UNIVERSITY OF CRAIOVA
FACULTY OF CHEMISTRY

Summary of
PhD Thesis

ELECTROPHORETIC STUDY OF HEMOGLOBIN AND SERUM PROTEINS – CLINICAL APPLICATIONS

PhD Advisor:
Professor Mircea Preda

PhD Candidate
Daniela Laura Pălărie

2008

Thesis is structured as follows:

Introduction

Theoretical considerations

Chapter 1. *Migration media and mechanisms of separation in electrophoresis*

Chapter 2. *Separation of proteins by electrophoresis*

Original contributions

Chapter 3. *Study of serum proteins electrophoresis – clinical applications*

Chapter 4. *Study of hemoglobin electrophoresis - clinical applications*

General conclusions

Chapter 5. *Appendices*

References

In *introduction* we presented a short evolution of electrophoresis and we specified the electrophoretic methods (paper electrophoresis and agarose gel electrophoresis) that have been used in our study performed on two categories of sanguin proteins: hemoglobin and serum proteins.

In the **chapter 1** a description of both migration media and mechanisms of separation in electrophoresis has been realized. We precizate the characteristic notions of electrophoresis (rate of migration, electrophoretic mobility, relative mobility, resolution of separation) and were discussed the factors that them influence: proper factors of particle (size, shape, net electric charge) and those characteristic of migration medium (viscosity, pH, ionic strength of buffer solution, intensity of the electric field, migration time, temperature, a.s.o.).

A systematic presentation of the evolution of migration media, and of the electrophoretic methods has been effected. Presentation began with free electrophoresis, the oldest method used for separation of proteins. Were exposed the utility of this method for the separation of labile biological systems but also its disadvantages.

Were presented the characteristics of the most important used supporting media (paper, cellulose acetate, polyacrylamide gel, starch gel, agarose gel, polyacrylamide-

agarose composite gels) and were discussed the mechanisms of separation and specific features for each electrophoretic techniques.

Were also exposed the electrophoretic methods based on specific mechanisms of separation (affinity electrophoresis, immunodifusion and immunoelectrophoresis, isoelectric focusing, isotachophoresis). We also presented the most advanced techniques, with a large area of applications (2D electrophoresis and capillary electrophoresis).

In the **chapter 2** were described the electrochemical properties of proteins and the mode of separation of serum proteins and hemoglobin by electrophoresis.

The more important specific features of the main categories of serum proteins (albumin, α_1, α_2, β_1, β_2, and γ - globulins) were described by emphasizing theirs clinical importance. We presented a series of comparative studies from the literature of speciality regarding the results obtained for the separation and determination of serum proteins by various electrophoretic techniques.

We discussed the structural specific features of the five categories of immunoglobulins (IgA, IgM, IgG, IgD, and IgE) and the manner of separation of immunoglobulins in electrophoretic field.

Were described the structure of hemoglobin and of its principal derivatives (Hb, HbO_2, HbCO, MetHb, and $HbCO_2$). Has been mentioned the utility of spectrophotometric method for the determination of the concentrations of hemoglobin derivatives. Were evidenced the structural differences of normal and abnormal hemoglobins, the manner of theirs influences on electrophoretic mobility of hemoglobins, and the mechanisms of separation used in hemoglobin electrophoresis.

The original contributions were presented in the chapters 3, 4, and 5.

Chapter 3 contains the studies concerning the serum proteins electrophoresis. We used a Sebia electrophoresis system on agarose gel and a Standard Apparatus for electrophoresis on paper.

The influence of the migration medium on electrophoretic separation has been analized by comparison of serum albumin values obtained by bromcresol green colorimetric method (A_{col}), agarose gel electrophoresis (A_{ag}), and paper electrophoresis (A_h). We concluded that the values of albumin obtained by electrophoresis depends on

migration medium that has been used, and are less than those resulted by colorimetric method. The following relation has been obtained: $A_{col}>A_{ag}>A_h$. The little values of serum albumin obtained by paper electrophoresis are due to the adsorption of proteins on paper. In the proces of electrophoretic separation and also in the other stages (staining, destaining, quantitative determination) appear little loss, fact that explains the less values for albumins obtained by electrophoresis as compared with those resulted by bromcresol green method. In order to obtain some informations about the homogenity of these supporting media we determined, for each migration medium, the correlation coefficient between values of albumin obtained by bromcresol green method and that obtained by corresponding electrophoretic method. A good correlation for agarose gel (r=0.94179) has been obtained, similar result with that subsequently reported by Snozek and his collaborators. For the paper, the low value of the correlation coefficient (r=0.88737) shows a high spreading of the experimental data, that is due to the nonhomogenity of the pore size of the paper.

