SPECTROPHOTOMETRIC ANALYSIS OF THE BLOOD PLASMA FOR DIFFERENT MAMMALS

IULIANA MOTRESCU, SERVILIA OANCEA*, ALINA RAPA**, A. AIRINEI****

*Biophysics Department, "I.I. de la Brad", University of Agricultural Sciences and Veterinary Medicine, 3, Aleea Sadoveanu, Iaşi, Romania, e-mail: imotrescu@yahoo.com
**Jhonson Academy Columbia SC, USA
***"P. Poni" Institute of Macromolecular Chemistry, Iaşi, Romania

Abstract. In this paper an analysis of the blood plasma spectrum for different mammals is performed in order to establish the relationship between protein concentration and their absorbance in the UV region. We found a direct dependence of these quantities correlating the spectra and the refractive index measured for the same protein concentrations. This suggests that the specific properties and protein concentration of plasma at different mammalian species influence the blood behavior.

Key words: plasma chromophores, absorption spectra, protein band.

INTRODUCTION

Blood plasma is the yellow, protein-rich fluid that suspends the cellular components of whole blood, that is, the red blood cells, white blood cells and platelets. Blood plasma is almost 55% of the blood's volume and contains 90% water, 8% proteins, 0.9% inorganic ions, and 1.1% organic substances. Besides water we see that proteins are the main constituents of the plasma.

Plasma proteins form three major groups and have various functions: albumin (60% of total plasma protein), fibrinogen (4%), and globulins (36%). In blood plasma, by far the most prevalent protein is albumin, approximately 32 to 35 grams per liter, which helps to maintain the osmotic balance of the blood. It is estimated that plasma may contain as many as 40000 different proteins.

It is a very complex and not fully understood mixture of proteins that performs and enables many housekeeping and other specialized bodily functions.

The optical absorption is a classic method of studying a protein and it consists in measuring the absorption function of wavelength.

Received November 2005;
in final form June 2006.

ROMANIAN J. BIOPHYS., Vol. 16, No. 3, P. 215–220, BUCHAREST, 2006

The UV spectrum contains information on the absorption and scattering properties of particle suspensions. This information can be used to interpret the spectrum in terms of the distribution of particle sizes, the particle shape and the chemical composition of the material in the sample. Strong plasma absorption in the UV region, due to the presence of numerous chromophores found in proteins, can provide information about these proteins from the blood plasma.

Many studies reported quantitative results on the blood plasma properties for human samples [4, 9]. The spectrophotometric analysis of the blood plasma was used to investigate small molecules in the human plasma and to determine protein concentration using FTIR [5]. Human plasma retinol-binding protein and transthyretin was also studied [6]. UV absorption spectra of retinol-binding protein were recorded from 200 to 600 nm within a Varian spectrophotometer and the protein concentration was estimated by using a molar absorption coefficient. In [3] the authors performed the complete UV–Vis (200–820 nm) spectrum for platelet-rich plasma (PRP) and platelets isolated from plasma, in order to develop a model for the quantitative interpretation of platelet spectral data. Analysis of blood plasma protein is also used to study Alzheimer's disease [7]. In [2] the authors studied a hypertensive factor, (known as parathyroid hypertensive factor), which has been identified in the plasma of some hypertensive humans. The substance was monitored by bioassay, as well as by UV absorbance at 210 and 280 nm and by protein assay.

There is little data on plasma properties and protein content for different animals. Specific properties of plasma of different mammalian species influence the blood behavior. In this work we studied the characteristic spectrum of the blood plasma, which is determined by the protein absorbance band in the UV domain in order to determine the concentration of protein.

