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RHEOLOGICAL AND PHYSICAL PROPERTIES OF BOVINE FIBRINOGEN-ENRICHED PLASMA

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Abstract: The rheological and physical properties of fibrinogen-enriched plasma and normal plasma samples were investigated. The density of the fibrinogen-enriched plasma ranged from 1036.65 to 1040.28 kg/m^3. The normal plasma density was1030.74 kg/m^3. For the fibrinogen-enriched plasma, the conductivity increased slightly with increasing temperature. Thermal conductivity value (0.53 $\text{Wm}^{-1}\,{}^{\circ}\text{C}^{-1}$) of the normal plasma was similar to the enriched plasma at 30°C. Thermal diffusivity values for the fibrinogen-enriched plasma were constant with increasing temperature. Thermal diffusivity value for the normal plasma was slightly higher compared to the enriched plasma samples. Specific heat of the enriched plasma samples ranged from 4143.5 to 4280.6 $\text{Jkg}^{-1}\,{}^{\circ}\text{C}^{-1}$. Temperatures within the range of 5 to 30°C had no significant influence on the specific heat of the enriched plasma sample. The shear stress of the enriched plasma samples decreased with increasing temperature. The apparent viscosity of the enriched plasma samples decreased with increasing shear rate. The plasma samples exhibited pseudoplastic characteristics. The apparent viscosity of the enriched plasma samples decreased with increasing temperature. Casson and power law models were fitted to all the plasma samples data. The influence of temperature on the apparent viscosity and yield stress of the enriched plasma samples were modeled.

INTRODUCTION

Bovine blood plasma is a complex electrolyte solution which consists of major proteins such as fibrinogen, globulins and albumin in water. Other elements present in the blood plasma during blood circulation are amino acids, vitamins, glucose, lipids, and hormones. The functional and nutritive properties of bovine plasma proteins make them essential in various health and nutritional applications. The blood plasma proteins possess suitable foaming, solubility and emulsifying properties. These properties make the plasma proteins useful in producing meat, cakes and confectionery products for human consumption (Tybor et al. 1975; Donnelly et al. 1978; Howell and Lawrie 1983; Lee et al. 1993; Myhara and Kruger 1998). The plasma proteins are also utilized in livestock feed formulation and in making pharmaceutical products (Donnelly et al. 1978; Shahidi et al. 1984; Coffey and Cromwell 2001; Quigley and Drew 2000; Moure et al. 2003).

Fibrinogen is an essential component of blood plasma which is converted to fibrin during the formation of blood clot through the enzymatic action of thrombin. Disseminated intravascular coagulation (DIC) is a disease that results in lack of fibrinogen in patients and may be congenital or acquired through severe bacterial infections, viral diseases, cancer, hemolytic-uremic syndrome, purpura fulminans, and giant hemangiomata (Hathaway et al. 1969). This condition results in intense utilization and reduction of fibrinogen. Fibrinogen concentrates and fresh frozen plasma can be used in the treatment of DIC (Franchini and Manzato 2004). Fibrinogen is also utilized in testing kits, diagnostic research, and surgery bandages (Harimex 2005).

Rheological and physical properties of bovine plasma are dependent on shear rate, temperature, concentration of proteins, and pH. Howell and Lawrie (1987) indicated that the viscosity of porcine and bovine whole blood plasma, porcine serum and porcine plasma fractions exhibited Newtonian characteristics between the temperature ranges of 20 to 73°C. The physical and rheological properties of swine blood and blood components were investigated by Rosentrater and Flores (1997) at temperatures between 5 and 35°C. The plasma was enriched with hemoglobin from the red cells as a result of the rupture of red cells during frozen storage of the blood. They indicated that all the fluids exhibited a pseudoplastic behavior and were affected by shear rate and temperature. Lee et al. (2003) showed that gamma-irradiation did not significantly affect the viscosity values of bovine and porcine plasma proteins.

Cross et al. (1991) evaluated the relationship between plasma fibrinogen concentration and tuberculin testing in red deer. They indicated that after intradermal injection of bovine purified derivative, the viscosity of red deer plasma increased with increasing plasma fibrinogen concentration. The effects of fibrinogen subfractions and ethylenediaminetetraacetic acid (EDTA) denatured fibrinogen on the viscosity of human blood plasma were investigated by Jensen et al. (2004). They concluded that the viscosity of the main fibrinogen subfractions did not differ from the native fibrinogen, and the use of EDTA as anticoagulant did not significantly affect the viscosity of the fibrinogen at 37°C.

