# OPTIMIZATION OF CAPILLARY GEL ELECTROPHORESIS FOR THE ANALYSIS OF SERUM

An Undergraduate Research Scholars Thesis

by

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# ABSTRACT

Optimization of Capillary Gel Electrophoresis for the Analysis of Serum

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Capillary gel electrophoresis (CGE) is a protein separation technique that could help identify disease biomarkers using microliters of human blood serum. Analytical techniques that separate serum proteins with high sensitivity and repeatability have the potential for significant clinical impact. This study examined multiple ways to improve current CGE methodology, such as the injection method, separation voltage, and serum concentration to buffer ratio. Electrokinetic injection with a 15kV separation and a 1:1.4 protein to buffer mass ratio were found to increase electropherogram resolution and repeatability. With these improvements, CGE may become an important future instrument for disease detection.

# ACKNOWLEDGMENTS

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# NOMENCLATURE

Apolipoprotein A-1 (Apo A-1)

Bovine Serum Albumin (BSA)

Capillary Electrophoresis (CE)

Capillary Gel Electrophoresis (CGE)

High Density Lipoprotein (HDL)

Sodium Dodecyl Sulfate (SDS)

# CHAPTER I

# INTRODUCTION

Human serum is dominated by high abundance proteins but also contains a large span of low abundance proteins. Low abundance proteins vary in concentration within the human population, providing unique serum chemistry profile for each individual. Biomarkers are chemical components that can be measured to identify and assess a disease (1). Capillary gel electrophoresis (CGE) is a method capable of detecting low abundance proteins, which could be potential biomarker candidates. Using CGE to detect the changes in the blood composition of an individual due to a disease, CGE analysis of human serum could lead to early diagnosis and preventative care.

Capillary gel electrophoresis presents alternatives to traditional analytical methods in the field of proteomics. The Beckman P/ACE MDQ Capillary Electrophoresis System separates proteins at a faster rate with higher efficiency and greater resolution compared to slab gel electrophoresis techniques (2). It draws the contents of a sample vial into a capillary where the biomolecules are sieved according to their molecular size. The light transmittance of the sieved proteins are recorded by an internal UV spectrometer and converted into absorbance using 32 Karat Software. These absorbance values are represented as peaks, which represent the relative area of the proteins in the generated electropherograms. Limitations of this analytical tool such as a lack of repeatability in protein absorbance, however, are present. It is therefore imperative to optimize the sensitivity and performance of CGE for possible clinical applications by improving current methods.

A possible factor that can reduce repeatability is the injection method for sample uptake. Electrokinetic injection is the primary mode of injection in CGE. It creates a voltage potential for sample uptake in CGE by placing the electrode and the capillary in the sample. Sample uptake is dependent on pressure, duration of the injection, and viscosity of the medium through the capillary (3). It is affected by variables such as the applied voltage for sample separation, mobility of the components, and the diameter of the capillary. Electrokinetic injection has been known to change sample composition after multiple injections. The change in sample composition and different voltages applied for sample separation can potentially have an effect on repeatability. Hydrodynamic injection has the potential to correct this error and improve electropherogram resolution. It uses the pressure difference between the two ends of the capillary for sample uptake into the CGE system and therefore does not affect sample composition.

Electropherogram repeatability and peak intensity may also be influenced by the proportion of ` serum protein to sodium dodecyl sulfate (SDS) mass. Sodium dodecyl sulfate is a negatively charged buffer that coats proteins and normalizes protein charges. The SDS-CGE technique separates macromolecular serum proteins strictly by size. A recommended 1:1.4 mass ratio of protein to SDS will be compared to a 1:1 volumetric ratio of serum to SDS sample buffer to determine which produces greater repeatability. When protein mass is greater than SDS mass it can lower peak resolution (4).

Effective analysis of serum includes identification of its proteins. If serum protein concentrations could be seen to correlate with stages of disease, this would help the understanding of the various roles serum proteins play in the body. With the identification of protein peaks, the

electropherograms of diseased patients can be compared to healthy individuals, which could be an effective medium for diagnosis. Several common proteins found in serum are analyzed in this study, including apolipoprotein A-1 (apo A-1), high density lipoprotein (HDL), and albumin. For example, defects in apo A-1 production are linked with HDL deficiencies including Tangier Disease (5).

The focus of this study is to optimize CGE as a method for sieving serum. The injection method, serum concentration to buffer ratio, and separation voltage will be examined for their effects on the repeatability and intensity of the electropherogram peaks. Electropherograms of serum protein standards will also be generated to serve as identifiers of peaks and to determine the optimal method.

# CHAPTER II

# METHODS

Bovine serum albumin standard and commercial human serum were analyzed using the Beckman/Coulter P/ACE MDQ Capillary Electrophoresis System. Electropherograms for each sample were generated using 32 Karat™ Software. Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC) approval was obtained prior to serum analysis, (IRB2014-0375M and IBC2013-161).

## I. Materials

The Beckman/Coulter SDS-MW Kit purchased from AB Sciex (PN: 390953) was used for this study. Contents of the kit include capillaries of 50 μm I.D. bare-fused silica, a proprietary formulation of SDS-MW Gel Buffer (pH 8), SDS-MW Sample Buffer (100 mM Tris-HCl pH 9.0, 1%SDS), SDS-MW Size Standard (10 to 225 kDa, 16 mg/mL), Acidic Wash Solution (0.1 N HCl), and Basic Wash Solution (0.1 N NaOH). Other materials that were used in this study include bovine serum albumin (BSA), human high density lipoprotein (HDL), human apolipoprotein A-1 (apo A-1), and commercial human serum. Lyophilized BSA powder was obtained from Sigma Aldrich ≥ 96% purity (PN: A9418). Lyophilized HDL powder was obtained from Sigma Aldrich (PN: L1567). Human apo A-1 was obtained from Academy Biomedical (PN: 11P-UP210). Commercial human serum sample was acquired from Proteogenex (ID #282013S).

## II. Experimental Methodology

*Injection Method*

For the electrokinetic injection method, six samples were prepared by mixing 25 µL 5 mg/mL solution of BSA in deionized water with 25 µL sample buffer. After being placed in a sonicating water bath for five minutes, each sample was analyzed using electrokinetic injection voltages of 2.5 kV, 5 kV, and 7.5 kV with the Beckman/Coulter P/ACE MDQ Capillary Electrophoresis System.

For the preliminary hydrodynamic injection, six samples were prepared by mixing 25 µL 5 mg/mL solution of BSA in deionized water with 25 µL sample buffer. After being placed in a sonicating water bath for five minutes, each sample was analyzed by applying a 0.5 psi hydrodynamic injection for a three-second duration using the Beckman/Coulter P/ACE MDQ Capillary Electrophoresis System.

After the preliminary data was obtained, six samples were prepared by mixing 25 µL 5 mg/mL solution of BSA in deionized water with 25 µL sample buffer and being placed in a sonicating water bath for five minutes. The six samples were divided into three durations of 0.5 psi hydrodynamic injection: 30-second, 60-second, and 90-second. Two electropherograms were generated for each duration using the Beckman/Coulter P/ACE MDQ Capillary Electrophoresis System.

*Sample Concentration-to-Buffer Ratio*

There are 60-78 g/L of proteins found within human serum. In order to determine the amount of sample buffer needed to sufficiently coat the proteins using a 1:1.4 protein to SDS mass ratio, an average value of 69 g/L of proteins was used. The protein content in 25 µL of serum was calculated using the 69g/L concentration, and the serum protein mass was then multiplied by 1.4 to find the necessary SDS mass. There are 10 grams of SDS per 100 mL SDS buffer in a 10% solution (9). In a 1% solution, which was used for this experiment, there would then be $1.0 \times 10^{-5}$ g of SDS in 1.0 µL SDS buffer solution. The solution volume with the needed SDS mass was calculated to be 280 µL for 25 µL serum. Due to sample vial size limitations, 6 µL serum with 60 µL SDS sample buffer was used to satisfy the 1:1.4 mass ratio.

Three different samples with varying serum concentrations were prepared: 6 µL serum with 60 µL SDS buffer, 33 µL serum with 33 µL SDS buffer, and 66 µL serum with no SDS buffer. Three aliquots of the 6 µL serum/60 µL SDS and 66 µL serum variations were prepared and two aliquots of the 33 µL serum/ 33 µL SDS variation were prepared. A total of eight samples were analyzed using the CE system.

A second analysis was performed for 5 mg/mL BSA in deionized water. The necessary SDS sample buffer volume required for the 1:1.4 mass ratio in the BSA sample was calculated using the previous calculations. The prepared sample consisted of 35 µL 5 mg/mL BSA in deionized water with 25 µL SDS sample buffer. The prepared sample was analyzed eight times using the CE System.

*Voltage Separation*

Six BSA standards were prepared by mixing 25 µL sample buffer with 35 µL BSA standard. Three human serum samples were prepared by adding 33µL serum to 33µL sample buffer. All samples were degassed using a sonicating water bath for five minutes.

The electrokinetic injection method was used to analyze the prepared BSA standard and serum samples using the Beckman/Coulter P/ACE MDQ Capillary Electrophoresis System. Three voltage separations of 10kV, 15kV, and 20kV were applied to the samples to determine which voltage would produce high-resolution protein peaks with a flat baseline. Two BSA samples and one serum sample were analyzed for each voltage separation.