We performed a comparative evaluation of concentrations of serum proteic fractions separated by agarose gel electrophoresis and paper electrophoresis by which we evidentiated the manner in that the physical-chemical properties of migration media influence the obtained results. Good correlations for albumin (r=0.974), α_2 - globulins (r=0.955), and γ - globulins (r=0.987), and moderate correlations for α_1 - globulins (r=0.872) and β – globulins (r=0.761) were obtained. The values of the correlation coefficients have been closely interpreted in concordance with the electrophoretic characteristics of proteic fractions, corresponding to these two electrophoretic techniques (migration rate, electrophoretic mobility, and relative mobility).

We studied the dependence of the bandwidth corresponding to the human serum albumin on migration time by using agarose gel electrophoresis. We remarked a relative increase of migration rate of albumin, due to the variation of migration time from 17 to 24 minutes, less than 1%. This increase of migration rate is due to the Joule heating effect that becomes more important if the migration time increases. The linear dependences of bandwidth on migration time and migration distance, obtained by our study, are in a good agreement with the result reported by Yarmola and his collaborators on R-phycoerythrin protein.

By using agarose gel electrophoresis, at alcalin pH, we evidentiated monoclonal components pertaining to the IgA, IgM, and IgG classes and we demonstrated the impossibility of their quantification by this electrophoretic technique by obtaining values less than one for resolution of separations for the pairs of bands: β_2-IgA, IgA-IgM, and IgM-IgG.

Chapter 4 contains the studies related to hemoglobin electrophoresis on agarose gel by using a Sebia system.

By using the electrophoretic method on acidic buffered (pH 6.0) agarose gel for the determination of glycated hemoglobin (HbA_{1c}) and an enzymatic method for blood glucose determination, we established a relation of linear dependence between mean blood glucose (GMS) and HbA_{1c}: $GMS = 32.51 \cdot HbA_{1c} - 79.17$. We remark that the relation derived by us is in a good agreement with the relations obtained by Nathan and Rohlfing, that were determined HbA_{1c} by HPLC.

Based on a study, performed in a time period of two years, regarding the incidence of β-thalassemia in the Dolj county, have been identified, by using hemoglobin electrophoresis on agarose gel at alcalin buffered solution, 47 persons with β-thalassemia minor from a lot of 177 suspected individuals.

We studied the influence of smoking on concentration of HbCO from venous blood by investigating four group of persons: healthy persons (separated in smokers and nonsmokers) and persons with β-thalassemia minor (also divided in smokers and nonsmokers). Percentage of HbCO from venous blood has been determined by multicomponent analysis of hemoglobin derivatives based on absorption spectra of the samples, obtained with an Ocean Optics S2000 spectrophotometer. Were obtained the following mean values of HbCO for the investigated groups: 1.32% for healthy nonsmokers, 6.15% for healthy smokers, 4.97% for β-thalassemic nonsmokers, and 10.12% for the smokers with β-thalassemia minor. The results obtained in the cases of healthy persons are in a good agreement with the corresponding one resulted by the Sagone study. The mean value of 4.97% obtained for nonsmoker individuals with β-thalassemia minor is a characteristic value for persons with hemolitic anemia. Determination of HbCO concentration for smoker persons with β-thalassemia minor

has been reported by our group for the first time in the literature of speciality. We remark that for this group were obtained the highest concentrations of HbCO, which that can be explained by addition of endogenous CO resulted by heme catabolism to the CO inhaled by smoking.

We studied the pH influence on the in vitro oxihemoglobin autooxidation by performing a cinetic analysis of some human biological samples constituted by hemoglobin solutions, at various pH values between 5.6 and 8.0. The absorption spectra of the samples were recorded immediately after their preparation and at further time moments. By applying the multicomponent analysis method for these absorption spectra, the temporal evolutions of the concentrations of hemoglobin derivatives were obtained. We remarked the quasiconstant maintaining of percentage of HbCO, a decreasing of HbO_2 concentration, while an increasing of the concentration of MetHb. The analysis of the temporal evolution of MetHb concentration allowed the determination of the apparent rate constants of the processes of autooxidation of HbO_2. The values of the apparent rate constants were depended on the pH of the samples, having the values between $0.771 \cdot 10^{-3}$ h^{-1} for pH=8.0 and $3.502 \cdot 10^{-3}$ h^{-1} for pH=5.6.

We analysed how the pH of blood influences the concentration of MetHb by effecting a comparative study on two categories of persons: nontreated diabetic persons (with HbA_{1c} > 9%), and nondiabetic persons. For the nondiabetic persons were obtained MetHb concentration situated in the range of 1.09-2.30%, the mean value being 1.69%. In the case of diabetic persons were resulted values for MetHb between 2.20% and 3.47%, with a mean value of 2.76%. The mean value of MetHb, greater in the case of diabetic persons has been explained by their less value of pH that is due to the metabolic ketoacidose.

The conclusions of our researches have been presented for each study and, shortly repeated in the section *general conclusions.*

Has been evidentiated the importance of electrophoresis in determination of concentrations of hemoglobins, and of serum proteins and were established correlation between the values of these blood proteins and the concentrations of other biochemical parameters.