The investigation of the rheological and physical properties of bovine blood plasma is important in designing of blood plasma processing machines such as pipelines, pumps, centrifuges, spray dryers and freeze dryers. These properties may also be required in the design of health-related machines such as blood oxygenators and dialysis devices. Rheological and physical properties of bovine plasma and fibrinogen-enriched bovine plasma were investigated at different temperatures.

MATERIALS AND METHODS

Bovine Blood Plasma Samples

Normal bovine plasma and bovine fibrinogen-enriched plasma were supplied by Harimex Inc., Strathmore, AB. Upon receipt, the samples were stored in a freezer at a temperature of -20°C until they were used. The plasma samples were thawed at room temperature (23°C) for 3 hours before they were used. The fibrinogen concentrations in the samples were not supplied by the company or determined at our laboratory. The fibrinogen-enriched plasma was tested at temperatures of 5, 15, and 30°C. The normal plasma sample was tested at a temperature of 4°C. These temperatures were selected according to the recommendations of the supplier.

Density of Plasma Samples

A 25-ml pycnometer density bottle was used to determine the density of the plasma samples. To determine the density, a sample was placed in a beaker, brought to the required temperature and kept constant. The mass of the pycnometer bottle and cover was measured. The plasma sample was immediately poured into the bottle and covered with a stopper. The excess liquid on the bottle was wiped away, and the mass of the bottle, cover and sample was determined. Four replications were conducted on each sample test. The density of a plasma sample was then calculated from the sample mass and known volume of the bottle.

Thermal Properties of Plasma Samples

The thermal properties analyzer (KD2) developed by Decagon Devices, Inc., Pullman, WA, was used to determine the thermal properties of the bovine plasma samples. The samples were placed in a beaker after thawing and a digital refrigerated circulating bath (Neslab Instruments, Inc., Portsmouth, NH) was used to bring the samples to the desired temperature and to keep it constant. Four measurements were made of each sample test. The KD2 device has an accuracy of 5% for thermal conductivity and 10% for thermal diffusivity measurements. The range of measurement for thermal conductivity is 0.1 to 2 $Wm^{-1}C^{-1}$ and that for diffusivity is 0.1 to 1.0 mm^2/s. The thermal conductivity and diffusivity values of the samples were produced by the device. The specific heat capacity of the samples was calculated from the knowledge of density, thermal conductivity and diffusivity using the equation below:

$$C_p = \frac{k_t}{\rho \alpha} \tag{1}$$

where, C_p is specific heat capacity ($Jkg^{-1}\,^{o}C^{-1}$), k_t is thermal conductivity ($Wm^{-1}\,^{o}C^{-1}$), ρ is density (kg/m^3), and α is thermal diffusivity (m^2/s).

Apparent Viscosity of the Plasma Samples

The apparent viscosity of the plasma samples was measured using a programmable rheometer (Model DV-III, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Each sample was poured into a Brookfield UL adapter consisting of a precision cylindrical spindle and a tube. A ULA-40Y water jacket was attached to the tube. The water jacket was connected to the refrigerated circulating bath which allowed a precise control and maintained the temperature of the sample constant. Figure 1 shows the programmable rheometer.

The rheometer was programmed to make measurements at 7 shear rates: 12.23, 18.35, 24.46, 36.69, 48.92, 61.15, and 73.38 s^{-1}. All the samples were allowed to achieve a constant temperature before measurements were carried out. About four replications were made on each sample test.

Data Analysis

A statistical package, SPSS 11.5.0 for Windows, SPSS Inc., Chicago, IL, was used to analyze the results. Mean, standard deviation, and one-way analysis of variance were conducted on the data at 5% level of significance. Duncan multiple range test was used to compare the means.

To determine whether the apparent viscosities of plasma samples were Newtonian or non-Newtonian, the ratio of the plasma sample's viscosity measured at two different speeds was determined. In determining the ratio the viscosity value at the lower speed is placed in the numerator and the one at the higher speed is placed at the denominator. The value of the ratio exceeding 1.0 indicates a pseudoplastic material and a value less than 1.0 will indicate a dilatant material. For Newtonian material, the ratio will be equal to 1.0 (Brookfield Engineering 2003). A factor of 2 was established for the computation of the viscosity ratios. The viscosity measurements were plotted against shear rates which also gave an indication of the characteristics of the plasma samples.