*Protein Standard Identification*

As performed before, 6 µL serum was combined with 60 µL sample buffer. The sample was placed in a sonicating water bath for five minutes and analyzed eight times using the CE System.

To ensure HDL proteins were sufficiently coated by SDS, a 1:1.4 protein to SDS mass ratio was maintained. As it is known that there is 1 g SDS per 100 mL SDS buffer in 1% stock buffer solution (2), 0.28 mL SDS buffer was added to each milliliter of HDL stock solution. Human serum concentrations of HDL are approximately 50 mg/dL (8). The 200 mg/dL HDL stock solution was diluted to this concentration by using a 1:3 volumetric ratio of HDL stock solution to diluted water and sample buffer, which required 2.72 mL diluted water and 0.28 mL SDS buffer per 1mL HDL stock solution.

The solution was scaled to 72 µL by combining 18 µL HDL stock, 5 µL SDS buffer, and 49 µL diluted water. The sample was placed in a sonicating water bath for five minutes and analyzed eight times using the CE System.

For analysis of apolipoprotein A-1, 52.6 µL 1 mg/mL apo A-1 was combined with 7.4 µL sample buffer. The sample was placed in a sonicating water bath for five minutes and analyzed three times using the CE System.

For analysis of sample buffer, 66 µL sample buffer was placed in a sonicating water bath for five minutes and analyzed three times using the CE System.

# CHAPTER III

# RESULTS

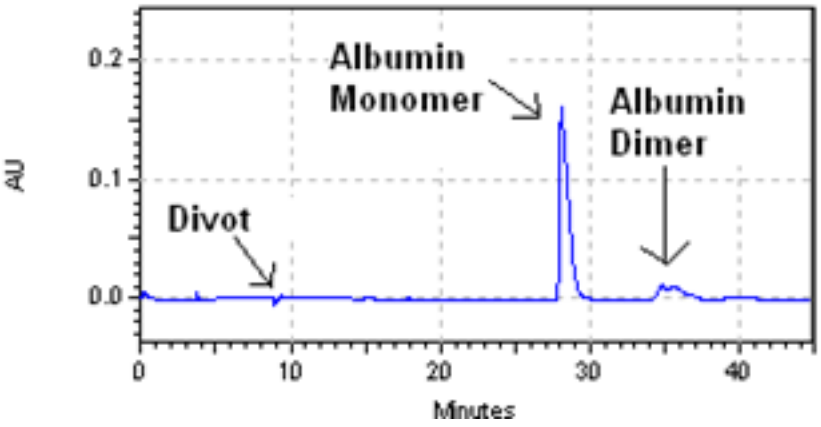


**Figure 1. Peak Identifications for the Bovine Serum Albumin electropherograms**

The divot is an artifact of the capillary gel electrophoresis system and is consistently shown on electropherograms. The divot and the albumin peak were chosen as features of interest as they are common features in albumin standard electropherograms and were used for all following results.

*Injection Method*

Six electropherograms using electrokinetic injection of voltages 2.5 kV, 5kV, and 7.5 kV were generated and analyzed.

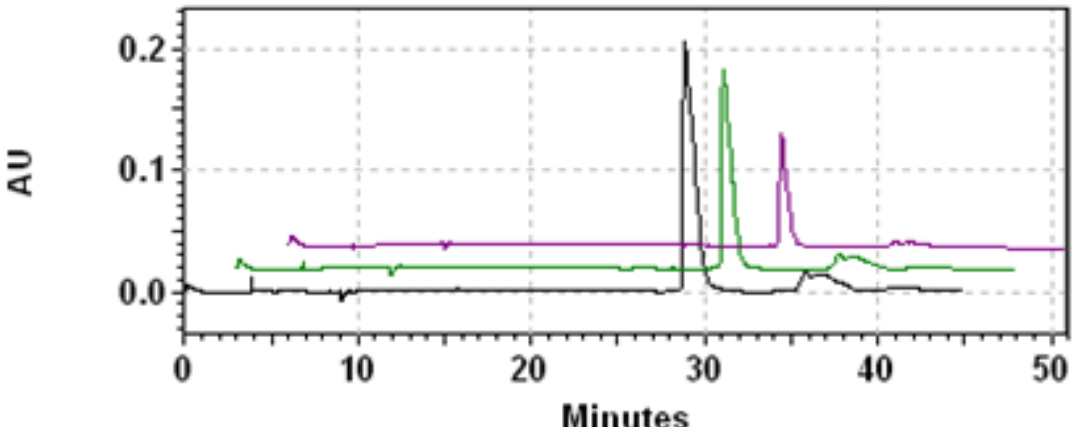


**Figure 2. In descending order, three electropherograms stacked on the x-axis of albumin were analyzed at 2.5 kV, 5 kV, and 7.5 kV electrokinetic injection voltage.**

The electropherograms are stacked for clarification as to which peaks are associated for each separation.

