

Abstract

Online preconcentration techniques in capillary electrophoresis (CE) improving sensitivity of electrophoretic separations and are in the scope of interest of many research groups. Therefore, proposal of new techniques for preconcentration and their theoretical description is desirable for their wider application. In this thesis, new preconcentration techniques are proposed and models of ionic strength effects in separation systems of CE and hydrophilic liquid interaction chromatography (HILIC) are presented and new set of pI markers for isoelectric focusing (IEF) was showed with their utilization for characterization of IEF pH gradient.

Part of this thesis is keen on proposal of new preconcentration techniques and their theoretical description. We presented two preconcentration techniques, while first is based on sweeping with neutral complexing agent, the second one utilizes pH boundaries that are induced by disturbing phenomena of system zones. Both techniques are described theoretically by means of numerical simulations and by experiments. Both techniques are even utilized for real drug sample or for mixture of similar analytes. For system peak preconcentration technique, flow chart of how to design such method is presented.

Second part of this thesis have rather more fundamental subject and that is ionic effects in separation systems of CE and HILIC mode of HPLC. In CE, topic of ionic effects is focused on ionic strength corrections in determination of dissociation constants and limiting mobilities. We presented new software AnglerFish, that implements theory of ionic strength for easy utilizable and precise correction of these parameters on zero ionic strength of solutions, while also having other advantages compared to standard approaches. For HILIC separation system, we showed how retention of various amino acids, dipeptides and their N-blocked analogues changed with type and concentration of present cation in the buffer for two different stationary phases. Surprisingly, increasing concentration of some buffer cations decrease retention of analytes, due to the competition on stationary phases.

Finally, we predicted, found and characterized set of 14 pI markers for IEF. pKa values and limiting mobilities of these markers were determined experimentally. These markers were utilized for characterization of IEF pH gradient by simulations and experiments.