Abstract

This work is focused on mixed mode chromatography, a characterisation of 2 mixed mode columns in particular - Acclaim^{TM} WAX-1 and Acclaim^{TM} WCX-1, both of these stationary phases yielding a combination of reversed phase and ion exchange separation mechanisms. The characterisation was carried out thanks to two newly developed buffer mixing system optimalizations, that can be universally used on the high performance liquid chromatography instrument. While the first column introduces a protonable secondary amine group to the separation process, the latter contains a weak carboxylic group also charged in a range of pH, bearing an opposite charge. The characterisation process was done by measuring retention of analytes of various polarity and charge, in a defined pH range (2.5 – 7.5 in 0.5 steps).

The behaviour of the stationary phases was proven to change with a shift in mobile phase pH, as it affected the dissociation/protonation of the ion exchange groups present in the column. The WAX-1 column starts to lose its positive charge above approximately the pH of 5 while the WCX-1 column appears to gain a negative charge near and above a pH of 3. This was determined thanks to data evaluation from graphs where retention time dependences on pH were plotted for various analytes. While the retention of neutral compounds remained the same, the acid-base equilibrium of weak electrolytes was affected by the change in pH and proven to give their dissociation constants obtained from appropriate data fitting. For aniline, the WAX-1 column with a positive charge showed a greater approximation, while for benzoic, 4-hydroxybenzoic and nicotinic acids WCX-1 column with a negative charge showed a better estimate. These estimates were further compared to those obtained by a pH gradient pKa determination technique, using the same, previously developed buffer mixing systems.

Keywords