Versatile Separation Capabilities of the PROTEAN® i12™ IEF System

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Introduction

Two-dimensional (2-D) gel electrophoresis is a powerful and widely used analytical tool for resolving and separating complex biological samples (Garfin 2003). The technique employs two sequential electrophoretic separation steps. The first step or dimension is isoelectric focusing (IEF), a separation based on protein charge. The second dimension is SDS-PAGE, a separation based on protein size. Currently, the first dimension is most commonly performed on IPG (immobilized pH gradient) strips, as this provides the best reproducibility and ease of use (Görg et al. 2004). IPG strips are available from Bio-Rad in different lengths (7, 11, 17, 18, and 24 cm) and each of them is available in a variety of pH ranges including broad ranges (pH 3–10 and pH 3–10 nonlinear), narrow ranges (pH 3–6, pH 4–7, pH 5–8, and pH 7–10), and micro ranges (pH 3.9–5.1, pH 4.7–5.9, pH 5.5–6.7, and pH 6.3–8.3). Broad-range IPG strips are best for an initial view, but narrow- and micro-range IPG strips offer higher resolution separations and often allow the visualization of many proteins that cannot be distinguished on broad-range IPG strips. In many cases, it is desirable to analyze the same sample on multiple pH ranges.

IPG-IEF is conducted at high voltage (typically several thousand volts) and the current is typically limited at 50 µA per IPG strip. Due to these unique running conditions, IPG-IEF is conducted with specialized equipment. Currently available instruments for IPG-IEF are designed to run several IPG strips simultaneously and use a single power supply to apply voltage across all of the IPG strips. The current limit specified is an average current per IPG strip. If IPG strips of differing conductivity are run on the same instrument, they will experience different current-voltage profiles in an uncontrolled manner. It is therefore recommended that one use only samples of similar composition in the same run. Since individual IPG strip pH ranges have differing intrinsic conductivities, it is also recommended that one restrict a single run to IPG strips of the same pH range.

Prefractionation of samples by liquid-phase IEF in the MicroRotofor™ cell is employed prior to 2-D electrophoresis to reduce sample complexity, allow increased protein loading, and facilitate detection of less abundant proteins (Davidsson et al. 2002, Rachinsky et al. 2007). However, this strategy generates fractions of differing conductivities that must be run on different pH-range IPG strips. Analysis of these fractions using current equipment must be done on multiple instruments or sequentially in a time-consuming manner.

Bio-Rad's PROTEAN i12 IEF system represents an improvement over traditional IEF systems by offering individual lane power control. The individual lane control feature of the PROTEAN i12 IEF system allows each IPG strip to be run independently, making it possible to process different samples and different types of IPG strips in a single run. This feature greatly shortens the time needed for protocol optimization for a specific sample. In this study, we demonstrate the versatility and flexibility of the PROTEAN i12 IEF system using soybean protein samples prefractionated on the MicroRotofor cell.

Methods

Protein Extraction

Soybeans were hydrated overnight in aerated water and homogenized with a mortar and pestle in a solution composed of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, and 40 mM DTT. For each gram of tissue, 9 ml of solution was used. The homogenized sample was centrifuged at 14,000 g for 15 min and the supernatant was stored at –80˚C until use.

MicroRotofor Cell Fractionation

Soybean protein samples (1.26 mg) were diluted in 7 M urea, 2 M thiourea, and 0.2% biolyte 3–10 to a final volume of 3 ml and 2.5 ml were loaded onto the MicroRotofor cell (Bio-Rad Laboratories, Inc.) with 0.1 M H_3PO_4 at the anode and 0.1 M NaOH at the cathode. The run was conducted at 1 W constant power until the voltage stabilized. Total fractionation time was 50 minutes. Ten fractions were harvested at the end of the run. After measuring the pH of each fraction using pH paper, the samples were used directly for the following 2-D experiments.

