3 Conclusions

This Thesis gives an overview of high-performance liquid chromatography in various separation modes for a characterization of selected proteins.

Various affinity stationary phases were prepared for analysis of pepsin, seminal plasma proteins and selected glycoproteins. Choice of the affinity system suitable for a particular purpose is always a complex problem in which number of aspects must be considered. Properties of the proteins to be separated and ligands used had to be taken into an account. The separation system should not influence the biological activity of proteins separated. From this point of view it is clear that for any each individual system is necessary to find specific conditions for I) immobilization of ligand, and II) separation conditions.

Stationary phases with immobilized 3,5-diodo-L-tyrosine exhibited sufficient selectivity, proved to be useful for analysis of porcine pepsin A and are applicable to practical samples of human pepsin. Reversed-phase C18 column is suitable for analysis of pepsin fragments obtained after α-chymotrypsin digestion. A study of interaction of various forms of pepsin and pepsinogen are essential because of their high importance as clinical diagnostic markers and as stomach enzyme.
Affinity chromatography with heparin immobilized to Toyopearl support proved to be useful for the analysis of binding properties of boar, bull and human seminal plasma proteins. Different interactions with heparin and phosphorylcholine in different species were confirmed. Study of the structure of seminal plasma proteins, their characterization and recognition of all their functions will significantly help to understand a complex process of fertilization.

Concanavalin A is well-known plant lectin with a high affinity toward α-D-mannoside and α-D-glucoside residues. This property can be utilized for analysis of selected glycoproteins. Stationary phases with Con A immobilized were used for affinity chromatography of selected glycoproteins and allergens.

For the study of the composition of mixed aggregates of whey proteins two different options were employed: conversion of the aggregates back into the native protein molecules (refolding) or full denaturation of the whey proteins followed by quantification in the denatured state (denaturation). The denaturation approach led to the establishment of the ratio BLG/ALA. A value of 1.29 was found. A study of food proteins composition and their mutual interactions is important for a preparation of foods with improved structures.