Since the viscosity ratio of all the plasma samples were greater than 1.0, the data was analyzed using Casson and power law models. Casson's two-parameter model is given as:

$$\sqrt{\sigma} = \sqrt{\sigma_0} + \sqrt{\eta}\sqrt{\gamma} \qquad (2)$$

where, σ is the shear stress (Pa), σ_0 is the yield stress (Pa), η is the apparent viscosity (Pa s) and γ is the shear rate (1/s).

A two-parameter model for the power law is expressed as:

$$\sigma = k\gamma^{n} \qquad (3)$$

where, k is the consistency index (Pa s^n) and n is the flow behavior index.

The parameters in these models were determined using TableCurve 2D Windows v2.01 (Jandel Scientific, San Rafael, CA).

RESULTS AND DISCUSSION

Density of Plasma Samples

The results of the density measurements of the fibrinogen-enriched plasma and bovine plasma are presented in Table 1. Density of the fibrinogen-enriched plasma ranged from 1036.65 to 1040.28 kg/m^3. There was no significant difference between the enriched plasma samples at temperatures of 5 and 15°C at 5% level. The density of the normal plasma was the lowest (1030.74 kg/m^3) and it was significantly different from the enriched plasma at all temperatures. It appears the density of the enriched plasma decreased with increasing temperature from 15 to 30^{o}C.

Rosentrater and Flores (1997) gave the density of normal swine blood plasma at temperatures of 5 and 15^{o}C as 1062.85 and 1054.75 kg/m^3, respectively. They indicated that the fluids had a linear relationship with temperature. The density values obtained in these tests were lower than the values obtained by Rosentrater and Flores. The density of each fluid decreased with increasing temperature. Density of pure water at 4, 15 and 30^{o}C are given as 1000.00, 999.10 and 995.65 kg/m^3, respectively.

Thermal Properties of Plasma Samples

The results of the thermal properties of the plasma samples are presented in Table 2. Thermal conductivity values for the enriched plasma increased slightly with increasing temperature. There was no significant difference between the thermal conductivity values at temperatures of 5 and 15^{o}C at 5% level. The thermal conductivity value for the normal plasma was similar to the value of the enriched plasma at 30^{o}C. Rosentrater and Flores (1997) indicated that thermal conductivity values of normal swine plasma increased with temperature from 5 to 20^{o}C. Zhang et al. (2003) measured the thermal conductivity of blood plasma at 27^{o}C using a dual-thermistor probe and gave the thermal conductivity value as 0.592 Wm^{-1} ^{o}C^{-1}. Spells (1960) determined the thermal conductivities of rat blood at 30.4^{o}C, human blood at 36.8^{o}C and human blood plasma at 36.0^{o}C as 0.506, 0.506 and 0.577 Wm^{-1} ^{o}C^{-1}, respectively.

The thermal diffusivity value for the enriched plasma samples was constant at 0.12 mm^2/s with increasing temperature from 5 to 30^{o}C. There was no significant difference between the values. The thermal diffusivity value for the normal plasma was slightly higher than the values for the enriched plasma. Rosentrater and Flores (1997) calculated and gave the thermal diffusivity of whole swine blood to be 0.071 mm^2/s at a temperature of 15^{o}C. Zhang et al. (2003) gave the thermal diffusivity of blood plasma at 27^{o}C as 0.141 mm^2/s. Balasubramaniam and Bowman (1977) measured the thermal diffusivities of human whole blood and human blood plasma at 21.0^{o}C as 0.119 and 0.121 mm^2/s, respectively.

Specific heat of the enriched plasma ranged from 4143.5 to 4280.6 Jkg^{-1} ^{o}C^{-1}. Specific heat of the normal plasma was the lowest compared to the values of the enriched plasma. There was no statistical difference between the specific heat values for the enriched plasma. There is an indication that temperature had no influence on the specific heat of the plasma samples.

Apparent Viscosity of Plasma Samples

Figure 2 shows the variation of temperature with shear stress for the plasma samples. The figure shows that the shear stress of all the samples increased with increasing shear rate. As the temperature of the enriched plasma samples increased, the shear stress of the samples decreased. It appears that both temperature and shear rate have effects on the shear stress.