2-D Electrophoresis

The first dimension was conducted using 11 cm ReadyStrip™ IPG strips pH 3–6, pH 5–8, and pH 7–10 depending on the pH of the samples. Fifteen to 40 µl of each fraction were diluted in a rehydration solution consisting of 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 0.2% (w/v) Bio-Lyte® ampholyte pH 3–10 (or ReadyStrip 7–10 buffer for pH 7–10 IPG strips), 50 mM DTT, and 0.001% (w/v) bromophenol blue dye to a final volume of 185 µl and loaded onto the strips via passive rehydration. The strips were rehydrated overnight and focused with a program of 250 V for 30 min followed by 8,000 V for 35,000 Vh under a current limit of 50 µA.

Following isoelectric focusing, the IPG strips were equilibrated for 15 min in equilibration buffer I composed of 6 M urea, 50 mM Tris-HCl, pH 8.8, 2% (w/v) SDS, 1% (w/v) DTT, 30% (w/v) glycerol, and 0.001% bromophenol blue. This was followed by another 15 min in equilibration buffer II composed of 6 M urea, 50 mM Tris-HCl, pH 8.8, 2% (w/v) SDS, 2.5% (w/v) iodoacetamide, 30% (w/v) glycerol, and 0.001% bromophenol blue. Second-dimension separations for 11 cm IPG strips were performed on Criterion™ 8–16% Tris-HCl gels. Equilibrated IPG strips were applied to the second-dimension gels and run using Tris-Glycine-SDS buffer at 200 V for 55 min.

Gel Staining and Imaging

After running, all gels were washed three times for 5 min with deionized water and stained with Bio-Safe™ Coomassie stain (Bio-Rad) for 1 hr. After destaining with water, the gels were scanned on a GS-800™ calibrated densitometer using standard settings (Bio-Rad).

Results

MicroRotofor fractionation

Adjacent fractions collected from the MicroRotofor cell differed from each other by 0.5 to 1.0 pH units, as judged using pH paper, and covered most of the pH range from pH 3 to pH 10 (Table 1). Narrow-range IPG strips for subsequent 2-D analysis were chosen based on sample pH (Table 1). The acidic, neutral to near-neutral, and basic fractions were focused on pH 3–6, pH 5–8, and pH 7–10 ReadyStrip IPG strips, respectively.

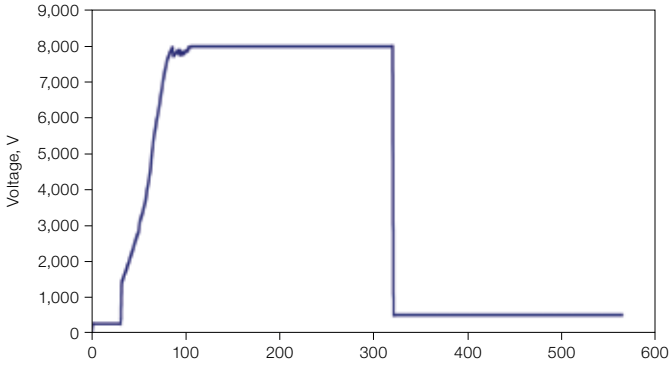
Table 1. MicroRotofor fraction pH and corresponding IPG strips used.

Fraction #	pH	pH range of IPG strips
1	3.0	3–6
2	4.0	3–6
3	4.5	3–6
4	5.5	5–8
5	6.0	5–8
6	7.0	5–8
7	7.5	5–8
8	8.5	7–10
9	9.0	7–10
10	>9.0	7–10

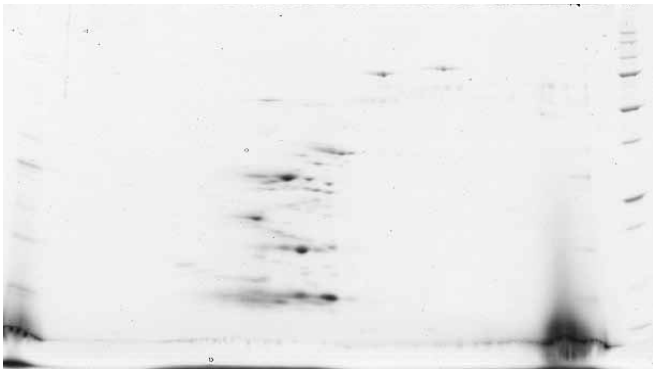
PROTEAN i12 IEF System Performance with Different Samples and Different IPG Strips in a Single Run

