## Introduction

High-performance liquid chromatography (HPLC) is currently one of the most common analytical separation techniques. Due to its ability to separate a wide range of a structurally different low- and high molecular compounds of polar as well as non-polar nature, HPLC found application in analytical laboratories in the field of medicine, pharmacy, biology, industry and the environment.

Although the conventional packed columns are used in most of the chromatographic applications, stationary phases in the form of particles have their limitations and disadvantages. Quality of the packed columns is influenced by a number of parameters, for example relatively complicated process of filling [1]. Variances in the particle size and irregularities in their shape have a negative impact on the chromatographic process. Common problems of the packed columns are an inhomogeneous distribution of the particles of various sizes and their settling in the column with consequent creation of dead volumes. Highly uniform spherical particles prepared by suspension polymerization can partly solve some limitations, however, disadvantages arising from the principle of the packed columns still remain.

Promising alternative to the packed columns are monoliths, made of a block of porous material. The structure of monolithic columns is characteristic by communicating macropores which allow the flow of mobile phase with the analyte, and small mesopores which allow the access of the molecules to be separated to the active sites of stationary phase. Character of the porous monolith structure, the specific surface area, quantity, size and shape of the pores substantially affect the properties of the monolithic columns.

Monolithic stationary phases are usually prepared by *in situ* polymerization inside the columns or capillaries. The polymerization is a complex process in which a number of factors affect the structure of resulting monolith. The polymerization mixture is usually composed of five components, while both the quantity and chemical structure of each of them more or less influence the monolith structure. The way of initiation and the temperature of polymerization are other factors affecting the structure of polymer.

Characterization of the structure of monolithic columns is necessary for understanding of chromatographic processes, which take place in them. It is also important to understand the process of polymerization and various factors that affect the structure of arising monoliths. For systematic development of a new monolithic materials it is necessary to elucidate the relations between the preparation of monolithic stationary phases, their structure and subsequent chromatographic properties. Therefore, this PhD thesis was written, to describe the relationship between the preparation, structure and resultant chromatographic properties of monolithic columns.

## The objectives of PhD thesis

- Methodological mastering of the preparation of HPLC monolithic stationary phases of classical proportions.
- Application of the experience on the preparation of molecularly imprinted monolithic columns.
- Morphological characterization of prepared stationary phases by physical methods.