Numerical simulations are presented for ampholyte-based isoelectric focusing in 2D microgeometries. In this study, model proteins are focused in the presence of 25 biprotic ampholytes under an applied electric field. Each protein is considered as a simple polypeptide having ten charge states, while the biprotic ampholytes are selected to generate a shallow pH range of 6 to 9. Straight and contraction-expansion microchannels are considered here, and a nominal electric field of 300 V/cm is maintained for separation of proteins. Six distinct values \( \Delta pK \) between 1 and 3.5 are investigated for ampholytes to form pH profiles in a 1 cm long microchannel. Simulation results show that relatively larger values of \( \Delta pK \) difference are required to form stepless pH profiles in the system. The peak heights and differential resolution of focused proteins are much higher for lower values of \( \Delta pK \) for which a stepped pH profile is evident. For each protein, the time it takes for the two edges of a peak to merge decreases linearly, while the focusing time goes up exponentially with increasing \( \Delta pK \). Both focusing and merging times are higher for contraction-expansion microchannel than those of straight microchannel. For a particular value of \( \Delta pK \), the contracted “zoom” region of contraction-expansion channel is able to form more tightly focused bands than the expanded region.

**Summary and Conclusions**

- Generalized 2D IEF Model for Simultaneous Focusing of Carrier Ampholytes and Proteins
- Stepwise pH Profile, 6.21-8.3, is formed in a 1 cm channel for Natural pH Gradient Case
- Proteins are Highly Resolved in the Throat Region, While Proteins in the Wider Regions are Poorly Focused
- Merging Time and Focusing Time Increase with Dissociation Constant Values
- Stepwise pH Provides Tight Focusing and High Resolution of Protein

**References**


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