

High performance liquid chromatography (HPLC) is currently one of the most widely used analytical separation techniques. Although the most of applications use standard chromatographic columns packed with particles, these stationary phases has some disadvantages and limits. A promising alternative are monolithic columns. In contrast to the packed stationary phase consisting of many particles, monoliths are composed of one block of porous material. Monoliths are characteristic with communitating macropores, instead of the interparticle spaces of packed columns, which allow the flow of mobile phase carrying the analyte molecules, and small mesopores providing sufficient surface for active sites of stationary phase. The character of the porous structure of the monolith, its specific surface area, quantity, size and shape of pores significantly affect the properties of monolithic columns. The aim of this thesis is to manage the preparation of monolithic chromatographic columns by *in situ* polymerization within the columns and a description of factors influencing the morphology of the resulting monoliths. Second part of the work is focused on the characterization of the morphology of prepared monoliths, which is essential for the understanding of chromatographic processes that run through them. Describe the factors influencing the internal structure of the monolith formed during the preparation and understanding of the influence of the internal structure on the chromatographic process brings feedback which is essential for systematic preparation of monolithic columns suitable for various applications.