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THERMOANALYTICAL INVESTIGATION OF BLOOD

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The aim of this study is to understand some properties and thermal behavior of blood, giving a possible alternative tool which differs from the traditional blood diagnostic methods, and to improve investigations in hematology and artificial bloods. Wistar rat blood samples (WRBS); SHR rat blood samples (SHRBS) and human blood samples (HBS) were analyzed. TG curves showed two decomposition stages for HBS at around 100 and 230°C (T_{onset}), while three mass degradation stages for WRBS (70, 110, 270°C) and SHRBS (70, 120, 270°C) could be observed. DSC peaks showed five endotherms for HBS at 65, 82, 194, 201 and 309°C and three endotherms for WRBS at 83, 184 and 313°C.

Keywords: *blood, differential scanning calorimetry, thermogravimetry*

Introduction

Blood is a viscous, slightly alkaline fluid that accounts for approximately 7% of body mass [1]. Blood is a specialized connective tissue consisted of cellular elements in an aqueous salt solution (Fig. 1).

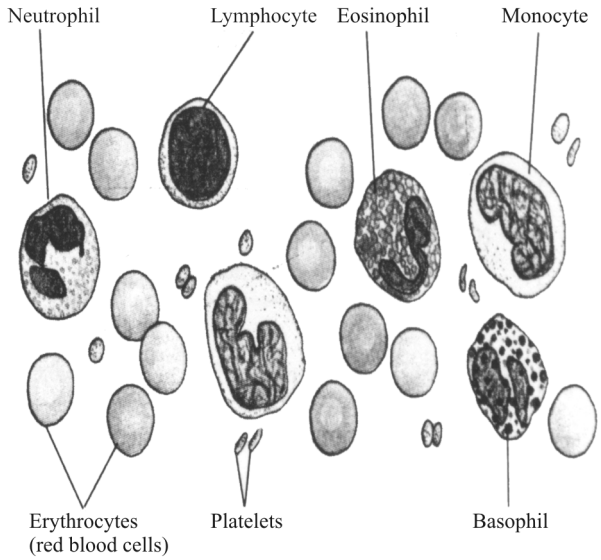

Fig. 1 Cells and platelets of circulating blood [1]

The principal functions of blood are conveying nutrients from the gastrointestinal system to all of the cells of the body and subsequently delivering the waste products of those cells to specific organs elimination [1, 2]. Some further functions of blood circulation are: carrying hormones and other regulatory agents to and from the cells and tissues of the body; carrying oxygen by the hemoglobin within erythrocytes from the lungs for distribution the cells of the organism; carrying CO_2 from the cells to the lungs. Moreover, blood functions in regulating body temperature, to maintain the acid/base balance and osmotic balance of the body fluids [1, 3]. Blood acts as pathway for migration of white blood cells, between various tissues of the body.

By blood centrifugation two fractions can be obtained: one aqueous part called to plasma and a solid part consisted of cells. Approximately 91–92% of plasma is water while the rest contains dissolved substances: 7–8% of proteins (albumin, globulin and fibrinogen), electrolytes, and substances containing nitrogen, blood gases and others (Table 1). In general, the amount of plasma is about 55% with regard to the total blood volume and of the remaining 45% is consisted of cells: erythrocytes (red blood cells), leukocytes (white blood cells) and platelets [2, 3].

A normal adult has about 35 mL of red cells per kg of body mass [2]. Red blood cells are packed with hemoglobin, a large tetra protein (M≈68 000) containing four polypeptide chains, each of them covalently bond to an iron containing heme.

Blood also contains a number of colorless cells. The leukocytes are true cells with nucleus and cytoplasm. Their number is far smaller than that of erythrocytes [3].

Since XVIII century, blood transfusions have been a solution to complete the loss of blood caused by injuries, surgeries, childbirth and hemorrhages. In addition, the cells of the blood and of the blood cell

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Table 1 Composition of blood plasma [2]

Component	%
Water	91–92
Proteins (fibrinogens, globulin, albumin)	7–8
Other solutes	1–2
Electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-})	
Non-protein nitrogen containing substances (urea, uric acid, creatine, creatinine, ammonium salts)	
Nutrients (glucose, lipids, amino acids)	
Blood gases (oxygen, carbon dioxide, nitrogen)	
Regulatory substances (hormones, enzymes)	

forming tissues have been widely studied in the last decades [1]. After the 1980s, due to several public health crises, including recurred blood shortages and increasing number of HIV and hepatitis infections, led reserchers to intensify their studies and to develop safe artificial blood substitutes [4].

An artificial blood would have a great number of advantages over donated blood. Sources of artificial blood would not be limited, it would be free of lethal viruses, and it might have a longer shelf-life than the natural blood has. Recent studies in blood substitutes are still based on modified hemoglobin because free hemoglobin is too toxic for the body [5, 6].

