Electroendosmosis (EEO)

Active water transport towards the ends of the IPG strip occurs during IEF. This is exacerbated by the presence of charged molecules in the sample, or breakdown products found in poor grades of urea, all of which accumulate at the ends of the IPG strips.

The detrimental effects of this EEO is to cause macromolecules to focus extremely slowly at the ends of the strips. Also, if enough water transports to the ends, the center of the IPG strip will become dry and burn.

The best recourse is to de-salt your samples beforehand (see the 2D: Principles and Methods guide (above) for detailed discussions on Sample Preparation) and to use IEF grades ofurea.

[T Berkelman, 25 Aug 99] Glycerol or isopropanol do seem to help suppress cathodic streaking in some cases. I'm not really clear on why this is so, but it may be supression of electroendosmosis. One thing you do notice with these reagents is that the current is lower and higher voltages are often possible. Thus, the run is quicker and overfocusing is less likely.

I know of a couple groups using isopropanol at 15%. I've also heard of glycerol being used at 10%. Your best bet with using pads under the ends of the IPG strips is to use relatively thin filter paper, like the Munktell paper that comes with the DryPlates. They should be cut to about 3 mm x 8 mm and slightly dampened. Following rehydration, you just pick up each end of the strip with forceps and slip the pad under. It's really not that big a deal, but may be somewhat time-consuming to do to 12 strips. I actually find that it's rarely necessary, with the exception of preparative loads on narrow ranges. The pads don't have to necessarily go in directly following rehydration. They are of the most benefit towards the end of the run, so if it's more convenient to place them later, by all means do so.