Protein phosphorylation is one of the most important post-translational modifications in nature. The process of protein phosphorylation/dephosphorylation regulates almost all cellular processes and plays a key role in the maintaining of cell homeostasis. Abnormal phosphorylation is associated with many serious human diseases including cancers.

The phosphorylation of main gastric aspartic proteases pepsins and their precursors varies among animal species. Pepsins and pepsinogens of several species contain phosphate groups, while others have no phosphate groups. Phosphorylation of human pepsins and pepsinogens is probably associated with different gastric diseases. Gastric cancer patients show higher phosphorylation degree of pepsinogens compared to healthy subjects. To utilize this finding for diagnostic purposes, it is requisite to elaborate simple and reliable methods for the study of pepsin and pepsinogen phosphorylation, which could be applied to limited sample amounts.

This thesis focuses on the elaboration of appropriate methodical approach for the study of protein phosphorylation, particularly phosphorylation of human pepsins, which are characterized by a very low content of basic amino acid residues and a higher content of acidic amino acid residues. The attention was paid to the elaboration of affinity chromatography methods for the selective separation of phosphorylated pepsins and for the isolation of phosphopeptides derived from these proteins. In the first part of this thesis, we selected sorbents suitable for the separation of phosphoproteins by high-pressure immobilized metal affinity chromatography. In the next part, we elaborated methods for the separation of phosphorylated and non-phosphorylated forms of pepsins using selected high-pressure sorbents. The elaborated methods were applied to human pepsins isolated from gastric juice of a healthy individual and from gastric mucosa of a gastric carcinoma patient. In the last part of this thesis, we elaborated an approach for the identification of pepsin phosphorylation site. First, we optimized affinity chromatography methods for the selective enrichment of phosphopeptides from pepsin digest. The enriched peptides were then detected using tandem mass spectrometry and the phosphorylation site in pepsin was identified using database searching. Then we applied the elaborated procedure to human gastric juice proteins and successfully identified the phosphorylation site in human pepsin A.