In this aspect, thermal analyses of natural blood helps to understand its properties and behavior, moreover can help to recognize some blood anomalies. Based on the provided information one is able to produce artificial blood being more and more similar to the natural blood and eliminating danger for human health.

Experimental

Human blood samples (HBS)

Peripheral human blood was taken by volunteers, healthy young women students, (20–30 years) from State University of Rio de Janeiro. Blood was taken from donors by pricking a finger and pressing the blood directly onto a pan (a platinum pan for TG/DTA analyses and an aluminum pan that was immediately closed for DSC analyses). Blood did not coagulate during this short period (10–15 s); therefore no anticoagulants were used [7].

Wistar rat (WRBS) and SHR rat blood samples (SHRBS)

Blood of male Wistar and SHR (spontaneous hypertension rats) rats obtained from colonies and maintained in the State University of Rio de Janeiro from birth to 6 months old have been studied. The University Standing Committee on Animal Research had approved the protocols. The investigation conforms to the 'Guide for the Care Use of Laboratory Animals' published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Rats were kept in a polypropylene cage, in a temperature-controlled (21±1°C) and humidity-controlled (60±10%) room and submitted to a 12 h light and dark cycle (artificial lights, 7 a.m.–7 p.m.) and to an air exhaustion cycle (15 min h^{-1}). They received a balanced diet and unlimited amount of water.

After 6 months rats received ether/thiopental anesthesia and were killed with KCl injection in their heart. The blood was collected from left ventricle for posterior TG/DTA and DSC analyses.

Coupled thermogravimetry-differential thermal analysis (TG-DTA) experiments

The analyses have been performed on a TA 2960 simultaneous TG-DTA model (TA Instruments, New Castle, DE, USA). Rat and human blood samples (8–12 mg) were heated from 30 to 800°C, at a 10 K min^{-1} of heating using nitrogen atmosphere. Three parallel thermal runs for each group of blood (human, Wistar rat and SHR rat) were carried out.

Differential scanning calorimetry (DSC)

DSC measurements have been performed on a TA Instruments, model 2010. 10–12 mg Wistar rat and human blood samples were heated from 25 to 400°C applying a 10 K min^{-1} of heating rate and flowing nitrogen atmosphere.

Results and discussion

TG and DTG curves of human blood samples (HBS) are presented in Fig. 2. TG curve show two main mass loss stages. The first and main mass loss stage starts at room temperature and completes around 100°C causing 78% of mass loss, suggesting water evaporation from the plasma as well as some decomposition of reminders of the plasma and its constituents (Table 1).

The degradation of the solid part of blood (red blood cells, white blood cells and platelets), proteins (e.g. hemoglobin and fibrinogen), lipids and other constituents; probably occurs between 230 and 420°C, showing a second decomposition process accompanied with 14% of mass loss.

The residue of HBS at 800°C is 4.0±1.7%, which is probably related to the presence of metals, like iron, calcium, magnesium, potassium and sodium [8]. DTG curves show at least three consecutive processes be-

low 200°C with the maximum rate at 70 and another peak between 250–450°C (T_{max}: 310°C). The DTA curve (Fig. 2) indicates endotherm processes all along in the investigated temperature range (T_{peak1}: 70°C; T_{peak2}: 300°C).

The DSC curve of HBS sample is given in Fig. 3. Two main endothermic peaks can be seen (one at 194 and the other at 201°C). They can be attributed to some decomposition and/or suggest melting during (T_m) of some organic compounds [9].

Figure 4 depicts TG/DTG and DTA curves of male Wistar rat blood sample (WRBS). While the TG curve of human blood shows only two decomposition stages, the TG curve of Wistar rat blood sample shows three main mass loss stages. In the first stage 50% mass loss occurs up to 70°C, probably related to blood plasma decomposition. The second one appears between 70–140°C showing 26% of mass loss. The third stage, which lasts from 270 to 400°C one can observe 16% of further mass loss, likely due to the presence of proteins, lipids and other compounds. However, it cannot be affirmed that these substances

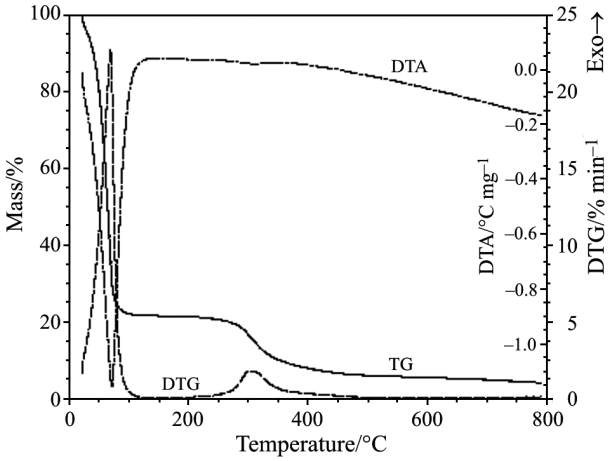

Fig. 2 TG/DTG and DTA curves of human blood sample (HBS)