MATERIALS AND METHOD

Blood samples from healthy animals at the Faculty of Veterinary Medicine of the University of Agricultural Science and Veterinary Medicine in Iaşi were collected in test tubes containing EDTA as an anticoagulant. About 20 mL of blood from sheep and dogs and about 100 mL from cows and from horses are sampled for every test. We collected only 20 mL from pig, sheep and dog because it is hard to collect much blood from these animals. The sampled blood is centrifuged to separate blood plasma (4000 g during 5 minutes). To analyze the blood plasma spectrum for different mammals, we prepared solutions putting 10 μL of blood plasma from different mammals in 5 mL of distilled water.

Solutions of different other concentration from horse blood plasma in distilled water were prepared in order to study the relationship between maximum absorption spectrum and the protein content.

Spectrophotometric analysis in the UV domain of the blood plasma was performed with a spectrophotometer Specord M-42. Measurements of plasma refractive index were performed with an Abbe refractometer.

RESULTS AND DISCUSSION

The protein absorbance band in the UV domain (about 280 nm) determines the characteristic spectrum of the blood plasma. Figures 1, 2, 3, 4 present the characteristic spectrum of the blood plasma for different mammals in UV domain.

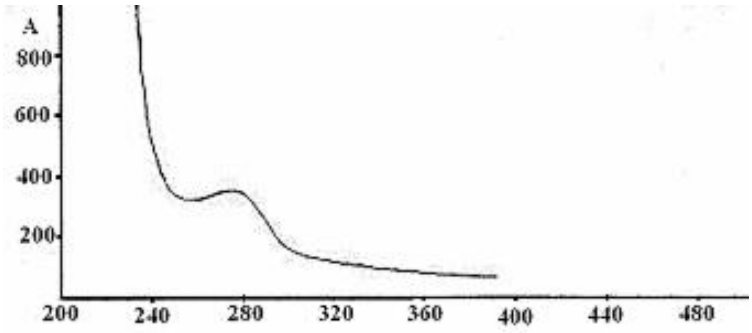

Fig. 1. Plasma spectrum in UV domain (horse blood).

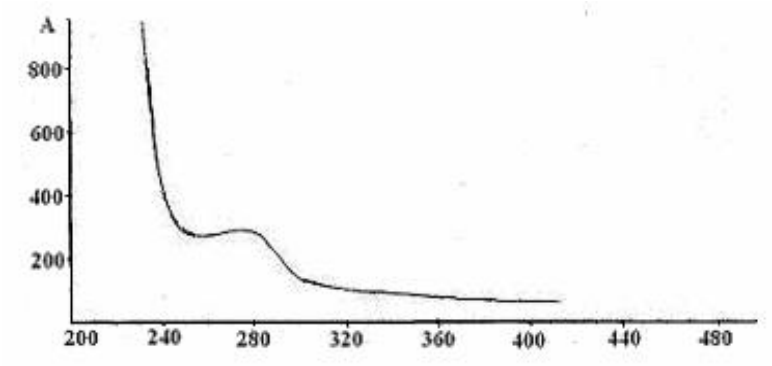

Fig. 2. Plasma spectrum in UV domain (cow blood).

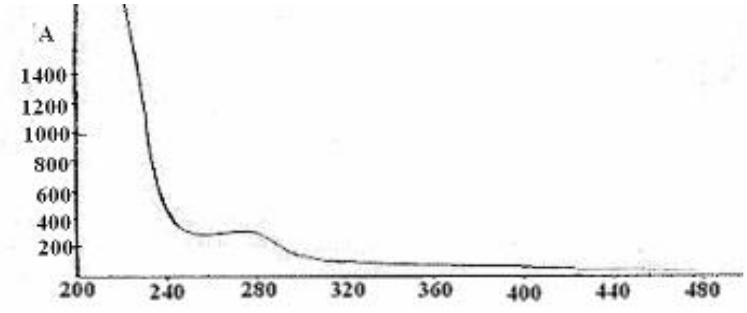

Fig. 3. Plasma spectrum in UV domain (sheep blood).