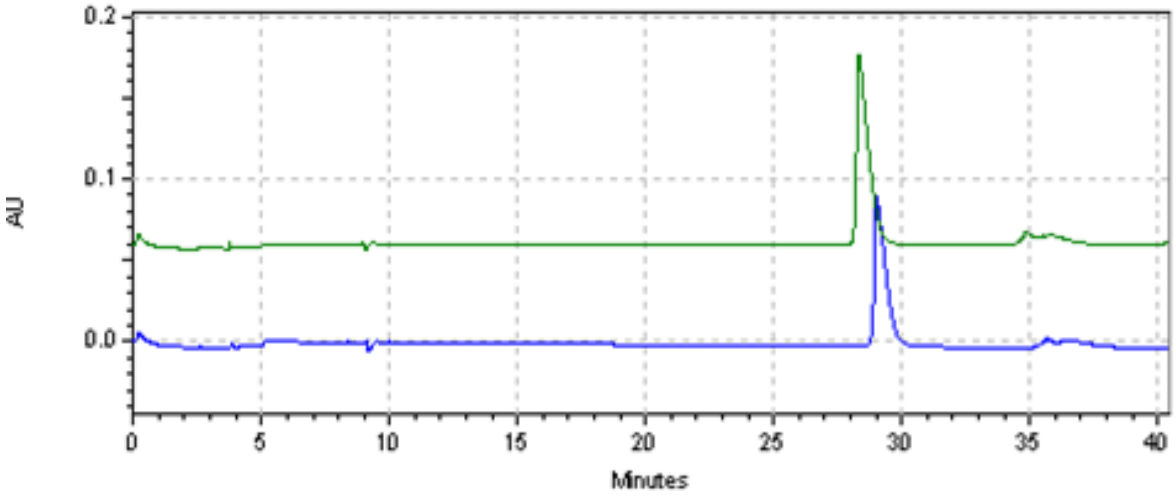


**Figure 3. Albumin electropherograms, a & b in ascending order, generated using 2.5kV electrokinetic injection.**

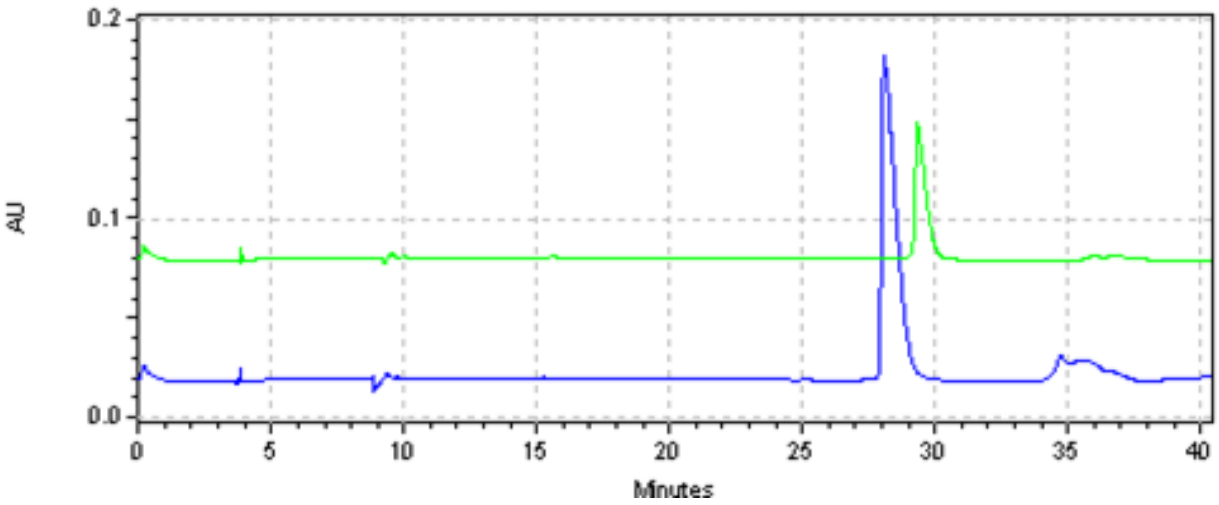


**Figure 4. Albumin electropherograms, a & b in ascending order, generated using a 5.0 kV electrokinetic injection.**

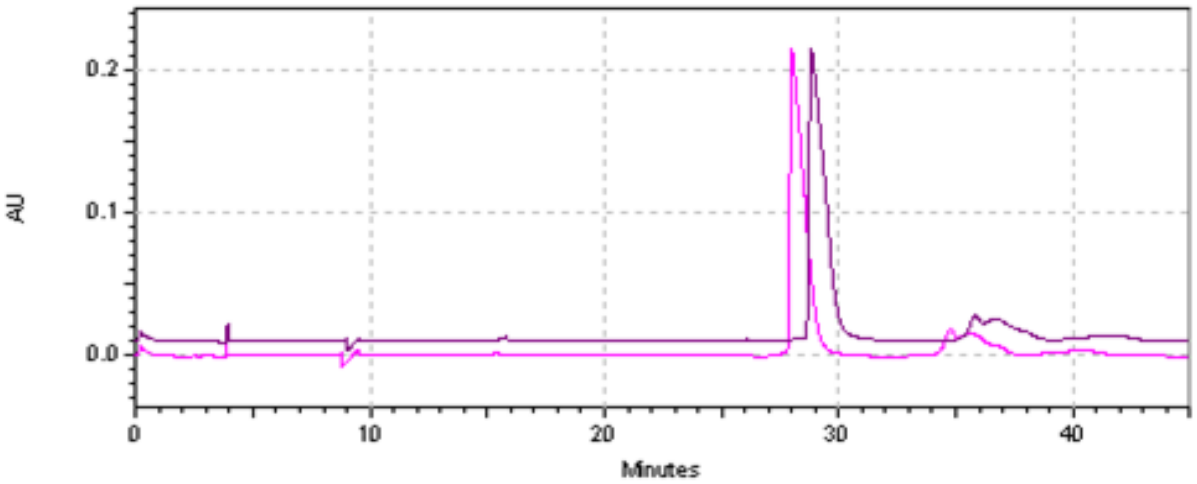


**Figure 5. Albumin electropherograms, a & b in ascending order, generated using 7.5 kV electrokinetic injection.**

One of the goals of the experiment was to observe repeatability of the electrokinetic injection method results. Figures 3-5 display the electropherograms comparing the generated results of each voltage injection. 2.5 kV and 7.5 kV electropherograms show repeatable results with small deviations in peak size and the presence of all protein peaks. One difference that was observed in

Figure 4 was the absence of the albumin dimer in trace b compared to the trace a using the same injection voltage.

**Table 1. Times of separation analyzed at the divot, albumin monomer, and albumin dimer peaks with 2.5 kV, 5kV, and 7.5 kV injection voltage**

| Figure and Electropherogram | Voltage (kV) | Divot Time (min) | Albumin Monomer Time (min) | Albumin Dimer Time (min) |
|---|---|---|---|---|
| 3a | 2.5 | 8.99 | 28.42 | 34.92 |
| 4a | 5 | 8.69 | 27.49 | 34.01 |
| 5a | 7.5 | 8.62 | 27.43 | 34.03 |
| 3b | 2.5 | 8.84 | 27.73 | 34.14 |
| 4b | 5 | 9.07 | 28.73 | n/a |
| 5b | 7.5 | 8.81 | 28.25 | 35.06 |
| | Std Dv | 0.171 | 0.534 | 0.514 |
| | Avg. | 8.837 | 28.008 | 34.432 |
| | RSD% | 1.941 | 1.909 | 1.493 |

**Table 2. Peak areas analyzed at the divot, albumin monomer, and albumin dimer peaks with 2.5kV, 5kV, and 7.5 kV injection voltage**

| Figure and Electropherogram | Voltage (kV) | Divot Area | Albumin Monomer Area | Albumin Dimer Area |
|---|---|---|---|---|
| 3a | 2.5 | 47531 | 2488435 | 171136 |
| 4a | 5 | 62123 | 4880581 | 328741 |
| 5a | 7.5 | 124417 | 3144267 | 470609 |
| 3b | 2.5 | 31703 | 3322128 | 203545 |
| 4b | 5 | 14199 | 1709594 | n/a |
| 5b | 7.5 | 107338 | 4862742 | 459738 |
| | Std Dv | 43184.62 | 1272132.92 | 139445.66 |
| | Avg | 64551.83 | 3401291.17 | 326753.8 |
| | RSD% | 66.90 | 37.40 | 42.68 |

Table 1 shows that the timing of the peaks are repeatable with a standard deviation of 0.534 regardless of the voltage used in electrokinetic injection. The divot time had a range of 0.45 minutes. Albumin monomer peak time had a range of 1.3 minutes. Albumin dimer time of separation had a range of 1.05 minutes. Table 2 shows the lack of repeatability in the separation of peak areas with different injection voltages: The divot area had a range of 110218, the albumin monomer area had a range of 3752816, and the albumin dimer area had a range of 299473. Separation b from figure 5's albumin dimer peak time and area were not used during this analysis. The peak is an outlier in this set of data.

Six electropherograms using hydrodynamic injection of 0.5 psi for 30 seconds were generated and analyzed.

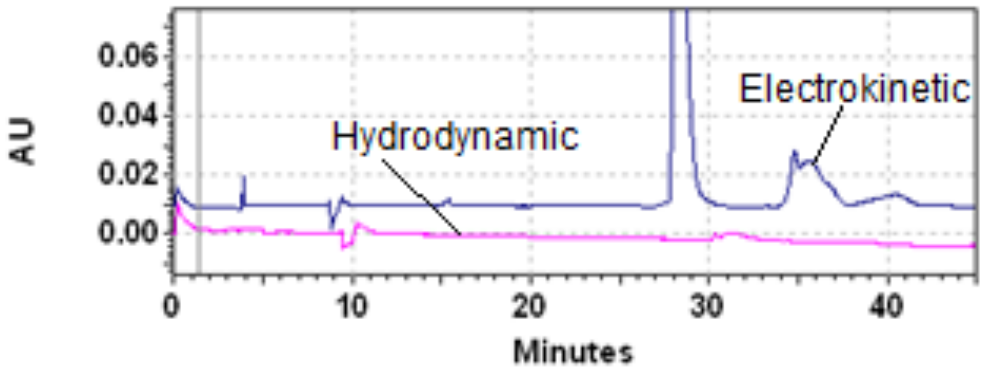


**Figure 6. Comparison of peak intensity between hydrodynamic and electrokinetic injection**

The intensity of the peaks using hydrodynamic injection was relatively low. Figure 6 shows a comparison between 0.5 psi hydrodynamic injection over a 30 second duration and 5kV electrokinetic injection over a 20 second duration.. The albumin peak in the electropherogram of

the hydrodynamic injection is barely detectable with an absent albumin dimer. In comparison, the electrokinetic injection shows a prominent albumin peak as well as the albumin dimer.

**Table 3. Comparison of the times labeled where the peaks of interest were found.**

| Separation # | Divot time (min) | Divot Area |
|---|---|---|
| 1 | 9.463 | 152280 |
| 2 | 9.57 | 121681 |
| 3 | 10.133 | 7190 |
| 4 | 10.063 | 18911 |
| 5 | 9.567 | 78432 |
| 6 | 9.771 | 399049 |
| Std Dv | 0.281 | 143550.885 |
| Avg | 9.761 | 129590.5 |
| RSD% | 2.875 | 110.773 |

The divot was the most pronounced peak of interest in the electropherograms. The divot is consistently found in every electropherogram generated through CGE. Its composition is still unknown. Its migration time had a range of 9.463 to 10.133. The area had a range from 7190 to 399049.

Six electropherograms using hydrodynamic injection using 0.5 psi with a varied duration of either 30, 60, or 90 seconds were generated and analyzed.

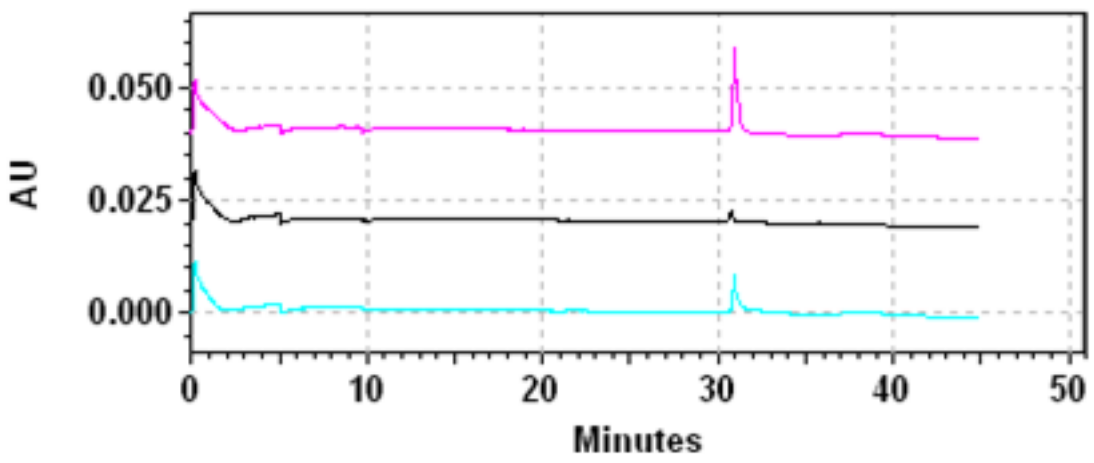


**Figure 7. In descending order, electropherograms of albumin using hydrodynamic injection of 0.5 psi for a duration of 90, 60, and 30 seconds were produced.**

**Table 4. Times of the peaks of interest Divot and Albumin with various durations of 90, 60, and 30 seconds**

| Separation # | Injection Duration (s) | Divot Time (min) | Albumin Time (min) |
|---|---|---|---|
| 1 | 90 | 11.07 | 38.15 |
| 2 | 60 | 11.77 | 42.93 |
| 3 | 30 | 10.17 | n/a |
| 4 | 90 | 9.73 | 30.92 |
| 5 | 60 | 9.66 | 30.75 |
| 6 | 30 | 9.86 | 30.89 |
| | Std Dv | 0.856 | 5.569 |
| | Avg | 10.377 | 34.728 |
| | RSD% | 8.249 | 16.035 |

In Table 4, the times of the divot had a range of 9.66 minutes to 11.77 minutes. The albumin peak showed deviation from the differing injections with a time range of 30.75 to 42.93 minutes.

