Applicability of Electrophoresis in Wildlife Forensics

Briana Smith and R. Christopher O'Brien, Ph.D.

University of New Haven – Department of Forensic Science

SUMMER UNDERGRADUATE RESEARCH FELLOWSHIP

INTRODUCTION

For this project we used sodium dodecyl sulfate polyacrylamide gel electrophoresis, better known as SDS-PAGE. SDS denatures the proteins so they can be separated by molecular weight rather than size. Some advantages to using SDS-PAGE are that it is efficient, fast, and economic because no sophisticated equipment is needed. This project will focus on how SDS-PAGE can be applied to wildlife forensic science. Specifically, this project will research speciation by SDS-PAGE of oligomeric proteins [1]. We will be using fibrous cartilage from the pectoral fins of short-fin mako sharks (*Isurus oxyrinchus*), blue sharks (*Prionace glauca*), and thresher sharks (*Alopias vulpinus*) for samples [Figure 4]. We will also be using a Mini-PROTEAN Tetra Cell to run the SDS-PAGE [Figure 1].

Figure 1. Mini-PROTEAN Tetra Cell and Casting Frame

APPLICABILITY

In addition to running the SDS-PAGE, we are also working with a graduate student named Beth Markello. Our research will be a proof of concept for her project. She is using capillary electrophoresis, or CE, to study the proteins in shark fin cartilage. This research will also be shared with the Center for Wildlife Forensic Research here at UNH because it shows how electrophoresis can be applied to wildlife forensics.

MATERIALS & METHODS

Throughout the summer we collected shark fin samples [Figure 2] from shark fishing tournaments in New York and Rhode Island. Before we could run any samples we had to extract the proteins from the cartilage. We used fibrous cartilage from dried shark fins that had been previously skinned and separated [Figure 3].

1	Cut an approximately 3.0 g piece of cartilage and put it in a 15 ml falcon tube with dH2O until rehydrated
2	Pulverize sample in Magic Bullet Blender, then sonicate and centrifuge until sample is separated
3	Use Bradford Assay to determine amount of proteins in solution
4	Prepare sample by mixing 5 µl with 4.75 µl Laemmli sample buffer and 0.25 µl β-mercaptoethanol
5	Heat sample solution for 10 minutes at 70°C
6	Secure gels with glass plates in frame and put system in the tank
7	Fill the inner chamber with running buffer and load samples into the wells of the gels with a pipette
8	Pour 550 ml of running buffer into the outer chamber and top off the inner chamber with running buffer
9	Connect the Mini-PROTEAN Tetra Cell to the power supply and run for 30-40 minutes at 200V

Once we ran the electrophoresis, we disconnected the cell from the power supply and disposed of the running buffer. After the running buffer was disposed of, we removed the gels from the system. We used stain-free gels, so instead of staining we used an imager that makes the protein bands visible by activating a reaction between the proteins and trihalo compounds in the gels. Finally, the protein bands became visible and we began our analysis.