The apparent viscosity of the plasma samples at different temperatures is presented in Figure 3. The apparent viscosity of all the plasma samples decreased with increasing shear rate. The characteristics of the graphs indicate a nonlinear relationship between the apparent viscosity and the shear rate. Also, the apparent viscosity of the enriched plasma samples decreased with increasing temperature. Viscosity ratios of the enriched plasma samples at 5, 15, and 30°C ranged from 1.07 to 1.12, 1.15 to 1.23 and 1.03 to 1.04, respectively. The normal plasma had viscosity ratios ranging from 1.06 to 1.15. Therefore, the plasma samples exhibited pseudoplastic characteristics.

Tables 3 and 4 show the parameter estimates, R^2 and standard errors for Casson and power law models for the plasma samples. The power law model best described the enriched plasma data at 5°C with higher R^2 (0.9991) and lower standard error (0.05145) compared to the Casson model. The Casson model fitted well the enriched plasma data at 15 and 30°C with a higher R^2 and lower standard error than the power law. The Casson model provided a best fit for the normal plasma sample at a temperature of 4°C with R^2 of 0.9998 and standard error of 0.00119 compared to the power law model.

For the Casson model, the yield stress and apparent viscosity parameters decreased with increasing temperature. The consistency index decreased with increasing temperature for enriched plasma using the power law. The flow behavior index did not show any pattern with temperature. The enriched plasma at 15°C showed more pseudoplastic behavior, and the enriched plasma at 30°C was closer to unity. The viscosity values obtained using Casson model were lower than the values obtained using the power law model.

A plot of the natural logarithm of the apparent viscosity against the inverse of the absolute temperature displayed a curvilinear behavior instead of linear (Figure 4). The effect of temperature on the apparent viscosity was modeled using the apparent viscosity values obtained from the Casson model as follows with R^2 of 0.9999 and standard error of 0.00045:

$$\eta = \frac{1}{3.75646T - 1027.7892} \qquad (4)$$

where, T is absolute temperature (K).

Using the consistency flow index values obtained from the power law model, the influence of temperature on the consistency index was modeled as follows with R^2 of 0.9998 and standard error of 0.00326:

$$k = \sqrt{4.07512 - 0.01327T} \qquad (5)$$

where, T is absolute temperature (K).

Yield stress values obtained from the Casson model showed a linear relationship between the absolute temperature. The relationship was described by an equation with R^2 of 0.996 and standard error of 0.00326 as:

$$\sigma_0 = 0.872547 - 0.0028727T \qquad (6)$$

where, T is absolute temperature (K).

Jensen et al. (2004) reported that increasing the temperature of purified fibrinogen solutions from 20 to 37°C resulted in 30% reduction in viscosity. They gave the viscosity of native fibrinogen at 37°C and concentration of 1.94 g/l as 0.000668 Pa s, which is lower than the viscosity values obtained in these tests. Howell and Lawrie (1987) determined the apparent viscosity of 6% bovine plasma at a temperature of 20°C as 0.0761 Pa s. Rosentrater and Flores (1997) reported the consistency index of porcine blood plasma as 0.03 Pa s at temperatures of 5 and 15°C. Zhou et al. (1999) determined the viscosity of broiler chickens plasma at temperatures of 20 and 30°C as 0.0009921 and 0.0009735 Pa s, respectively.

CONCLUSIONS

From the experimental results, the following conclusions can be made:

1. The density of the fibrinogen-enriched plasma ranged from 1036.65 to 1040.28 kg/m^3. The normal plasma density was1030.74 kg/m^3.

2. For the fibrinogen-enriched plasma, the conductivity increased slightly with increasing temperature. Thermal conductivity value (0.53 Wm^{-1} $^{o}C^{-1}$) of the normal plasma was similar to the enriched plasma at 30°C.

3. Thermal diffusivity values for the fibrinogen-enriched plasma were constant with increasing temperature. Thermal diffusivity value for the normal plasma was slightly higher compared to the enriched plasma samples.

4. Specific heat of the enriched plasma samples ranged from 4143.5 to 4280.6 Jkg^{-1} $^{o}C^{-1}$. Temperature had no significant influence on the specific heat of the plasma sample.

5. The shear stress of the enriched plasma samples decreased with increasing temperature. The apparent viscosity of the enriched plasma samples decreased with increasing shear rate. The plasma samples exhibited pseudoplastic characteristics. The apparent viscosity of the enriched plasma samples decreased with increasing temperature. The Casson and power law models were fitted to all the plasma samples data. The influence of temperature on the apparent viscosity and yield stress of the enriched plasma samples were modeled.

REFERENCES

Balasubramaniam, T.A. and H.F. Bowman. 1977. Thermal conductivity and thermal diffusivity of biomaterials: A simultaneous measurement technique. Journal of Biomechanical Engineering 99(3): 148-154.