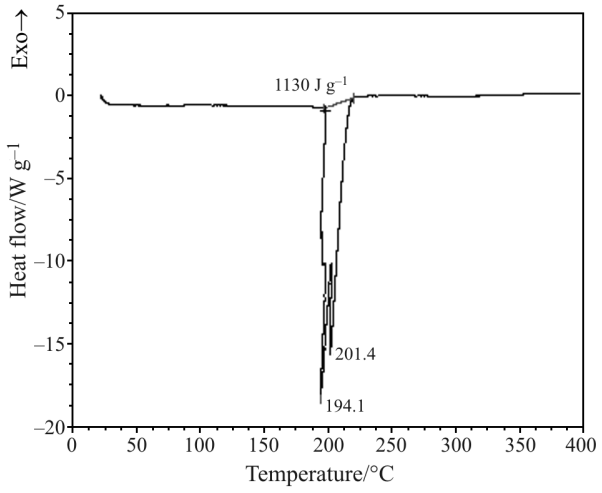

Fig. 3 DSC curve of human blood sample (HBS)

are similar to the constituents of the human blood sample. At 800°C 4.4±2.2% of residue was found, which are inorganic compounds (containing e. g. calcium, sodium and magnesium). DTG curve presents four decomposition stages with maximum rates at around 90, 120, 140 and 310°C. At the same time, three endothermic events occurred at around 70, 120 and 300°C as it can be seen in the DTA profile.

TG curve of SHR rat blood sample (SHRBS) shows also three mass decomposition stages (Fig. 5). Between the thermoanalytical curves of rat blood samples many similarities can be observed. In the first stage 65% of mass loss occurs up to 70°C. The second stage appears between 70–140°C accompained by 15% of mass loss. In the third stage, one can observe 12% of decomposition, starting at 270 and completing at 400°C. WRBS shows 5.4±1.2% of residue at 800°C. DTG curve presents four decomposition stages with maximum rates at around 90, 120, 140 and 310°C; and the DTA profile shows three endothermic events at 90, 150 and 300°C.

By the comparison the DSC curves of HBS (broken line) with the DSC profile of WRBS (continuous line) some interesting differences can be observed (Fig. 6). Two endothermic events (194 and 201°C) can be seen for HBS, however, only one event (184°C) is representative for WRBS. Since the resulted heat flow values are small, the indicated areas of Fig. 6 are enlarged in Figs 7 and 8.

The events enlarged in Fig. 7 are probably due to thermal denaturation of protein compounds. HBS shows 2 endothermic events at 65.3 and 82.0°C; while, WRBS shows only one event at 83.5°C. It may correspond to an irreversible change of the tertiary protein structure but is not accompanied by the breakage of polypeptide chain. A large number of intermolecular hydrogen bonds are breaking in this temperature range which destabilizes the protein molecule [7].

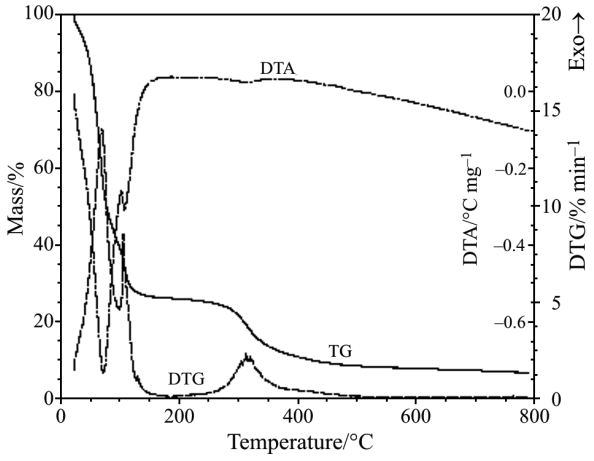

Fig. 4 TG/DTG and DTA curves of Wistar rat blood sample (WRBS)

