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Polyacrylamide gel electrofocusing and the ampholyte shift*

A slow, cathodic migration of the established pH gradient commonly occurs during polyacrylamide gel electrofocusing. RIGHETTI AND DRYSDALE[1] reported that ampholyte shifting hindered the stabilizing of pH gradients and was possibly caused by chemical reactions affecting carrier ampholyte concentrations or conductivity during acrylamide polymerization. Others have identified protein–ampholyte artifact complexes in previously polymerized gels[2,3], indicating that carrier ampholyte reactivity is not dependent upon polymerization reactions.

Synthetic carrier ampholytes have been substituted effectively for N,N,N′,N′-tetramethylenediamine (TMED) to accelerate polymerization[4]. Neither TMED nor carrier ampholytes are believed to bind to the acrylamide gel matrix during free radical polymerization, although numerous side-reactions of either required accelerator are possible.

We have simplified the riboflavin catalyzed photopolymerizing system effecting acrylamide gel formation in the absence of TMED, carrier ampholytes, and sample. A photopolymerization mixture containing 3.5 % dimethyl sulfoxide (DMSO) and riboflavin, acrylamide, and N,N′-methylene bisacrylamide according to WRIGLEY[5] was used. DMSO improved the firmness as well as the resilience of the acrylamide gels. DMSO did not react with the gel matrix as cathodic electroosmotic flow and ampholyte shifting, regardless of ampholyte and sample addition times, were observed to be equivalent to the electroendosmosis reported in persulfate catalyzed gels with ampholytes added by perfusion[6].

Phenolic compounds appeared to aggravate the ampholyte shift, possibly by inhibiting free radical polymerization. Excessive electroosmosis and soft gels were observed. Ionized microsolute remaining after polymerization could contribute to electroosmotic flow in a charged gel matrix. Hydrogen bonding between phenolics and carrier ampholytes also could modify the relative acidity and basicity of the ampholytes. In neither case would unidirectional migration be expected.

Cathodic migration of ampholytes resulting in a pH gradient shift is apparently independent of catalysts, accelerators, ampholytes, samples, and other added constituents. The ampholyte shift has been observed in the absence of phenolics and other free radical polymerization inhibitors. Agents flooding out inter- and intramolecular hydrogen bonding have had little effect. Polymerization in the absence of carrier ampholytes, TMED, and samples precludes an ampholyte–acrylamide charged gel matrix.

Carrier ampholyte modifications and reactions have not adequately explained pH gradient migration. The ampholyte shift is explained more readily as an electroosmotic flow phenomenon inherent to the polyacrylamide gel matrix. The explanation may be two-fold. First, although polyacrylamide gels are relatively inert and contain few (if any) ionizable groups[7], partially charged free amide groups are present. The carbonyl oxygen, the most electronegative element of the amide group, imparts a partial negative charge to the fixed gel support matrix. The resul-

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tant electroosmotic flow of the fluid phase to counteract the fixed matrix charge would be cathodic. Because the partial charge is weak, one would expect the electroosmotic flow to be slow. Second, amide hydrolysis at the pH extremes normally used for electrode solutions with pH 3–10 carrier ampholytes could slowly release free carboxyl groups. Few fully ionized carboxyls would be required to produce an equivalent cathodic electroosmotic flow. Thus, electroosmotic flow (negligible for short electrophoretic runs) should be considered carefully in reporting and interpreting polyacrylamide gel electrofocusing results involving long run times.

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