**Table 5. Areas of the peaks of interest the Divot and Albumin using various hydrodynamic injection durations of 90, 60, and 30 seconds**

| Separation # | Injection Duration (s) | Divot Area | Albumin Area |
|---|---|---|---|
| 1 | 90 | 42329 | 161225 |
| 2 | 60 | 2822 | 61200 |
| 3 | 30 | 5119 | n/a |
| 4 | 90 | 11620 | 32700 |
| 5 | 60 | 1415 | 33859 |
| 6 | 30 | 2017 | 135151 |
| | Std Dv | 1548.321 | 59670.670 |
| | Avg | 10887 | 874827 |
| | RSD% | 145.571 | 70.344 |

In Table 5, the areas of the divot have a range of 40914 units. The albumin monomer peak had a range of 128525 units.

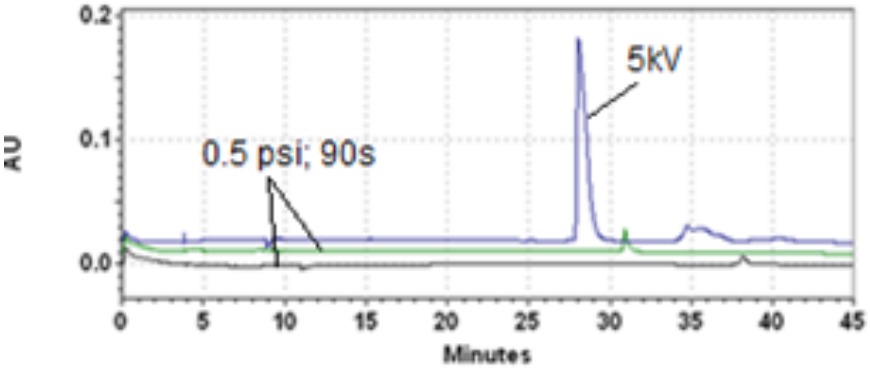


**Figure 8. In ascending order separations both using 0.5 psi and a 90 second duration of hydrodynamic injection, and separation a from Figure 4 using 5kV electrokinetic injection voltage.**

Figure 8, shows a final comparison of the electropherograms resulting from the optimum electrokinetic injection parameters versus the optimum hydrodynamic injection parameters.

*Sample Concentration-to-Buffer Ratio*

For the serum concentration analysis three electropherograms, one of each concentration, were compared. The first separation of each concentration was used. Figure 9 shows the differences in profiles stacked along both the x and y-axis. Figure 10 displays the same electropherograms magnified to illustrate the profile differences in greater depth.

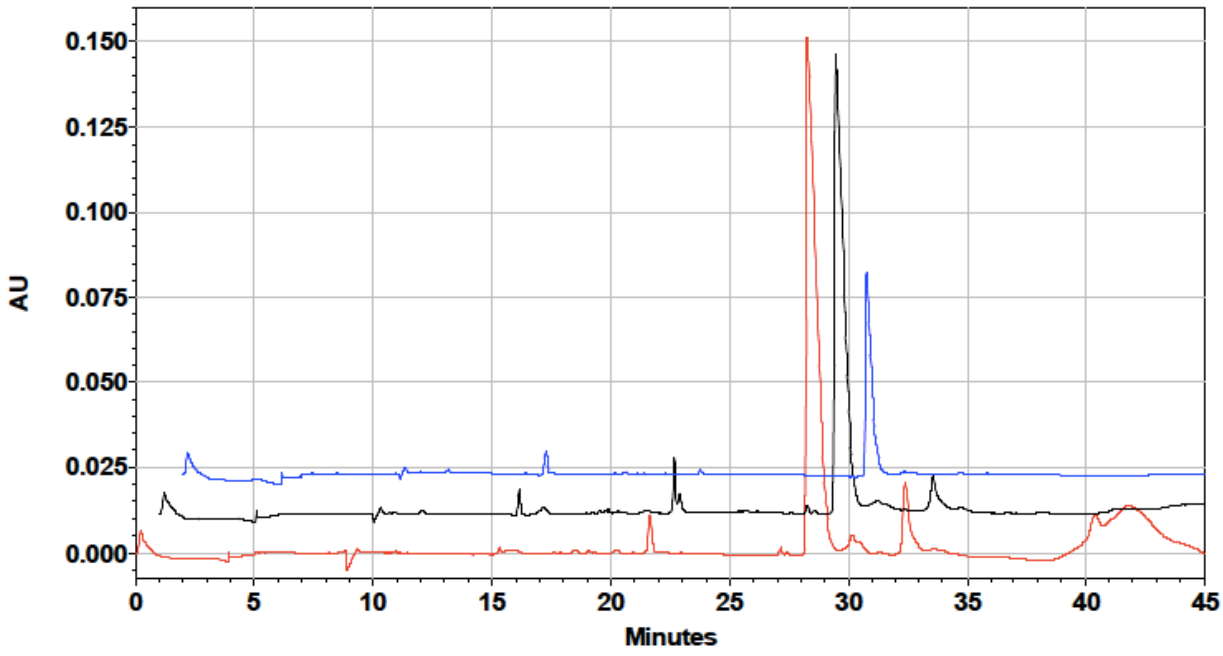


**Figure 9. Electropherograms of the first separation of each concentration ratio. In ascending order: 1:1.4 mass ratio, 1:1 volumetric ratio, and serum with no sample buffer. The electropherograms are stacked and offset along the x-axis.**

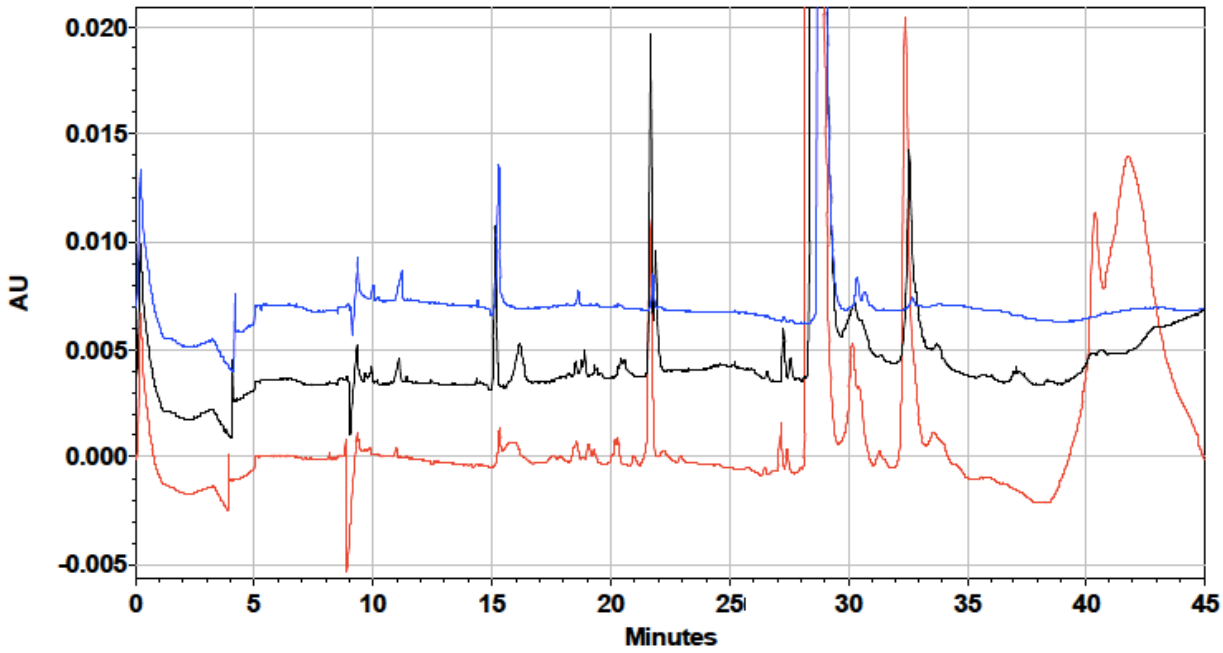


**Figure 10. Electropherograms of the first separation of each concentration ratio magnified to include data from the divot to albumin peak. In ascending order: 1:1.4 ratio, 1:1 volumetric ratio, and serum with no sample buffer.**

The three electropherograms of the 1:1.4 protein to SDS mass ratio were also stacked and compared to examine repeatability as seen in Figure 11. The 1:1.4 mass ration had the greatest albumin peak intensities of the three concentrations.