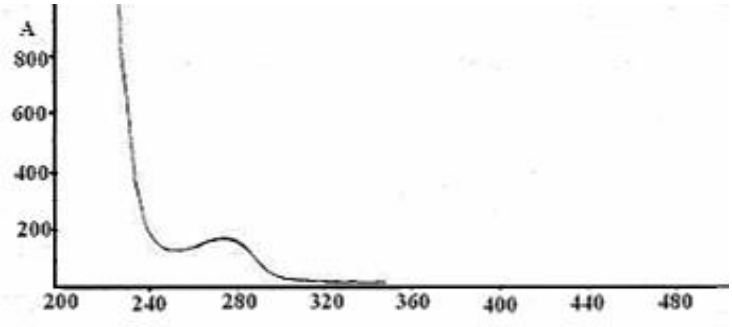

Fig. 4. Plasma spectrum in UV domain (dog blood).

From these spectra we can see that our measurements have shown some differences between the spectra from the different studied species. The highest value of the maximum absorption spectrum (protein content) was obtained for horse and the lowest for dog.

It is known that there is a linear dependence between the protein concentration and the refractive index. Our results obtained from sprectrophotometric measurements are in good accordance with the protein content obtained by measurements of the refractive index for non-diluted blood plasma of studied mammals, which are presented in Figure 5, by comparison with human blood plasma.

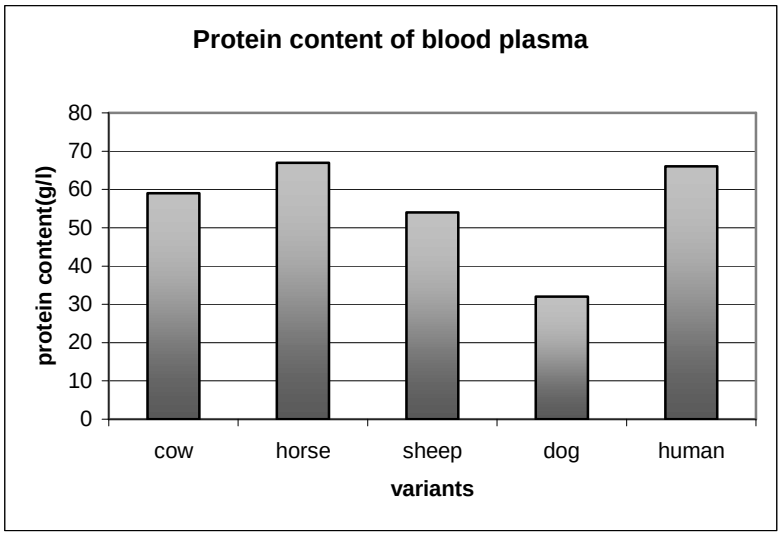

Fig. 5. Protein content of blood plasma from human and different species of mammals.

The value of the absorption band strongly depends on the blood plasma concentration. This dependence is presented in Figure 6.

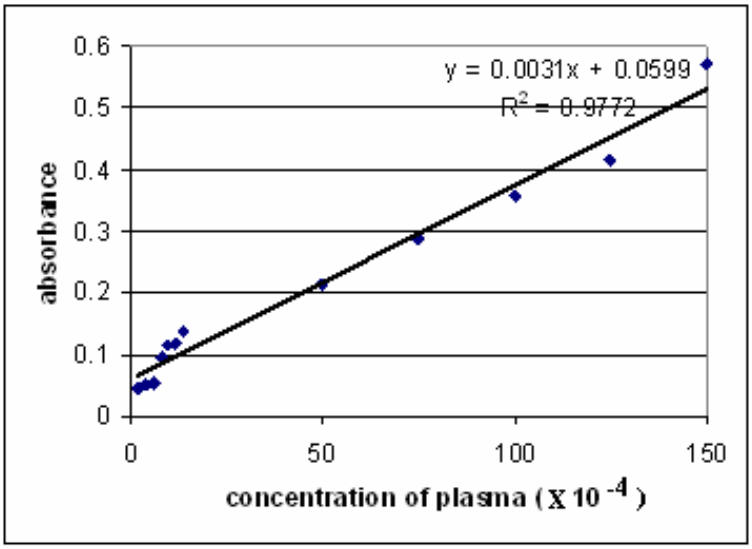

Fig. 6. Plasma absorption at 280 nm *versus* plasma concentration for horse blood (R^2 = correlation factor).

