Analytical Solution of Steady-state Protein Concentration in Carrier Ampholyte-based Isoelectric Focusing

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Abstract: Analytical solution to find the steady state protein concentration distribution in isoelectric focusing (IEF) is very challenging due to the nonlinear coupling between mass and charge conservation equations. In this study, approximate analytical solutions are obtained for steady state protein distribution in carrier ampholyte-based IEF. The final concentration profile for proteins is assumed to be Gaussian, but appropriate expressions are presented in order to obtain the effective electric field and pH gradient in the focused protein band regions. The validity and applicability of the analytical model are evaluated for three different cases by comparison with numerical results. In the first case, three proteins are separated in a narrow pH range (6-9) using 50 carrier ampholytes. In the second and third cases, the separation of proteins is studied in broad pH range (3-10) IEF using 100 carrier ampholytes. Results obtained from the approximate analytical models are in very good agreement with the numerical results for IEF separation of cardiac troponin I, albumin, and hemoglobin in both narrow and broad pH ranges. The sensitivity of the analytical model is also tested for different initial mass ratios of proteins to ampholytes. No appreciable differences are observed between approximate analytical and numerical results within the mass ratio range studied. The effect of a nominal electric field and/or a nominal pH gradient on protein focusing is also examined to demonstrate the effectiveness of the analytical model. Our results indicate that the use of both nominal electric field and pH gradient will result in erroneous peak concentrations for proteins.