COMPARATIVE ANALYSIS OF DIFFERENT ANIMAL AND HUMAN BLOOD SAMPLES BY UV-VIS SPECTROMETRY AND TESTING OF MAIN BIOCHEMICAL PARAMETERS

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Abstract
The paper presents a comparative evaluation of the UV-VIZ spectra for different blood types, with the identification of maximum absorption characteristic of oxyhemoglobin, respectively, a comparative analysis regarding some of the chemical analytical and clinical properties between animal and human blood types. The tests performed are the content of serum amylase, bilirubin, cholesterol (total, HDL, LDL), triglycerides, alanine aminotransferase, aspartate aminotransferase and uric acid.

Introduction
Blood is a vital fluid in the human or animal body that nourishes all body organs and tissues and eliminates unnecessary or residual substances in the body. A human adult has between 5 and 6 liters of blood, which represents 7-8% of the total body weight. During dehydration, for example in a marathon, the current blood volume decreases. [1-5]. Red cells are red-orange in color and make up about 45% of the blood. They are produced continuously in the hematogenous marrow from stem cells. [4-6]. The red blood cells play an important role in the transport of oxygen from lungs to tissue level and in taking up the carbon dioxide, which will be transported to the lung, where it will be eliminated. The red blood cells do not have a nucleus; their membranes are flexible and can stretch in several directions without breaking. Their number may change, it may increase or decrease and may be of a physiological or pathological nature. [6-9]. The color of the blood is conditioned by the amount of hemoglobin found in the blood cells and which, following centrifugation of the blood, separates as erythrocyte concentrate in a 33 ÷ 37% yield. Generally, erythrocyte concentrate contains 65% water, 33% protein (hemoglobin) and 1% mineral salts. [10-14].

Experimental
Horse blood (HB) was sampled from the carotid vein, lamb’s (LB) was sampled from the jugular vein. Cow’s blood (CB) was sampled from the mammary gland. Pig blood (PB) was sampled from the veins in the ears. Chicken blood (ChB) was sampled from the radial vein after poultry slaughter. Dog blood (DB) was sampled from the animal’s cephalic vein. Human blood (HB1-HB7) was sampled intravenously and capillary from the finger pulp from 7 patients of various blood groups. After sampling, the blood was allowed to coagulate completely at room temperature, followed by the removal of serum and clot. Determination of serum amylase, bilirubin, cholesterol (total, HDL, LDL), triglycerides, alanine aminotransferase, aspartate aminotransferase and uric acid involves firstly to prepare the patient, then intravenous sampling and separation in an anticoagulant vacutainer using a separating gel, then centrifuging the analyzed sample to separate the serum. The diluted serum
(about 0.5 mL) separated in this way was analyzed. UV-VIS analysis was performed with a Perkin Elmer, Lambda 25 spectrophotometer. The blood sample was diluted in a ratio of 1:1000 and then the spectra were recorded in the visible range of 400-700nm.

![Figure 1. Stages of blood analysis: A) sampling; B) centrifugation; C) analysis](image)

**Results and discussion**

Figure 2 presents the UV-VIS spectra of animal blood assortments: horse (HoB), lamb (LB), cow (CB), pig (PB), chicken (ChB), dog (DB) and human blood (HB). In the literature [13], the maxima at 542 nm and 576 nm are attributed to oxyhemoglobin. It is observed that the first peak of dog blood and horse blood (548 nm and 546 nm) respectively shows a slight bathochromic shift characterized by a darkening of color. In the cow blood (541 nm), pig blood (539 nm) and human blood (541 nm) there is a hypsochromic shift. The second maximum, the horse blood (590 nm), lamb blood (584 nm), cow blood (579 nm), pig blood (582 nm), cow blood (578 nm) shows a bathochromic shift compared to literature [13]. The composition of oxyhemoglobin in human and animal blood is similar, with a small difference of up to 6 nm. Serum amylase belongs to the category of hydrolases, enzymes that catalyze the hydrolytic degradation of starch, glycogen, poly- and oligosaccharides. Figure 3 (left) shows the values obtained for the 6 animal species and an arithmetic mean for the seven human patients. Values in humans are very close and relatively small in the normal range of 15 - 100 U / l. Pig blood was found to have a very high amylase value (14-fold than the upper normal limit). Large amounts of amylase can cause dysfunction in the body (pancreatitis, perforated peptic ulcer, pancreatic cancer) [15-17]. Bilirubin is produced by the catabolism of hemoglobin, is insoluble in water, and in the blood plasma circulates linked to the albumin serum. The oxidation of heme generates biliverdin that metabolizes bilirubin [1].

![Figure 2. UV-VIS spectra of animal and human blood samples](image)
In the case of lamb and cow blood (Fig. 3 right), bilirubin do not reach the minimum value (0.25 mg / dL). Low levels of bilirubin in human blood generate dysfunctions in the body, leading to jaundice. There are no traces of bilirubin in the chicken blood. Conjugated bilirubin, water-soluble, reacts with diazotized sulfanilic acid in acidic or neutral media to form a red colored complex.

