl/s.

The system, as configured, is easy handled, and can be easily cleaned. Thus, measurements in capillary part are done very fast in almost 3 minutes. Whole measurement - calibrating, blood running, recalibrating and plasma running including plasma and hematocrit centrifugation- takes about 20 minutes. To analyze data and print the report another 1 minute is required.

For that reason, the harvested data is transferred to Excel worksheet, a person who is familiar with that well-known data base software can easily manipulate all measurements.

Although, the LabView software allows to compute all these measurements, Excel worksheet is used for the sake of cooperation with other microviscosimeter equipped Hemorheology laboratory that use this pre-formatted worksheet which is shown in figure 8.1. In figures 8.2, 8.3 and 8.4, some sample of blood running results which was measured in the laboratory at Department of Anatomy, Istanbul Medical Faculty, University of Istanbul are shown on those pre-formatted worksheets.

Therefore, all data are kept in digital media, the results can be reproduced or re-evaluated any time. Besides, sending data will be possible using Internet both ways between laboratories.

The automated real time physiological viscometer is currently assembled in a laboratory room in the Department of Anatomy, Istanbul Medical Faculty - University of Istanbul, with the name of Viscosity Laboratory. It serves as a routine laboratory and accepts patients from Istanbul Medical Faculty Hospitals and Cerrahpaşa Medical Faculty Hospitals.

Currently, this system have been successfully used to diagnose elevated viscosity that would have been a valuable criteria for many clinical manifestations [50,75-84] .

Future work include several promising directions; such as discovering the best drug that will decrease elevated viscosity safely. Ginkgo Biloba (Maidenhair tree leaf) is the first candidate for that aim [85-88].

Three studies have already been started using this system;

(1) Fetal blood viscosity measurements and relevance with the premature birth.
(2) Blood viscosity changes in patients with diabetic ulcers.
(3) Blood and plasma viscosity values in the rheumatoid arthritic patients.

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