Brookfield Engineering. 2003. More solutions to sticky problems: A guide to getting more from your Brookfield viscometer. 2003. Middleboro, MA: Brookfield Engineering Laboratories, Inc.

Coffey, R.D. and G.L. Cromwell. 2001. Use of spray-dried animal plasma in diets for weanling pigs. Pig News and Information 22(2): 39N-48N.

Cross, J.P., G.E. Reynolds, C.G. Mackintosh and J.F.T. Griffin. 1991. Evaluation of relationship between plasma fibrinogen concentration and tuberculin testing in red deer. Journal of the American Veterinary Medical Association 198(10): 1785-1788.

Donnelly, E.B., A.M. Delaney and N. Hurley. 1978. Studies on slaughter animal blood plasma. I. Composition of bovine and porcine plasma. Journal of Food Science and Technology 2(1):31-38.

Franchini, M. and F. Manzato. 2004. Update on the treatment of disseminated intravascular coagulation. Hematology 9(2): 81-85.

Harimex. 2005. Products: Fibrinogen. http://www.harimex.nl/pharma7.html (2005/06/16).

Hathaway, W.E., M.M. Mull and G.S. Pechet. 1969. Disseminated intravascular coagulation in the newborn. Pediatrics 43(2): 233-240.

Howell, N.K. and R.A. Lawrie. 1987. Functional aspects of blood plasma proteins. V. Viscosity. International Journal of Food Science and Technology 22(2): 145-151.

Howell, N.K. and R.A. Lawrie. 1983. Functional aspects of blood plasma proteins. I. Separation and characterisation. Journal of Food Technology 18(6): 747-762.

Jensen, T., S. Halvorsen, H.C. Godal, P.M. Sandset and O.H. Skjønsberg. 2004. The viscosity of fibrinogen subfractions and of EDTA denatured fibrinogen do not differ from that of native fibrinogen. Thrombosis Research 113(1): 51-56.

Lee, S., S. Lee and K.B. Song. 2003. Effect of gamma-irradiation on the physicochemical propertites of porcine and bovine blood plasma proteins. Food Chemistry 82(4): 521-526.

Lee, C., J. Love and L. Johnson. 1993. Sensory and physical properties of cakes with bovine plasma products substitution for egg. Cereal Chemistry 70(1): 18-21.

Myhara, R.M. and G. Kruger. 1998. The performance of decolorized bovine plasma protein as a replacement fro egg white in high ratio white cakes. Food Quality and Preference 9(3): 135-138.

Moure, F., M. Rendueles and M. Diaz. 2003. Coupling process for plasma protein fractionation using ethanol precipitation and ion exchange chromatography. Meat Science 64(4): 391-398.

Quigley, J.D. and M.D. Drew. 2000. Effects of oral antibiotics or bovine plasma on survival, health and growth in dairy calves challenged with *Escherichia coli*. Food and Agricultural Immunology 12(4): 311-318.

Rosentrater, K.A. and R.A. Flores. 1997. Physical and rheological properties of slaughterhouse swine blood and blood components. Transactions of the ASAE 40(3): 683-689.

Shahidi, F., M. Naczk, L.J. Rubin and L.L. Diosady. 1984. Functional properties of blood globin. Journal of Food Science 49(2): 370-372.

Spells, K.E. 1960. The thermal conductivities of some biological fluids. Physics in Medicine and Biology 5(2): 139-153.

Tybor, P.T., C.W. Dill and W.A. Landmann. 1975. Functional properties of proteins isolated from bovine blood by a continuous pilot process. Journal of Food Science 40(1): 155-159.

Zhang, H., L. He, S. Cheng, Z. Zhai and D. Gao. 2003. A dual-thermistor probe for absolute measurement of thermal diffusivity and thermal conductivity by heat pulse method. Measurement Science and Technology 14(8): 1396-1401.

Zhou, W.T., M. Fujita and S. Yamamoto. 1999. Effects of ambient temperatures on blood viscosity and plasma protein concentration of broiler chickens (*Gallus domesticus*). Journal of Thermal Biology 24(): 105-112.

Table 1. Variation of plasma samples with temperature.

Material	Temperature (^oC)	Density (kg/m^3) Mean	SD
Enriched plasma	5	1040.28c	1.08
Enriched plasma	15	1040.79c	0.54
Enriched plasma	30	1036.65b	0.26
Normal plasma	4	1030.74a	0.26

Means with the same letter are not significantly different at 5% level.
SD = standard deviation

Table 2. Thermal properties of the plasma samples.