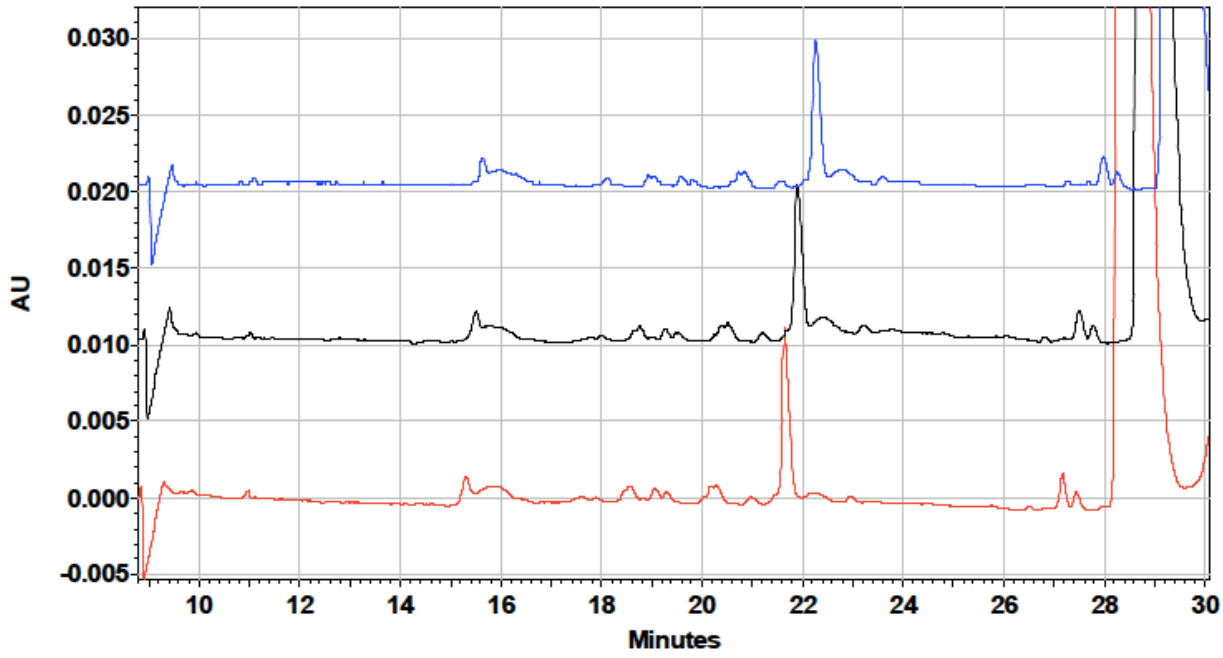


**Figure 11. Electropherograms of the three different separations for the mass ratio samples magnified to show the region between the divot and albumin peak.**

There were slight differences in migration time for the mass ratio samples as separation time increased. Relative standard deviation values were also calculated for the three concentrations and shown in Tables 6-8.

**Table 6. Relative standard deviation values for mass ratio samples electropherograms.**

| 6 µl Serum/ 60 µl Sample Buffer Samples | | | |
|---|---|---|---|
| Separation | Divot Migration Time (min) | **Albumin Peak Migration Time (min)** | **Albumin Peak Area** |
| 1 | 8.91 | **28.29** | 4186880 |
| 2 | 8.73 | **28.15** | 3843652 |
| 3 | 8.85 | **28.28** | 3954733 |
| **Standard Deviation** | **0.09** | **0.078** | 175136.5 |
| **Average** | **8.83** | **28.24** | 3995088 |
| **RSD%** | **0.01** | **0.003** | 0.043838 |

**Table 7. Relative standard deviation values for volumetric ratio sample electropherograms.**

| 33 µl Serum/ 33 µl Sample Buffer Samples | | | |
|---|---|---|---|
| Separation | Divot Migration Time (min) | **Albumin Peak Migration Time (min)** | **Albumin Peak Area** |
| 1 | 9.25 | **29.17** | 3577758 |
| 2 | 9.06 | **28.93** | 2541597 |
| **Standard Deviation** | **0.13** | **0.169** | 738676.5 |
| **Average** | **9.12** | **29.05** | 3059678 |
| **RSD%** | **0.01** | **0.006** | 0.023946 |

**Table 8. Relative standard deviation values for no sample buffer sample electropherograms.**

| 66 µl Serum Samples | | | |
|---|---|---|---|
| Separation | Divot Migration Time (min) | **Albumin Peak Migration Time (min)** | **Albumin Peak Area** |
| 1 | 9.39 | **29.61** | 1097767 |
| 2 | 9.04 | **29.25** | 689340 |
| 3 | 9.18 | **29.36** | 295742 |
| **Standard Deviation** | **0.18** | **0.184** | 401035.3 |
| **Average** | **9.21** | **29.41** | 6494283 |
| **RSD%** | **0.02** | **0.006** | 0.057763 |

The bovine serum analysis generated eight electropherograms; four of the separations were omitted from Figure 12 for various reasons. Absence of albumin peaks and late albumin peak migration times were the major contributing factors for removal.

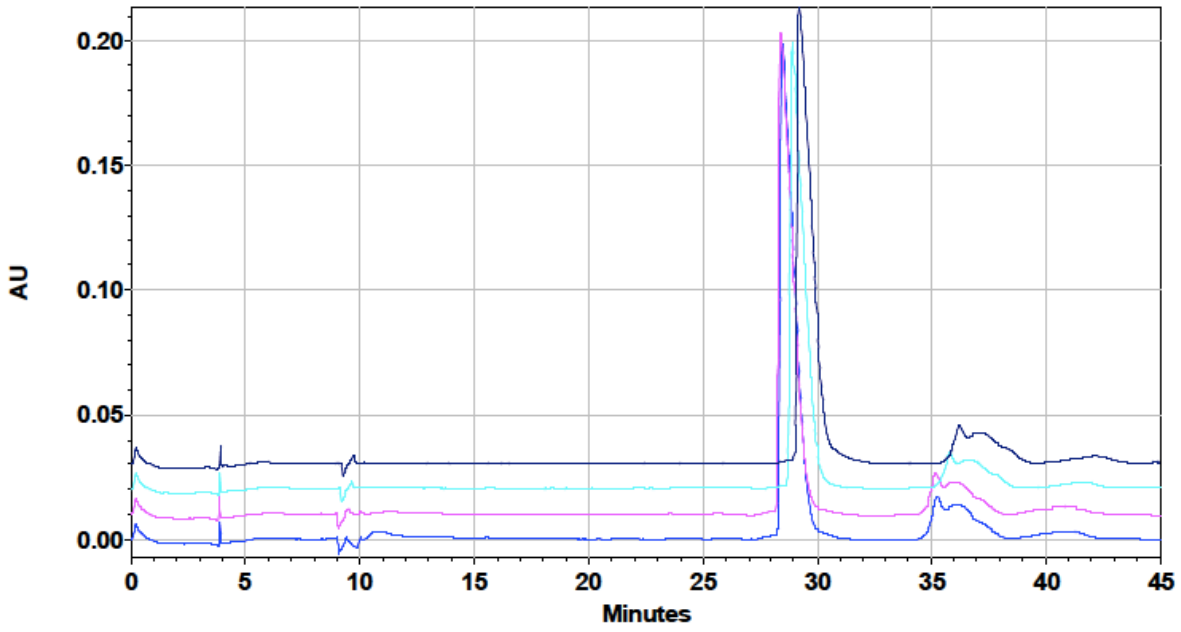


**Figure 12. Electropherograms of the first four separations of BSA stacked along the y-axis.**

Data from previously analyzed BSA was used to compare the 1:1.4 protein to SDS mass ratio to a 1:1 BSA to sample buffer volumetric ratio in Figure 13. Electropherograms were chosen based on albumin peak intensity and migration time. Four electropherograms from a sample of 35 µL 5 mg/mL BSA in deionized water with 25 µL SDS sample buffer were compared to four electropherograms from a sample of 25 µL 5 mg/mL BSA in deionized water with 25 µL SDS sample buffer.