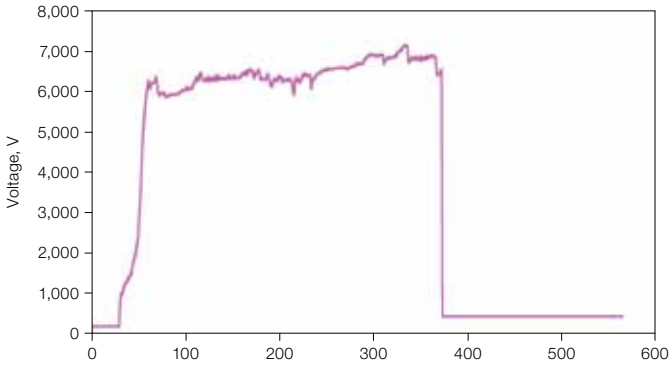
To test the flexibility of the PROTEAN i12 IEF system, we tested its performance by simultaneously running all the fractions from a MicroRotofor separation. As shown in Table 1, each fraction has a distinct pH. Protein composition and resistance are therefore expected to differ from fraction to fraction. Indeed, each sample gave a distinctive voltage profile when focused with a 50 µA current limit. In a traditional focusing system, the current through each IPG strip would have been uncontrolled, resulting in unpredictable results. On the PROTEAN i12 IEF system, with individual power control for each lane, there is no effect of the other concurrently run IPG strips on any of the individual runs. As shown in Figure 1, samples ranging from very acidic to very basic pH loaded on IPG strips with different pH gradients can all be focused in one run. In addition, the gel images show that different amounts of proteins were loaded on each strip. Therefore, the PROTEAN i12 IEF system can accommodate differences in sample pH, composition, and load, as well as different IPG pH gradients, at the same time.

Fraction #1 run on pH 3–6 strip.

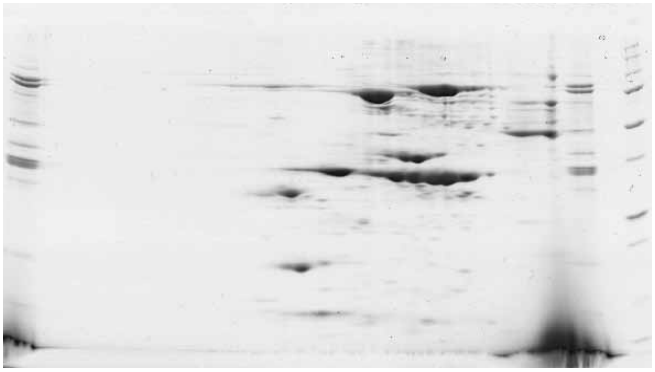


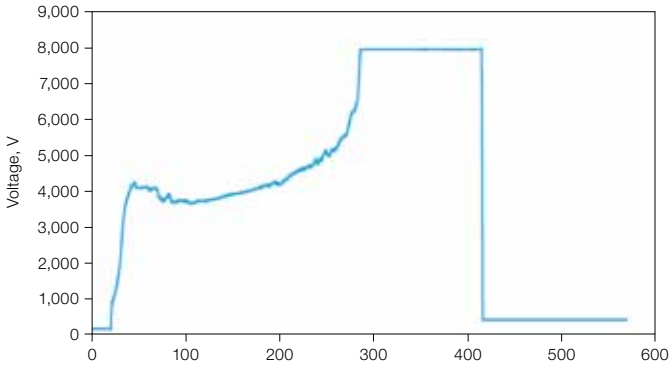
Fraction #2 run on pH 3–6 strip.