Material	Temperature (^oC)	Thermal conductivity ($Wm^{-1}\,{}^oC^{-1}$)		Thermal diffusivity (mm^2/s)		Specific heat capacity ($Jkg^{-1}\,{}^oC^{-1}$)	
		Mean	SD	Mean	SD	Mean	SD
Enriched plasma	5	0.51a	0.00	0.12a	0.00	4198.3a	176.3
Enriched plasma	15	0.52a	0.00	0.12a	0.00	4143.5a	40.1
Enriched plasma	30	0.53b	0.00	0.12a	0.00	4280.6a	40.1
Normal plasma	4	0.53b	0.02	0.13b	0.06	4096.8a	125.4

Means with the same letter are not significantly different at 5% level.

Table 3. Parameters, R^2 and standard error for Casson model for the plasma samples at different temperatures.

Material	Temperature (^oC)	Parameters σ_0 (Pa)	Ŋ (Pa s)	R^2	Standard error
Enriched plasma	5	0.07191	0.05860	0.9982	0.07442
Enriched plasma	15	0.04741	0.01804	0.9999	0.00615
Enriched plasma	30	0.00062	0.00938	1.0000	0.00142
Normal plasma	4	0.00168	0.00278	0.9998	0.00119

Table 4. Parameters, R^2 and standard error for the power law model for the plasma samples at different temperatures.

Material	Temperature	Parameters		R^2	Standard error
	(°C)	k (Pa s^n)	n		
Enriched plasma	5	0.14661	0.8417	0.9991	0.05145
Enriched plasma	15	0.06308	0.7876	0.9995	0.01243
Enriched plasma	30	0.00118	0.9599	0.9999	0.00206
Normal plasma	4	0.00533	0.8882	0.9993	0.00203

Figure 1. Brookfield programmable rheometer model DV-III.