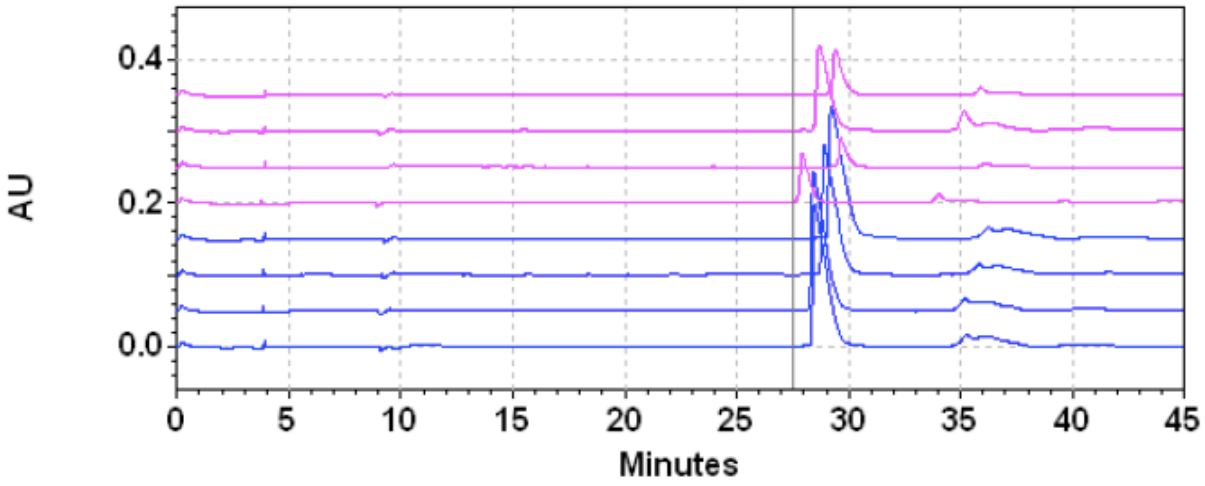


**Figure 13. Electropherograms of four 1:1.4 mass ratio of BSA separations (blue) compared to four 1:1 volumetric ratio of BSA separations (pink).**

*Voltage Separation*

In previous experiments, 15kV separations were applied in the analysis of prepared samples in accordance to the Beckman System Coulter Manual (6). One study has suggested, however, that the recommended voltage separation for CGE analysis should be 20kV due to the production of intense protein peaks with a faster migration time and flat baseline (7). In order to conduct comparisons in the proteomic profiles of the samples, BSA and commercial human serum

samples were prepared and analyzed using the two different recommended voltage separations. In addition, a 10kV separation was also performed to observe the effects of a lower voltage separation on a sample and to observe a potential increase in peak resolution due to the slower migration rate of the proteins.

10kV, 15kV, and 20kV BSA separations are shown in Figure 14. Each voltage separation was performed twice to see repeated results.

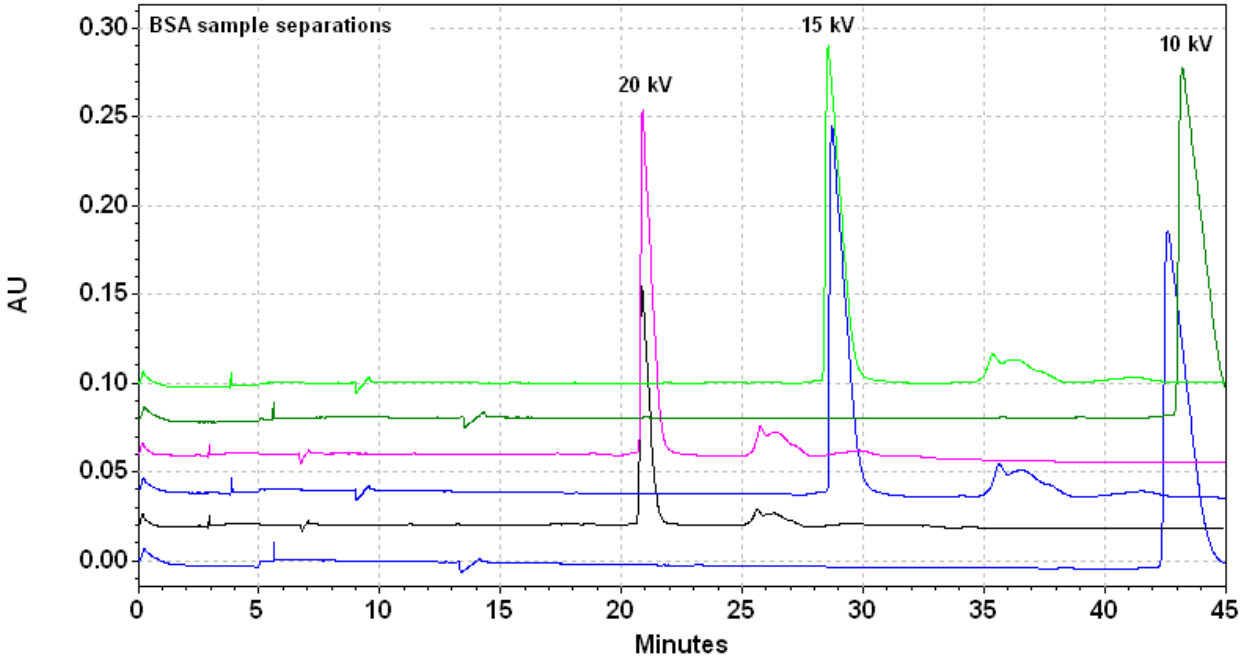


**Figure 14. 10kV, 15kV, and 20kV separations of BSA**

All BSA sample runs showed successful separations with intense albumin peaks and a flat baseline. However, albumin dimers were solely seen in the 15kV and 20kV separations due to the length of the separation time. With the extension of the migration time, the albumin dimer

would be observed in the 10kV separation electropherogram but parameters in this study were not changed to maintain consistency in the method.

Following the BSA sample runs, two commercial serum sample separations were performed for each voltage separation as shown in Figure 15. Successful separations were not seen in all samples. One electropherogram from each of the different voltage separations showed a lack of intensity or absence of low intensity serum protein peaks. The utilization of the same serum sample vial for each voltage separation could be a probable cause for this error.

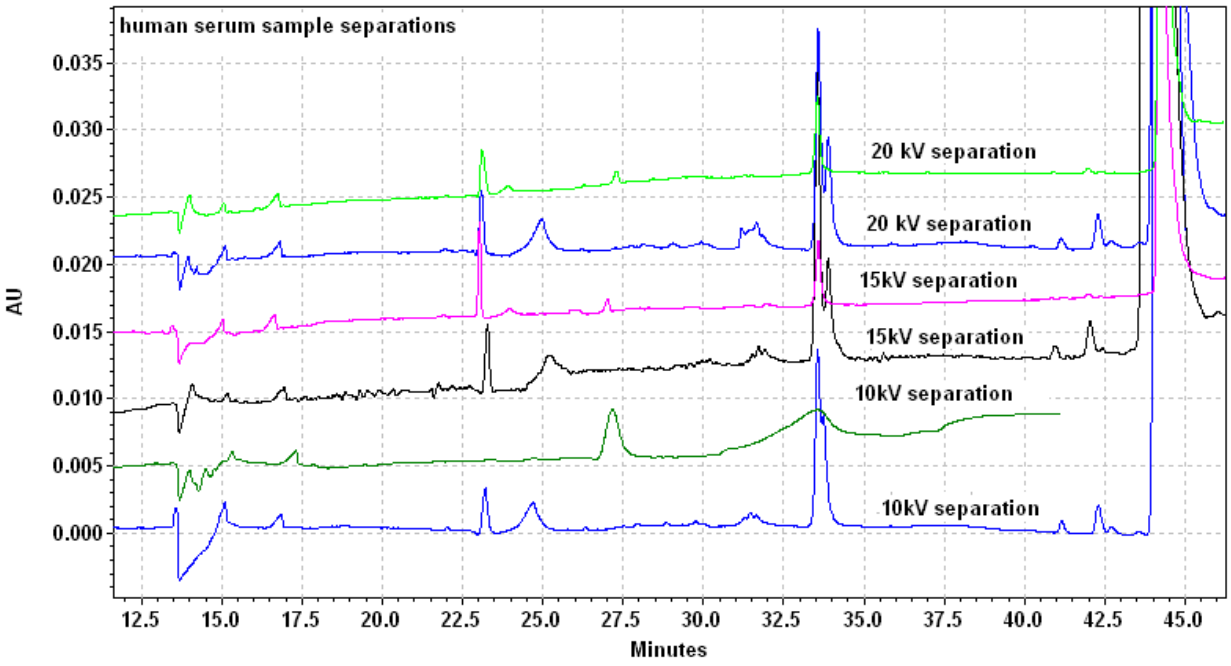


**Figure 15. 10kV, 15kV, and 20kV separation of commercial human serum sample**

The electropherograms with intense protein peaks from each of the different separation voltages were used to perform comparisons as shown in Figure 16, 17, and 18.