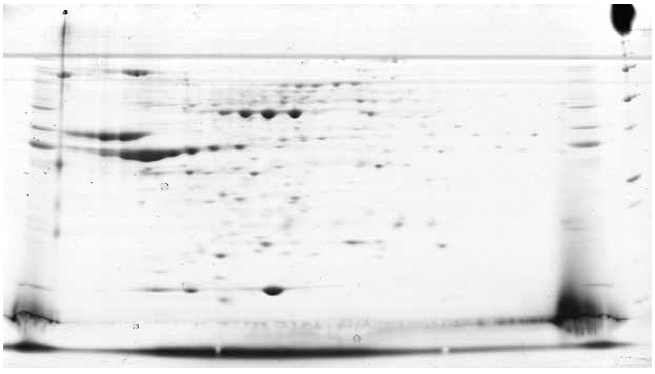


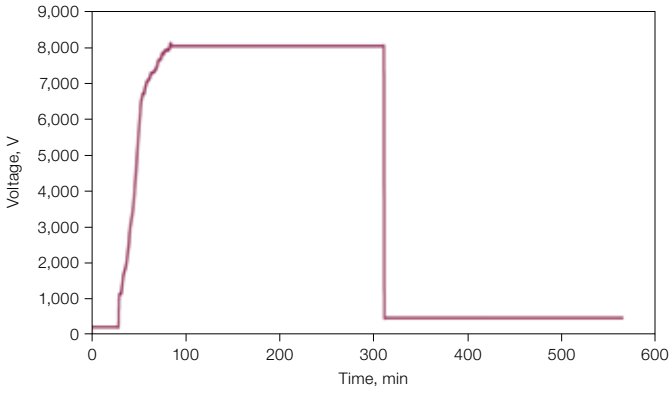
Fraction #3 run on pH 3–6 strip.



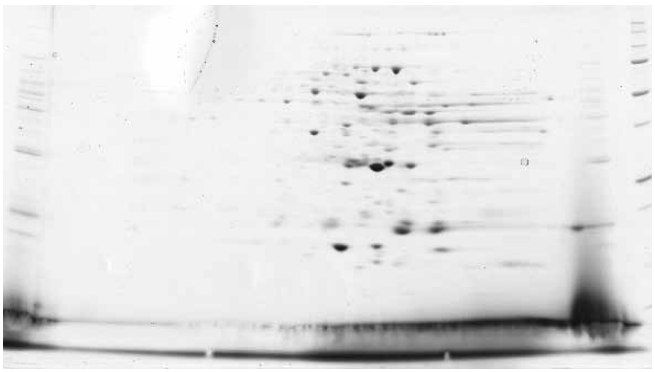


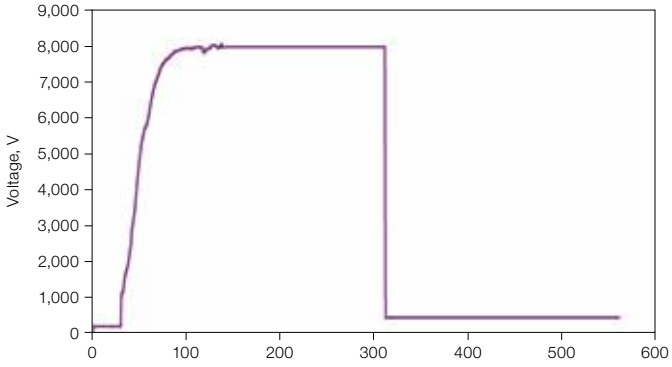
Fraction #4 run on pH 5–8 strip.



