EXPERIMENTAL DESIGN AND MEASUREMENT ERROR IN AFFINITY CAPILLARY ELECTROPHORESIS LIGAND BINDING STUDIES

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Abstract

In drug design, high quality methods are necessary to determine equilibrium constants precisely. There are several different methods which are used to determine equilibrium constants. Their basic principle is mostly the same. The change of the chemical response is measured in a range from ligand free analyte to the concentration of fully saturated analyte, for example this chemical response could be a change of the electrophoretic mobility in CE. Equilibrium constants are then determined by nonlinear fitting of the chemical response and the corresponding ligand concentrations to the binding equation. The resulting fit will be a hyperbola and the dissociation constant (K_D) can be calculated as a parameter of its fit. The determining process of binding constants is very error-prone. Measurement uncertainties of K_D-values higher than one order of magnitude are not uncommon. The overall error can be minimized when the measurement conditions are in accordance with the following aspects. The maximum-response-range (mrr) needs to be sufficiently large. The mrr should be greater than 0.5. The Data point range should cover at least 40 % of the binding hyperbola. The optimal data point positions cover the middle to the upper parts of the binding-curve. Therefore, the data points are to be set within the range of K_D to a tenfold of its value. If that is complied, six or more different data points are enough to calculate the binding constants adequately.