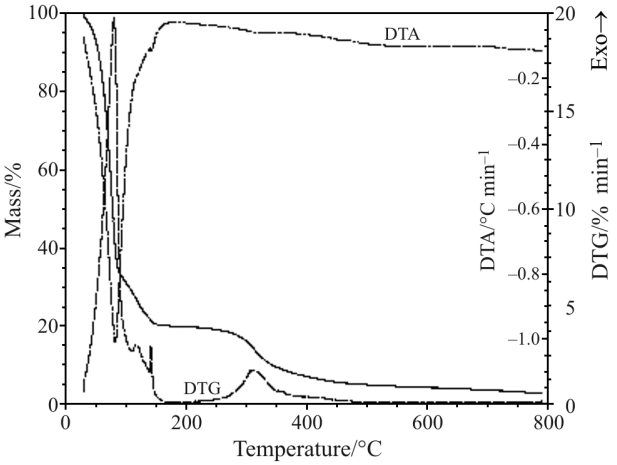

Fig. 5 TG/DTG and DTA curves for SHR blood sample (SHRBS)

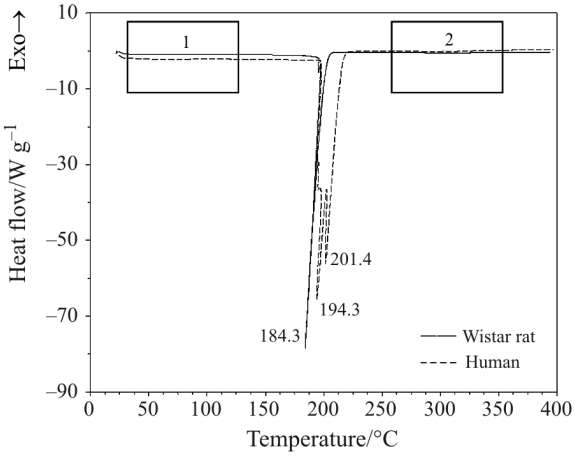

Fig. 6 DSC curves of human blood sample (broken line) and of Wistar rat blood sample (continuous line)

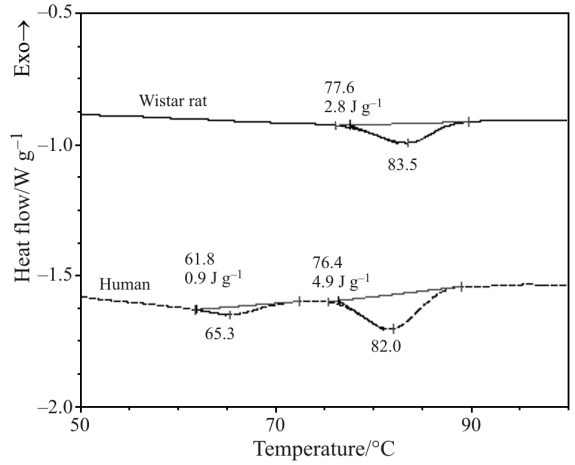

Fig. 7 DSC curve of human blood sample (broken line) and of Wistar rat blood sample (continuous line) – (Area 1 of Fig. 6)

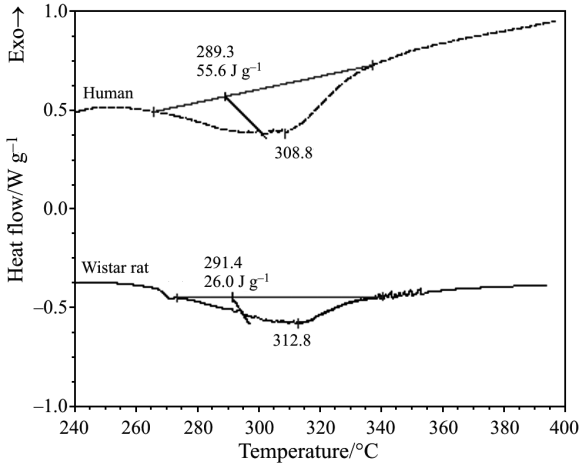

Fig. 8 DSC curves of human blood sample (broken line) and of Wistar rat blood sample (continuous line) – (Area 2 of Fig. 6)

At high temperatures more endothermic peaks for each blood samples can be observed. These events are probably relative to the degradation of fats [10]. Endothermic peaks at 308.8 and 312.8°C for HBS and WBS, respectively, can be seen.

Conclusions

Thermal analysis seems to be an interesting tool in the thermal study of blood samples. TG curves showed only two steps for human blood (HBS), while three stages were representative for rats blood (WRBS and SHRBS). Less residues remained in the TG analysis of human blood samples (4.0±1.7%), compared to Wistar rat (4.4±2.2%) and SHR rat (5.4±1.2%) blood samples. DSC peaks showed five endothermic events for HBS and three endothermic events for WRBS.

The TG/DTG and DSC profiles of human and rat blood samples showed similarities that permit correlation between them, however even more extended research must be done to obtain sufficient evidence on this matter. These methods might serve as a simple alternatives in blood diagnostics and in medical and physical investigations used in hematology, cardiology and biochemistry.

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