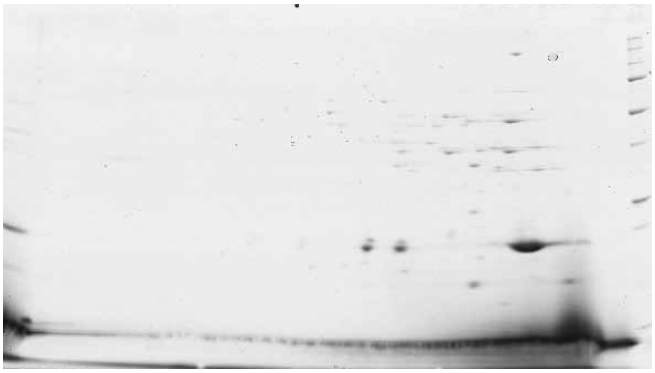


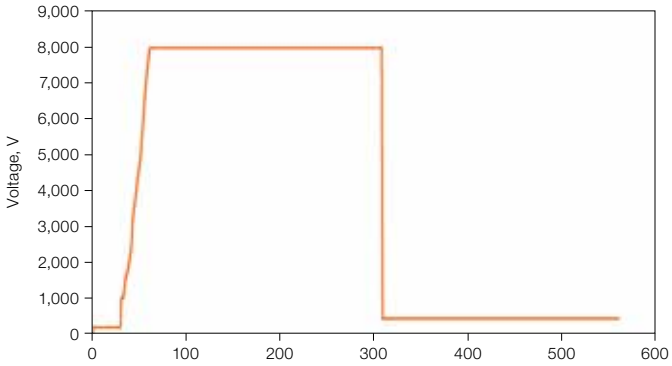
Fraction #5 run on pH 5–8 strip.



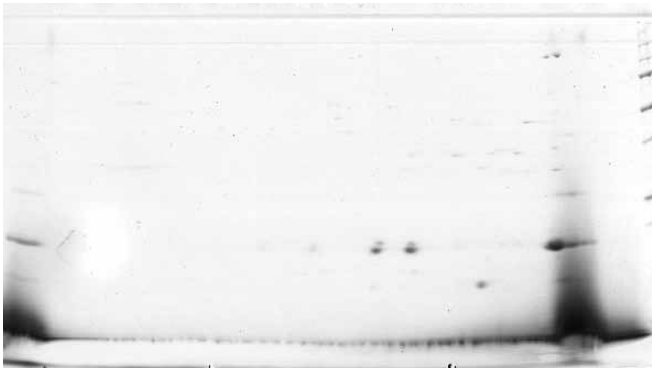


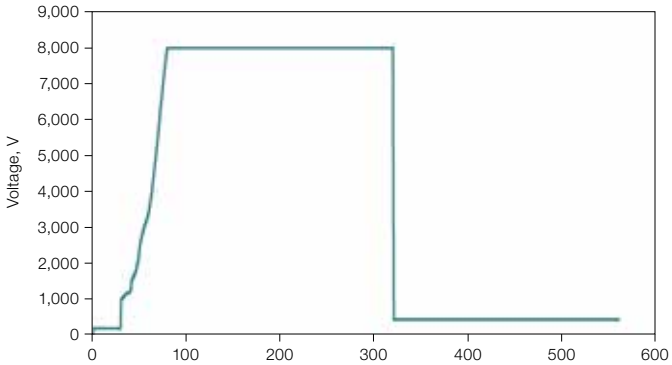
Fraction #6 run on pH 5–8 strip.



