OPTIMIZATION OF ISOELECTRICAL FOCUSING PROGRAMS IN 2D-SDS-PAGE PROTEIN ANALYSIS. ***P.A. Kowalski****, Dr. Laura E. Tubbs*, Dr. Paul A. Craig*, RIT, Department of Chemistry, Proteomics Laboratory, pxk9006@rit.edu, letsch@rit.edu, pac8612@rit.edu.*

Isoelectric focusing (IEF) is the method utilized to achieve the first dimension of separation in protein analysis, followed by SDS-PAGE, the second dimension. The basis of this method lies in the amphoteric nature of proteins; the weakly charged acidic and basic side groups will protonate or deprotonate, depending on localized pH conditions. The protein is said to be at its respective isoelectric point (pI) when it carries no net charge. When a current is applied to protein in solution, each protein migrates along an immobilized pH gradient (IPG) strip to their isoelectric point, and this is the function IEF serves. Many programming strategies are used to perform different types of separations, depending on the type of protein being studied, and its abundance. The focus of this project is to devise an IEF program, using the Biorad Protean™ IEF cell, which will focus the proteins being analyzed in the most efficient manner possible. The RIT Proteomics Lab Standard Operating Procedure calls for the use of an IEF program which entails a run time of 16 hours, 6 minutes. So far, new programs have been devised with run times spanning from as short as eight hours, to ten, and twelve hours. All programs have thus far worked with what appears to be a good degree of success, yielding reproducible and sharp, resolute protein spots in a consistent fashion. The next step in optimizing these IEF programs, in the context of definitively establishing if any of these shorter programs separate better, would be to do a spot count on the gels obtained and compare these to reference gels via Phoretix 2D software.