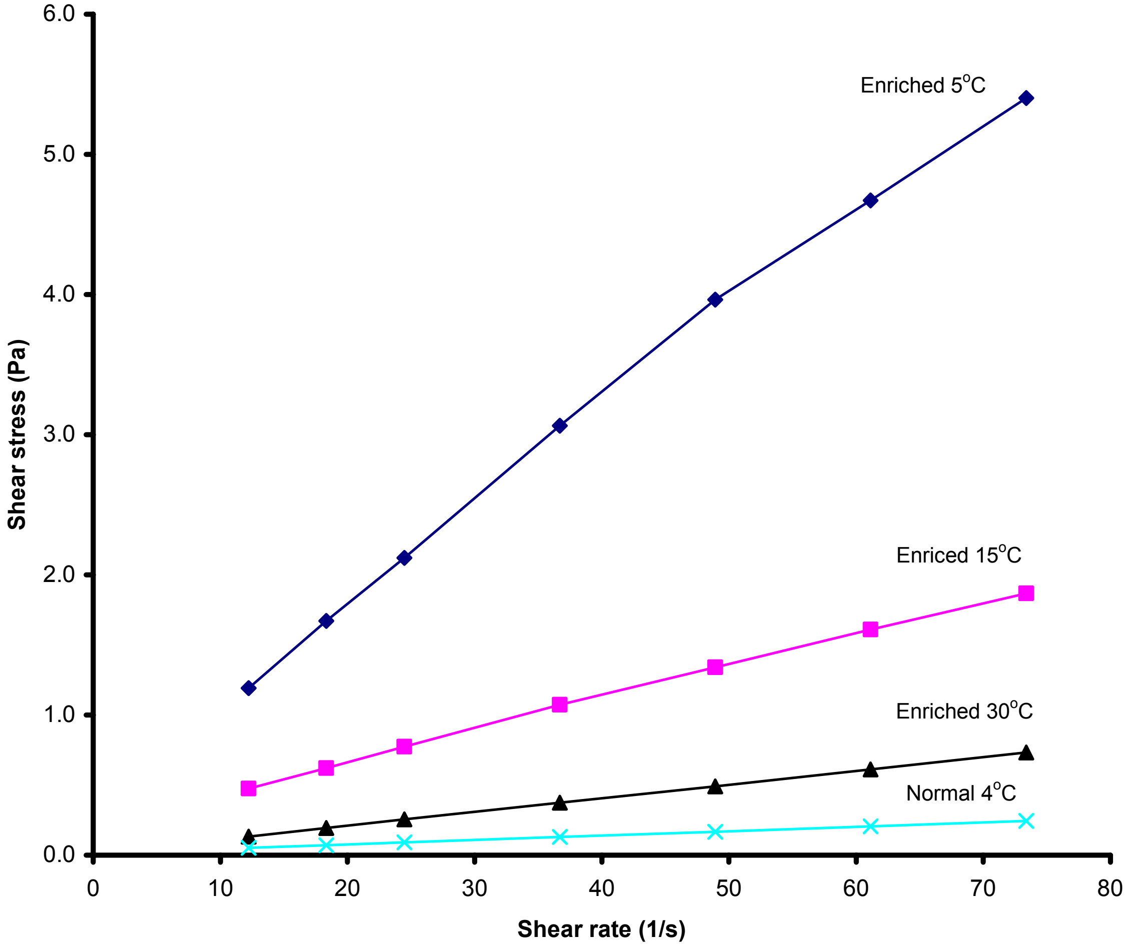

Figure 2. Variation of plasma samples temperatures with shear stress

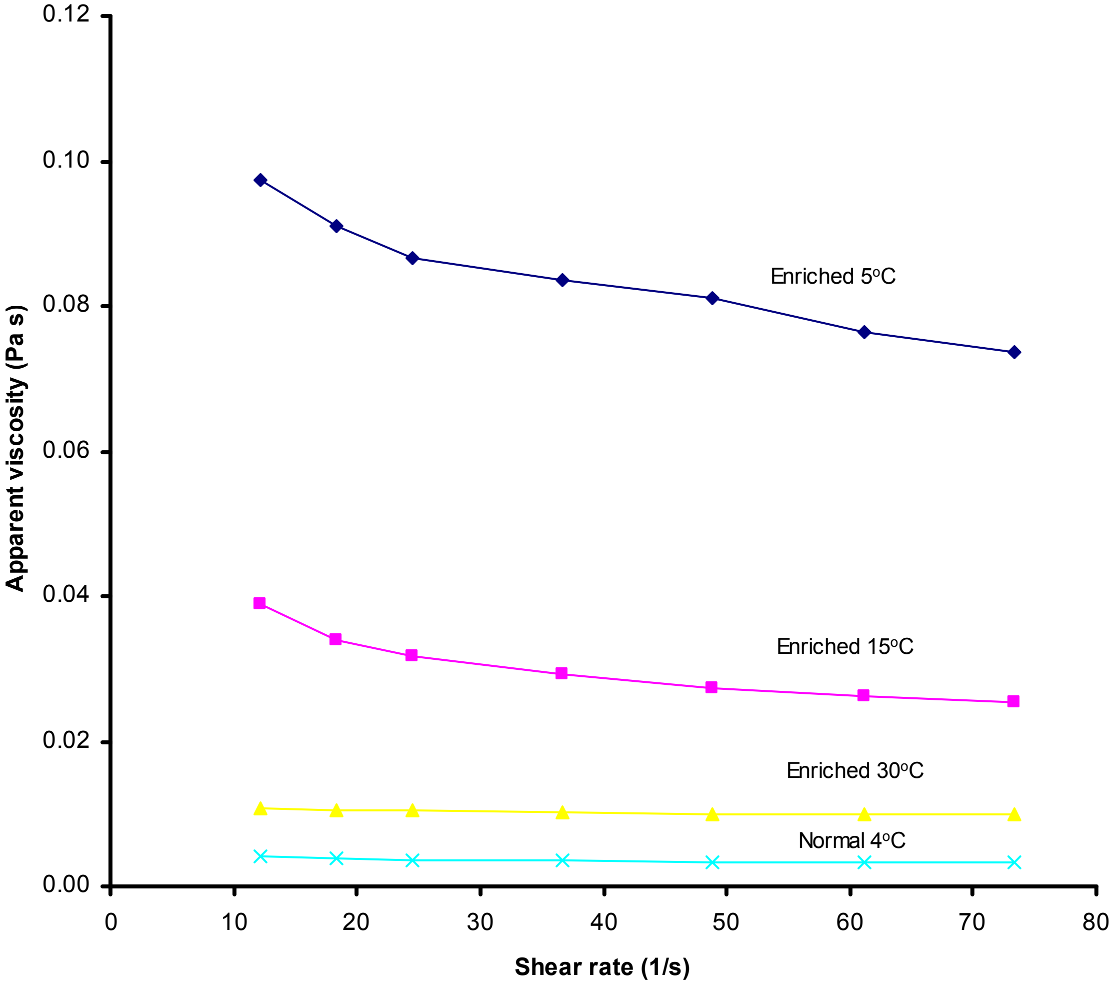

Figure 3. Apparent viscosity of the plasma samples at different temperatures