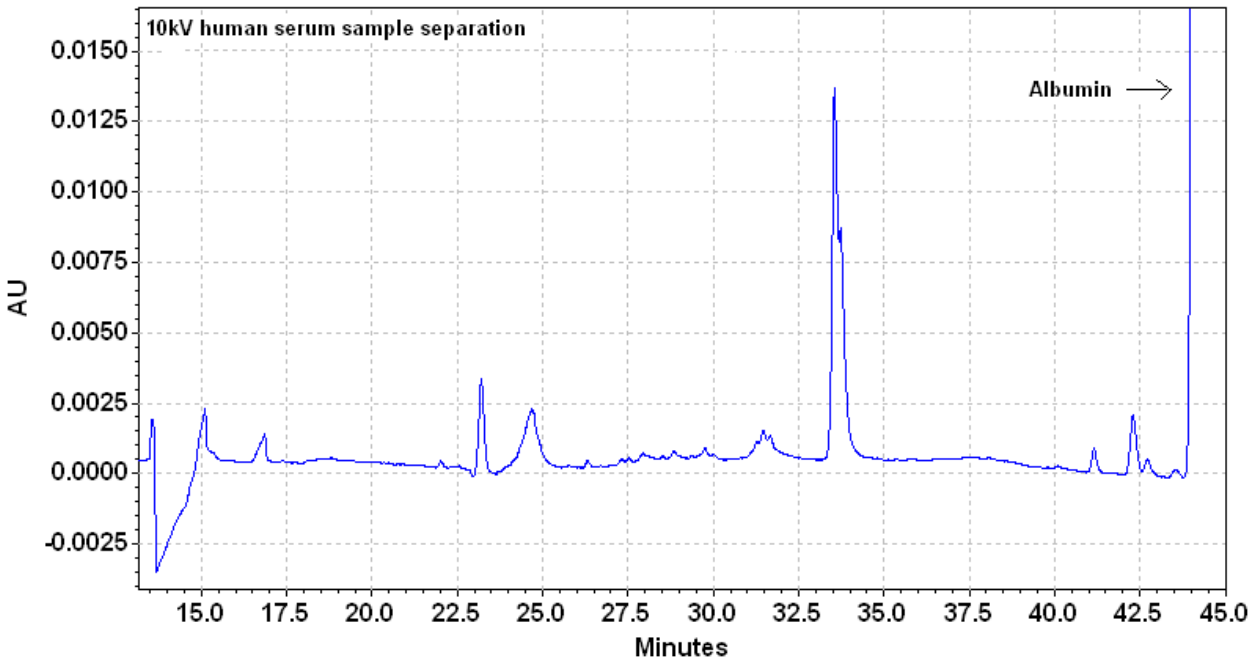


**Figure 16. Magnified electropherogram of 10kV separation for commercial human serum sample**

The 10kV separation showed the slowest migration time for the individual serum proteins with the albumin peak being present at the 44-minute mark. According to Figure 16, the 10kV separation had a flat baseline with no significant noise but did not show an improvement in resolution of the peaks. The doublet at the 33-minute mark was not as prominent in comparison to the other electropherograms, and some proteins peaks were found to have less intensity such as the peak at the 31.5-minute mark. This decrease in peak intensity, however, could have been due to the variance that is often seen in sample separations.

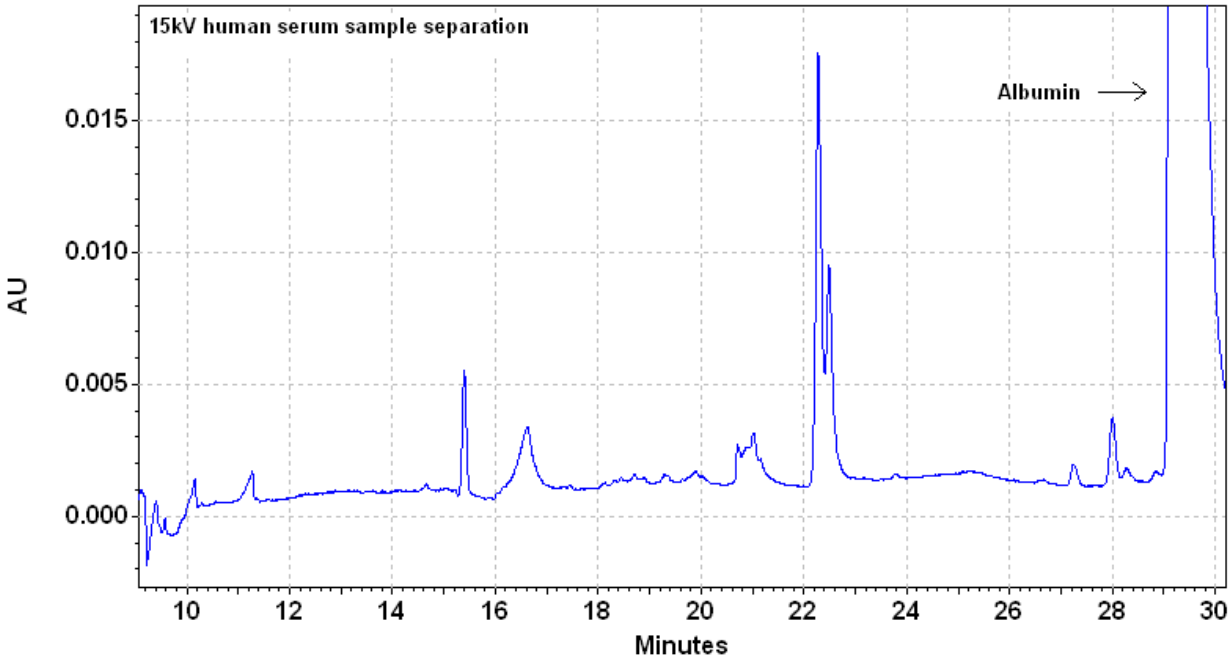


**Figure 17. Magnified electropherogram of 15kv separation for commercial human serum sample**

According to Figure 17, the 15kV separation showed an albumin peak at the 29.5-minute mark. The migration time of the individual serum proteins was found to be shorter than that of the 10kV separation, and the serum proteins were also found to be intense with a stable baseline and no significant presence of noise. A prominent doublet is shown at the 22.2-minute mark in comparison to the one shown in the 10kV separation.

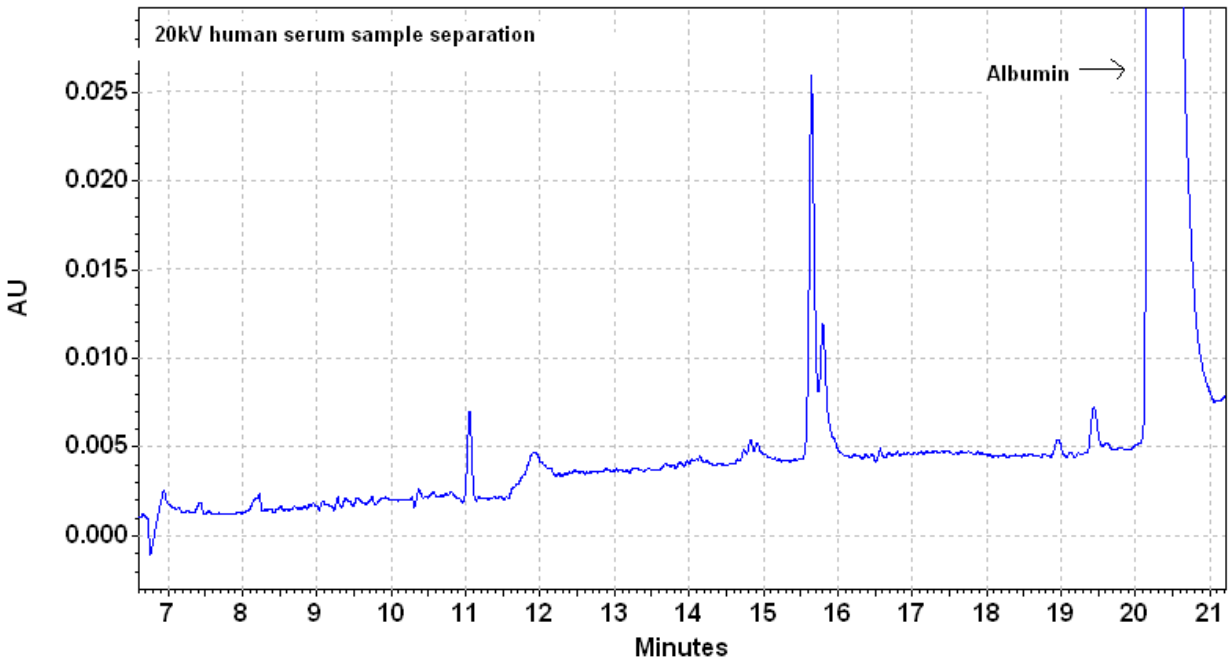


**Figure 18. Magnified electropherogram of 20kV separation for commercial human serum sample**

The 20kV separation had the shortest migration time for the individual serum proteins with a separation time that is approximately half of the 10kV separation. Despite successful protein separation and the prominent presence of the doublet at the 15.5-minute mark as shown in Figure 18, the 20kV separation is not recommended. The baseline showed a significant amount of noise, which could potentially conceal low intensity proteins and result in an ineffective analysis of the protein profile.

*Protein Standard Identification*

Using CGE methods determined by experiments previously listed, four samples were analyzed for comparison: sample buffer, apo A-1, HDL, and serum. These methods required using a 1:1.4 sample protein to SDS mass ratio, using a 5 kV electrokinetic injection, and using a 15 kV

separation voltage. The only exception to this was that while a 1:1.4 mass ratio was utilized for serum and HDL, it was not for apo A-1. Pure sample buffer contained no sample protein, so the ratio was not applicable.

