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Determination of Thermodynamic Binding Constants by Affinity Capillary Electrophoresis

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Abstract

A strategy to study thermodynamic binding constants by affinity capillary electrophoresis (ACE) is presented. In order to simplify mathematical treatment, analogy with acid-base dissociation equilibrium is proposed: instead of ligand concentration $[X]$, negative logarithm of ligand concentration (or activity), $pX = -\log[X]$, is used. On this base, and taking into account ionic activities, a general procedure for obtaining thermodynamic binding constants is proposed. In addition, the method provides electrophoretic mobilities of the free analyte and analyte-ligand complex, even when binding constants are low and thus, the complexed analyte fraction is also low. This is useful as a base to rationally analyze a diversity of situations, *i.e.*, different mathematical dependencies are obtained when analytes and ligands with different charges are combined. Practical considerations are given for carrying out a full experimental design.

Enantiomeric ACE separation based on the use of chiral selectors is addressed. 2-hydroxypropyl- β -cyclodextrin was chosen as a model ligand, and both enantiomeric forms of four pharmaceutical drugs (propranolol, pindolol, oxprenolol and homatropine methylbromide) were considered as model analytes. Practical aspects are detailed and thermodynamic binding constants as well as free and complexed analytes mobilities are determined.

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