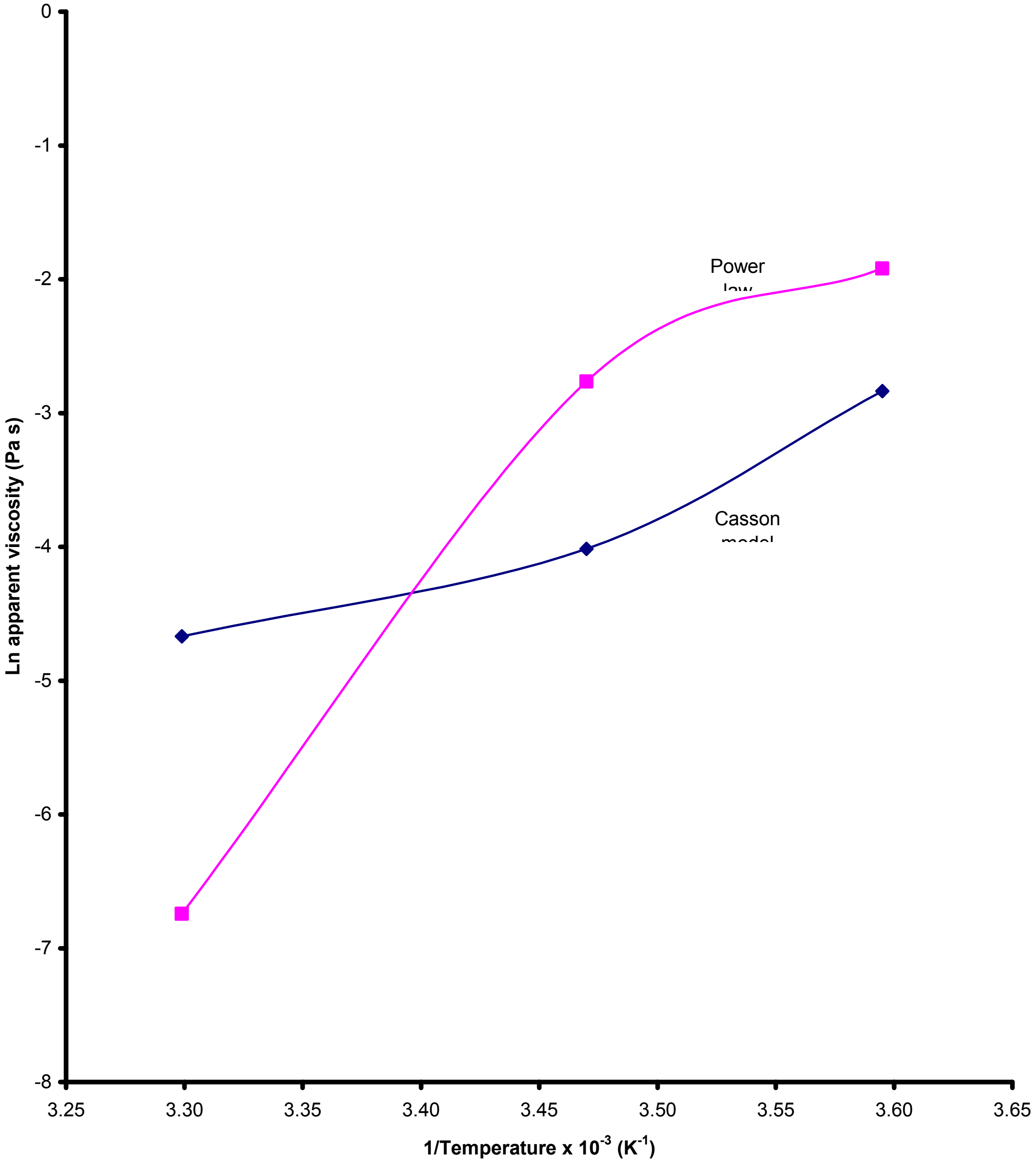

Figure 4. Typical plot of ln apparent viscosity against inverse of absolute temperature for Casson and power law models for the enriched plasma samples.