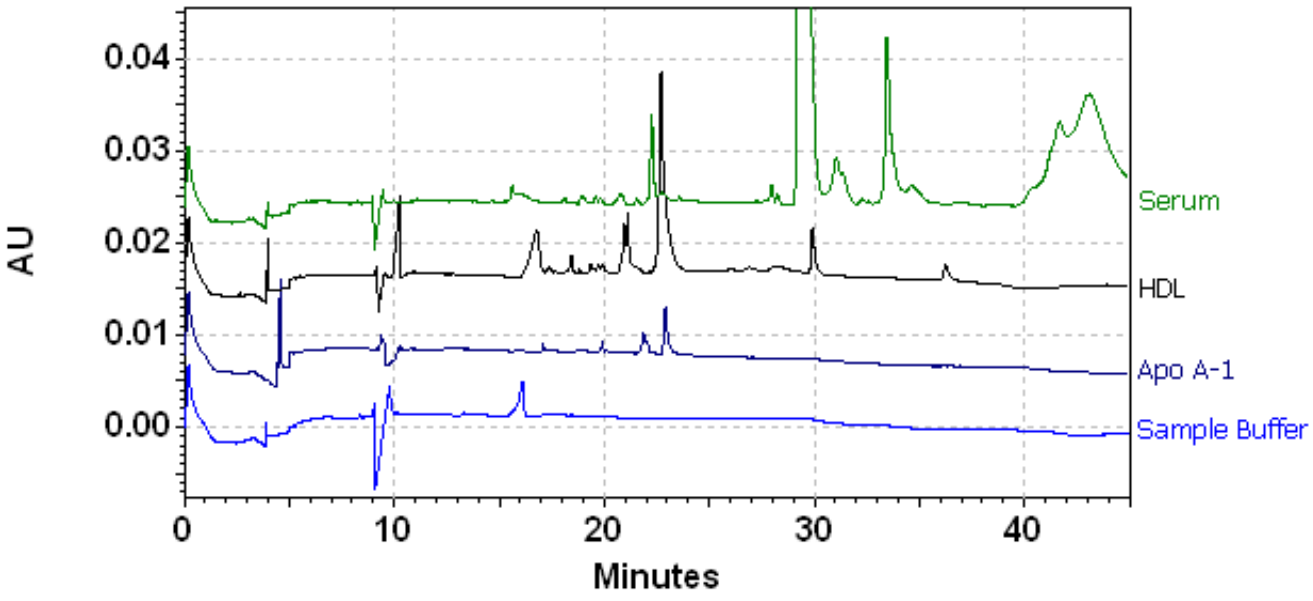


**Figure 19. In ascending order, sample buffer, apo A-1, HDL, and serum electropherograms stacked vertically with 15 kV separation.**

Peaks were compared between each sample. The standard buffer contains the fewest peaks, as it contains the fewest proteins. All other standards contained the standard buffer peak as they were prepared as a mixture with standard buffer.. In Figure 19, this expectation is upheld except for in the apo A-1. The presumed sample buffer peak at the 16-minute mark is present at approximately the same location in HDL and serum, yet it is missing in apo A-1.

Apo A-1 peaks are expected to be present in both HDL and serum electropherograms. In Figure 19 at the 23-minute mark of the apo A-1 electropherogram, there is a prominent peak that is

present at the 23-minute mark in the HDL electropherogram and also the 22-minute mark in the serum electropherogram. There is also another noteworthy peak at the 22-minute mark in the apo A-1 electropherogram that could potentially be the same protein as that at the 21-minute mark in the HDL electropherogram. It is uncertain if an analogous peak is shown in the serum electropherogram but is less prominent.

HDL peaks are expected to be present in the serum electropherogram. There is a range of peaks in Figure 19 from the 16-minute mark to the 23-minute mark in the HDL electropherogram that appears analogous to that in the serum electropherogram from the 15-minute mark to the 22-minute mark.

# CHAPTER IV

# CONCLUSION

Electrokinetic injection produced repeatable results with repeatable peak areas and separation times. Increasing voltage led to greater peak areas in the electropherograms. Wider peaks show less resolution therefore increasing voltage could mean a decrease in resolution and accuracy. 5kV Electrokinetic injection showed the best results for albumin. Electropherograms from 5kV injection had more intense peaks than 2.5 kV injection electropherograms and were less wide than 7.5 kV injection electropherograms. The results did not show great deviation, had prominent peaks with high resolution, and were repeatable.

Of the three peaks of interest found in previous electropherogram of the BSA standard, only the divot appeared pronounced in the preliminary hydrodynamic injection using 0.5 psi for over 30 seconds. The migration time of the divot did not deviate greatly, but its area was not repeatable. The electropherograms followed the baseline almost completely, but it didn't show any high intensity of peaks for albumin. Through the absence of albumin peaks and the varied peak area of the only peak of interest found, it is concluded that hydrodynamic injection was not successful using 0.5 psi over a duration of 3 seconds. As this was the first experiment using hydrodynamic injection, parameters of the experiment needed to vary to possibly produce repeatable electropherograms with clear peaks of all peaks of interest.

It was theorized that a longer injection time would allow for it to overcome the viscosity of the Tris gel in the capillary. Even at the maximum parameters of 0.5 psi and 90 second duration,

hydrodynamic injection produced weak peaks with high variability of peak time of separation. Figure 8 illustrates that the greatest intensity and resolution comes from electrokinetic injection using 5kV.

Even at the maximum parameters of the P/ACE MDQ Capillary Electrophoresis system, hydrodynamic injection is not comparable to the repeatability and intensity found in the peaks of electropherograms where the separations initially used electrokinetic injection. This may be due to the inability of hydrodynamic injection to overcome the viscosity of the gel inside of the capillary system (2). Electrokinetic injection using 5kV will continue to be used for further experiments.

The serum concentration analysis provided evidence that the 1:1.4 protein to SDS mass ratio does increase electropherogram intensity and repeatability. Relative standard deviation values were very low for the divot and albumin peak migration times and albumin peak area. In spite of the relatively low serum volume, the 6 μL serum samples also had the greatest albumin peak intensity. The 1:1 volumetric samples peak intensities were not as intense as the 6 μL samples, but were better than the samples completely lacking sample buffer. The samples with no added sample buffer had the lowest peak intensities and repeatability suggesting coating of proteins by SDS is necessary to achieve proper separation.

The mass ratio was tested with BSA in hopes of repeating the findings. The mass ratio generated electropherograms with high albumin peak intensities. The first four separations were used in the analysis. Some of the later separations lacked strong albumin peaks or had poor migration times.

This could have been due to only one sample being analyzed multiple times. However, compared to previous experimental data the BSA mass ratio samples had intensities higher than the 1:1 volumetric ratio samples. The migration times of the albumin monomer and dimer peaks were much more similar among the mass ratio samples as well.

Due to the high protein content in serum, a small volume is required for analysis. This is beneficial to its biomarker possibilities. A biomarker should be able to provide indication of a disease while requiring a minute sample for testing (1). Not only did this experiment prove the 1:1.4 protein to SDS mass ratio is advantageous, but also that as little as 6 μL of serum is necessary to obtain reliable results. The repeatability of the 1:1.4 protein to SDS mass ratios should be further tested, as well as the reliability of 6 μL serum samples. Perhaps even smaller volumes of human serum can be used for analysis and diagnostic purposes.

It is recommended to use a 15kV separation for CGE sample analysis. The 15kV separation produced proteomic profiles with high peak resolution and intense protein peaks while maintaining a flat baseline. Although the 20kV separation produced intense proteins peaks with high resolution, the noise created by the higher voltage application has the potential to conceal low intensity proteins that could be crucial in the determination of a disease biomarker.

Future studies should observe the repeatability of these results with a larger sample size. The possible variance in protein peak intensity should also be studied, as there is less time for individual proteins to not be detected in a faster migration time with the application of a higher voltage separation.

The three optimized experimental methods determined can be used for protein identification in serum. As displayed in Figure 19, similar peaks were found across HDL, apo A-1, and human serum electropherogram profiles, suggesting that the proteins these peaks represent are present in each sample compared for serum protein identification. By using standards such as HDL and apo A-1 and comparing their electropherograms to that of serum, it is possible to determine which peaks are caused by presence of HDL and apo A-1 in serum.

Certain electropherograms generated in the experiment for serum protein identification either lacked expected peaks or contained unexpected peaks. The apo A-1 electropherogram lacked the prominent peak featured in sample buffer at the 16-minute mark that both HDL and serum electropherograms contained. Repeated analyses of apo A-1 have yielded this same result. More samples will be analyzed to assess whether this remains true in a larger trial size. The reason for the disappearance of this peak is unknown. In the HDL electropherogram, there is a small peak that aligns with the intense albumin peak at the 30-minute mark. This could be the result of contamination of albumin in the HDL sample.

The intensity of the peaks should be disregarded in this study. Concentrations between each of the samples were not properly controlled. Although HDL and serum both had a 1:1.4 sample protein to SDS mass ratio, the concentration of the HDL was diluted to serum levels with the addition of water, whereas serum was diluted further with the addition of sample buffer. Furthermore, the apo A-1 analysis did not use the 1:1.4 sample protein to SDS mass ratio, which may have affected the peak intensity on its electropherogram. The results of this experiment are

based on the placement of peaks and not their heights. Further studies will focus on controlling these factors.

The injection method yielding the best electropherograms with clear, sharp, and repeatable peaks was electrokinetic injection of 5kV. The serum to sample buffer concentration buffer increased electropherogram intensity and repeatability the most in a 1:1.4 protein to SDS mass ratio. Electropherograms with flat baselines and intense protein peaks were obtained using a 15kV separation for sample analysis. Through these methods, Apo A-1 and HDL were identified as potential peaks in serum electropherograms

Optimizing CGE methodology increases serum electropherogram peak resolution and repeatability. This can lead to the discovery of potential biomarkers of disease, advancing the possibility of CGE use in clinical settings. Capillary gel electrophoresis could then be used for individualized health care. Future studies will further this technology's use in the medical field.

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