CONCLUSION

Analysis of the blood plasma spectrum for different mammals showed that there are some differences between the spectra from the studied species but the protein content is not in relation with the nutritional system. Some authors [1, 8] consider that the protein content and the dynamic viscosity of the blood plasma depend on the nourishment, so they should have smaller values for herbivores. As it is shown in Fig. 5, for horse and human sample we obtained the same value of the protein content and for dog a smaller value than for horse, cow and sheep.

We established a linear relationship between the protein content and the maximum absorption spectrum in the UV region.

The present study suggests that the spectrophotometric analysis of the blood plasma is a useful tool for determination of plasma protein content from different samples of mammal blood and also it is a less difficult method than the other ones. Due to the fact that the protein absorbance band at 280 nm determines the characteristic spectrum of the blood plasma and the absorption maximum strongly depends on the blood plasma protein concentration, this method permits the determination of a small content of protein from the plasma blood. This means we can use a very small quantity of plasma, which is diluted in distilled water.

REFERENCES

1. ALLEN, B.V., D.J. BLACKMORE, Relationship between paired plasma and serum viscosity and plasma proteins in the horse, *Research in Veterinary Science*, 1984, **36**, 360–363.
2. BENISHIN, C.G., R.Z. LEWANCZUK, P.K. PANG, Purification of parathyroid hypertensive factor from plasma of spontaneously hypertensive rats, *Proc. Natl. Acad. Sci. USA*, 1991, **88**(14), 6372–6376.
3. MATTLEY, Y., G. LEPARC, R. POTTER, L. GARCÍA-RUBIO, Light scattering and absorption model for the quantitative interpretation of human blood platelet spectral data, *Photochemistry and Photobiology*, 2000, **71**(5), 610–619.
4. NARAYAHAN, S., L. GALLOWAY, A. NONOYAMA, G.F. LEPARC, L-H. GARCIA-RUBIO, R.L. POTTER, UV-visible spectrophotometric approach to blood typing II: phenotyping of subtype A2 and weak D and whole blood analysis, *Transfusion*, 2002, **42**(5), 619–626.
5. PETIBOIS, C., G. CAZORLA, A. CASSAIGNE, G. DELERIS, Plasma protein contents determined by fourier-transform infrared spectrometry, *Clinical Chemistry*, 2001, **47**, 730–738.
6. RAGHU, P., RAVINDER, P., SIVAKUMAR, B., A new method for purification of human plasma retinol-binding protein and transthyretin, *Biotechnology and Applied Biochemistry*, 2003, **38**, 19–24
7. UENO, I., T. SAKAI, M. YAMAOKA, R. YOSHIDA, A. TSUGITA, Analysis of blood plasma proteins in patients with Alzheimer's disease by two-dimensional electrophoresis, sequence homology and immunodetection, *Electrophoresis*, 2000, **21**(9), 1832–1845.
8. WINDBERGER, U., A. BARTHOLOVITSCH, R. PLASENZOTTI, K.J. KORAK, G. HEINZE, Whole blood viscosity, plasma viscosity and erythrocyte aggregation in nine mammalian species: reference values and comparison of data, *Exp. Physiol*., 2003, **88** (3), 431–440.
9. ZAHAO, B., S.Y. THAM, J. LU, L. LEE, S. MOOCHHALA, Simultaneous determination of vitamins C, E and β-carotene in human plasma by high-performance liquid chromatography with photodiode-array detection, *J. Pharmaceut. Sci*., 2004, **7**(2), 200–204.