Cholesterol (C_{27}H_{46}O) is a sterol-based alcohol identified in the cell membrane and body tissues and transported in the blood. Total cholesterol should be between 110-220mg / dL. HDL (high density lipoprotein) ranging from 35 to 60 mg / dL. LDL (low density lipoprotein) the so-called "bad" cholesterol should be between 0-130mg / dL.

Figure 3. Variation of serum amylase (left) and bilirubin amylase (right) depending on blood type

Figure 4 Variation of total cholesterol (left), HLD-cholesterol (middle) and LDL-cholesterol (right) depending on the type of animal blood and human blood

Total cholesterol in humans (Fig. 4) slightly exceeds the upper limit, which can cause cardiovascular disease: risk of arteriosclerosis, coronary stenosis, myocardial infarction. Values below the minimum total cholesterol level found in horse, lamb and chicken's blood may cause malnutrition, hyperthyroidism, infections. The highest HDL-cholesterol value was found in cow's blood, and the lowest value was found in chicken's blood. The lowest amount of HDL-cholesterol results in hyperalphalipoproteinemia, chronic hepatopathy, anorexia, and at high levels cause hyperalphaproteinemia, arteriosclerosis. All blood types fall within the allowable limits for LDL-cholesterol. Excessive hypertension may cause hypercholesterolaemia, hyperlipoproteinemia, hypothyroidism, diabetes mellitus, cholestasis,
chronic renal failure, anorexia and low values cause hypolipoproteinemia, hyperthyroidism, chronic anemia, severe hepatocellular disease, acute stress, pulmonary diseases [15 - 17].

Triglycerides in adipose tissue and other tissues are the most important storage of energy reserves in the body. Normal values range between 40-140 mg / dL. Cow blood is below the minimum, and human, chicken and dog blood above the permissible limit (Fig. 5). Variations in human blood samples were also found, shown separately in Figure 5.

Figure 5 Triglyceride variation depending on human blood samples (left) and animal blood samples depending on human blood mean HB (right)

Alanine aminotransferase or glutamyl transaminase is an enzyme that is part of the transferase class and catalyzes the reversible transfer of the amino (NH₂) group from an α-ketoglutarate amino acid (alanine) resulting in the formation of pyruvic acid and glutamate. It is found in the liver, kidney, myocardium, pancreas. Normal values are considered between 0-40 U / l, only pig blood exceeds this limit (Fig. 6). Exceeding the maximum values can cause acute hepatitis, cirrhosis and alcoholism [15-17].

Figure 6. Variation of alanine aminotransferase (left) and aspartaminotransferase (right) depending on the type of animal blood and the mean value of human blood.

Aspartate aminotransferase is an enzyme that is part of the transaminase class and catalyzes the transfer of the amino group from aspartate to the ketone ketoglutarate group, with the formation of oxalocetic acid [10,11]. Limit values are between 0-35 U / L, only human blood falls within this limit (Fig. 6 right), the highest values being observed in horse blood and then in chicken. Values above the maximum may result in hepatic necrosis, CHCl₃ intoxication, and low values lead to chronic kidney dialysis [15-17].
Uric acid results from the degradation of nucleic acids, the ultimate product of purine metabolism. From the liver, it is transported by plasma to the kidneys, where it is filtered and excreted on about 70%. The uric acid residue is eliminated and degraded in the gastrointestinal tract. Normal uric acid values are between 2.5-7.0 mg / dL. All animal blood samples analyzed, apart from human blood and chicken, contain an amount of uric acid less than the admissible limit (Fig. 7 right). There is a rather large variation between the blood from different individuals (Fig. 7 left). Very high uric acid levels lead to kidney failure, gout, asymptomatic hyperuricemia; very low values lead to Wilson's disease [15-17].

Figure 7 Variation of uric acid depending on human blood samples (left) and animal blood samples depending on the mean of human blood SO (right)

Conclusion
UV-VIZ spectrophotometric analysis showed that oxyhemoglobin has two maxima in the 539-548 and 576-590 nm ranges, very close at all blood samples studied for both animal and human blood samples. The serum amylase in pig blood is very high. Bilirubin in the blood of the human samples is normal, while in lamb and cow blood is very small. Total cholesterol is within the normal range in human samples. Low concentrations of total cholesterol were found in the horse, lamb and chicken blood. In the chicken blood sample was found a high triglyceride concentration. The highest value of alanine aminotransferase was found in pig blood and the lowest in the chicken and dog blood. High concentrations of aspartat-aminotransferase were found in animal blood samples above the admissible limit for human blood. The highest concentration was found in horse blood while the lowest was in human blood followed by dog blood. Uric acid in all animal blood types except human and chicken blood has a very low concentration.

References
[17] Synevo Laboratory, References specific to the working technology used 2010.