Figure 2. Smith sampling a blue shark fin

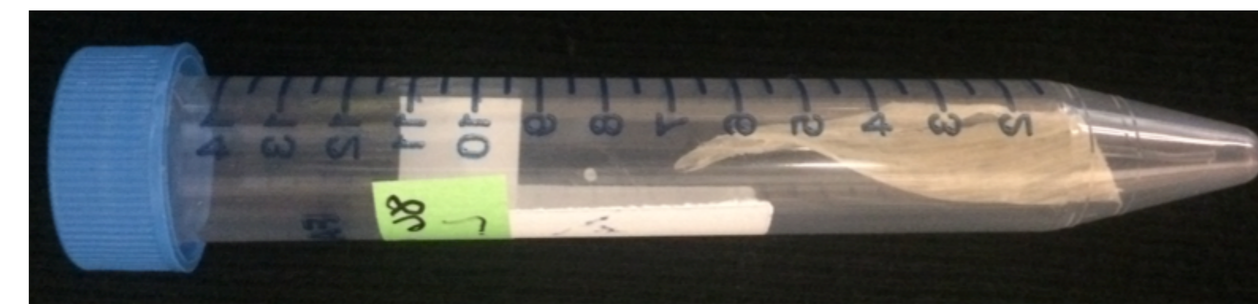

Figure 4. Sample of blue shark fin cartilage in a 15 ml falcon tube

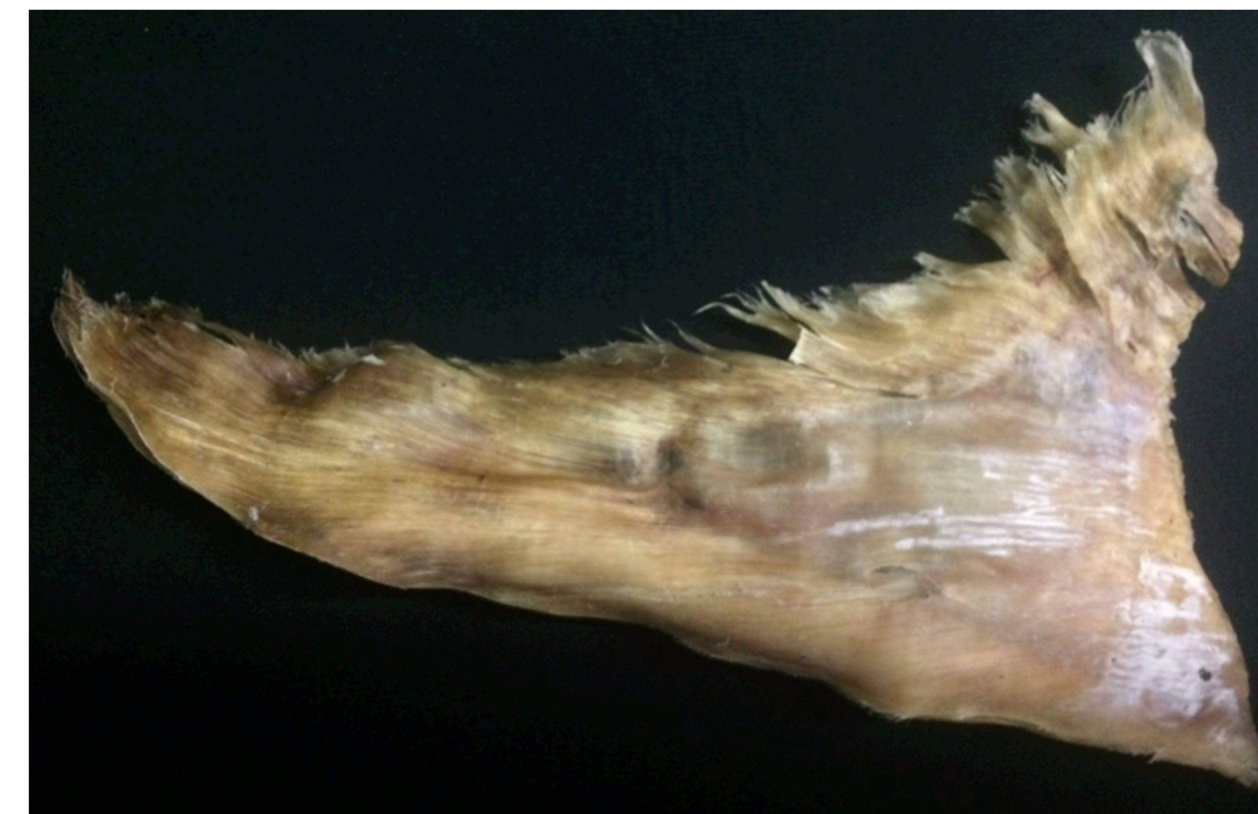

Figure 3. Skinned, separated, and dried short-fin mako shark pectoral fin

RESULTS

The results from one of the Bradford Assays we have conducted is shown below [Figure 5]. We have determined that there are proteins present in the solution, however, because the proteins will be further diluted with the sample buffer when running the SDS-PAGE we are trying to increase the amount of protein in solution before running any gels. We are trying to increase the amount of protein in solution because if there are not enough proteins present, the SDS-PAGE will not produce any visible protein bands. Which, in turn, would make analysis of the proteins present impossible.

Sample	Absorbance	K*Absorbance
0.05 Standard	1.319	1.3188
#1 unfiltered	1.181	1.1813
#1 centrifuged	1.035	1.0349
#2 centrifuged	1.195	1.1951
#2 filtered	1.314	1.3145
#1 below 50 K	0.67	0.6702
#1 above 50 K	1.308	1.3079
#2 below 50 K	0.761	0.7606
#2 above 50 K	1.735	1.735

Figure 5. Results from Bradford Assay

REFERENCES

[1] Cammack, R. Attwood, T. K. Campbell, P. N. Parish, J. H. Smith, A. D. Stirling, J. L. Vella, F.. (2006). Oxford Dictionary of Biochemistry and Molecular Biology (2nd Edition). Oxford University Press.

ACKNOWLEDGEMENTS

We would like to thank the SURF program at UNH and the Board of Governors for funding this research, as well as the Henry C. Lee College of Criminal Justice and Forensic Science for providing materials. We would also like to thank NOAA National Marine Fisheries for allowing us to collect samples at the tournaments.