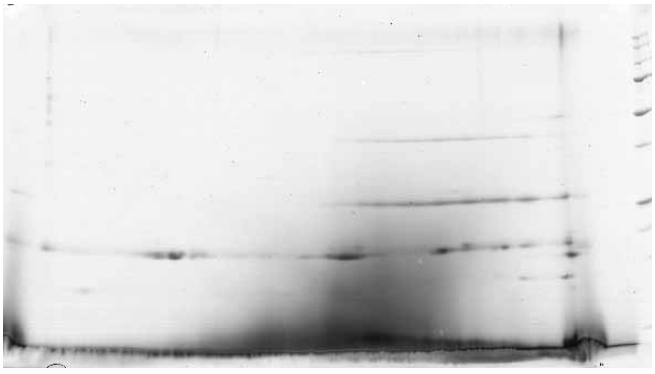


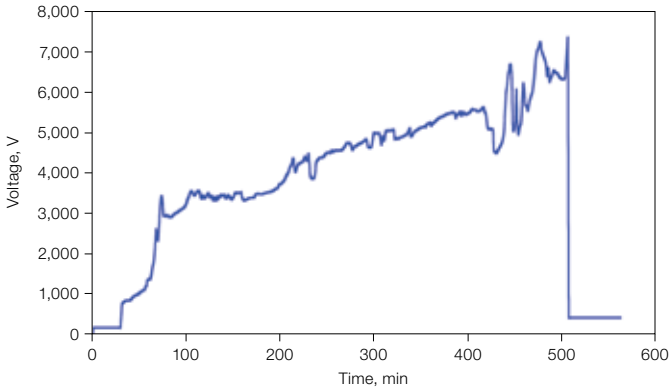
Fraction #7 run on pH 5–8 strip.



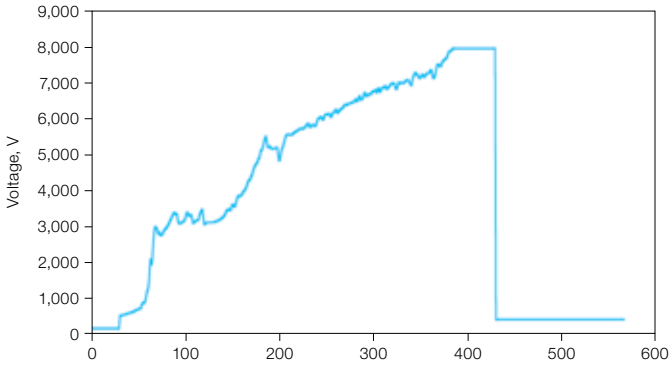


Fraction #8 run on pH 7–10 strip.





Fraction #9 run on pH 7–10 strip.



Fraction #10 run on pH 7–10 strip.

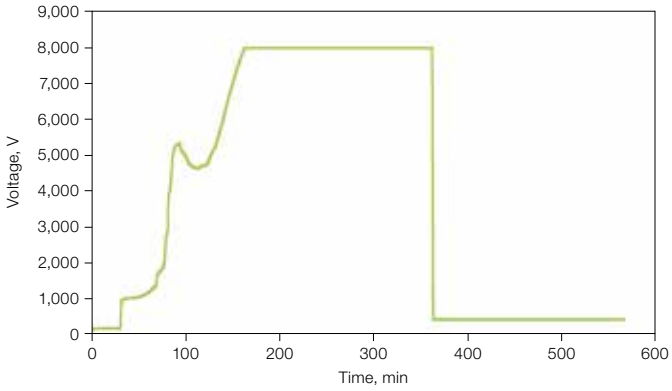


Fig. 1. 2-D gel images and voltage profiles of MicroRotofor cell focused in one run on the PROTEAN i12 IEF cell. All of the second-dimension gels were run on 8–16% Criterion Tris-HCl gels.

Conclusions

2-D electrophoresis is a widely used tool in proteomics for resolving up to thousands of proteins in a complex biological sample. A well-controlled first-dimension isoelectric focusing is critical for a successful 2-D process. Due to inherent differences in sample and strip resistance, it can be difficult to obtain reproducible results in a traditional first-generation IEF system that uses a parallel circuit setup to control the total current instead of sending the current into each individual strip. By controlling the current and voltage in each individual channel, the PROTEAN i12 IEF system can accommodate up to 12 different samples, strips, and protocols in a single run. In many cases, the focusing protocol for a particular sample may require extensive optimization of sample load, IPG strip pH gradient selection, and focusing protocol. Parallel circuit focusing systems can suffer when IPG strips are run concurrently, and the optimization process can take days to weeks, depending on the setup. With the individual lane power control of the PROTEAN i12 IEF system, different sample loads, IPG strips, and protocols can be set up in a single run. Therefore, the time required to optimize a focusing protocol can be considerably shortened. Bio-Rad's PROTEAN i12 IEF cell is designed to provide maximum flexibility to accommodate sample, strip, and protocol variations. By individually controlling each lane, the interlane effect can be completely eliminated and the system can offer outstanding controllability and time-saving capability.

References

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Bulletin 6139 Rev A US/EG *11-0944 0811 Sig 0211*