5-2015

Investigation of the Chemical Protection Capacity of Common Shoe Materials in Undergraduate Laboratories

Sarah E. Lawson
East Tennessee State University

Recommended Citation

Lawson, Sarah E., "Investigation of the Chemical Protection Capacity of Common Shoe Materials in Undergraduate Laboratories" (2015). *Undergraduate Honors Theses.* Paper 268. https://dc.etsu.edu/honors/268

Midway Honors Program

East Tennessee State University

Investigation of the Chemical Protection Capacity of Common Shoe Materials in Undergraduate Laboratories

Sarah Elisabeth Lawson

Sarah Elisabeth Lawson

Dr. Stacy Brown, Faculty Advisor

Terence Moore, Faculty Reader

Dr. Edward Onyango, Faculty Reader

Abstract

Investigation of the Chemical Protection Capacity of Common Shoe Materials in Undergraduate Laboratories

The objective of this study was to evaluate the chemical resistance of common shoe materials regularly worn in undergraduate chemistry laboratories by subjecting the materials to hydrochloric acid and sodium hydroxide. The materials tested were leather, canvas cotton, and polyester. Due to the lack of restriction on undergraduate laboratory footwear, the research discussed in this thesis is important to undergraduate universities. Currently, many universities across the nation only require undergraduate students to wear close-toed, close-heeled shoes in chemistry laboratories, and often the resistance of the shoe material to acids and bases may not be taken into careful consideration. Overall, the results of this experiment revealed that exposure to the different chemical concentrations of NaOH and HCl did not appear to negatively affect the structural integrity of the fabrics, but according to the mass spectrometry results gathered in this experiment, the three fabrics differed in individual complexities as well as in the compounds extracted following acid and base treatments.

Table of Contents

Introduction

A variety of different shoe materials are worn in undergraduate chemistry laboratories. Many students wear ballet flats, TOMS, leather shoes, boots, and tennis shoes into lab. Also, most undergraduate chemistry laboratories only require students to wear "closed-toed, closed-heeled shoes" in lab, and fail to consider the chemical stability of the shoe materials worn in lab. Chemical stability of shoe materials is important because when an individual drops something, it falls to the ground and breaks at the feet, possibly causing injury or burns. According to an article published in the *Journal of Chemical Health and Safety,* an anonymous online survey pertaining to lab accidents was conducted in the winter–spring semester of 2012. 1,704 undergraduate students enrolled in general chemistry or first semester organic chemistry at a single northeastern university were asked to participate in the survey (1). The purpose of the survey was to determine the number of lab related injuries and respiratory irritation symptoms present among the undergraduate students. Specific survey questions were used to determine the injury students experienced in any university course with a laboratory, in any chemistry course with a laboratory, and in the chemistry laboratory the respondents were currently enrolled in for the semester. Answer choices to the questions included bruises, chemical burns, cuts, heat burn and scalding. In order to determine specific behavior risks that occurred in the labs, a Behavioral Risk Factor Scale (BRF scale) was developed. The survey also determined the frequency at which accidents occurred in the lab by asking the students to estimate the number of times they had accidentally broken glassware, spilled or splashed a chemical onto bare skin, or spilled chemicals on the lab bench. Of the 1,704 students who took the survey, 265 valid records were analyzed, and of the valid records, 11.7% of the students reported being injured in the lab, and 39.4% of the students claimed their injury was due to chemical burns during their current semester chemistry course (1). Forty percent of the students claimed they had been chemically burnt during a university course offered at the university, and 31.4% claimed they had been chemically burnt during a previous chemistry department course. Below is a table that highlights a few of the behavioral risk factors the students were asked about in the survey, and the results for the frequency in which the factors occurred according to the survey (1). The risk factors shown in Table 1 are relevant to the research project completed in this thesis because they show the frequency at which chemical accidents can occur, which may result in injuries to the feet if proper footwear is not worn.

Table 1. Behavioral Risk Factor Scale for Undergraduate Chemistry Students (1)

Behavioral Risk Factor	**Sometimes**	**Often**	**Almost always**
Broke glassware	8.00%	1.00%	0.00%
Spilled chemical onto skin	19.00%	6.00%	1.50%
Spilled chemical onto bench top or workspace	25.00%	9.00%	3.00%

Laboratory safety includes the safety of a student's feet and is critical to the proper functioning of an undergraduate chemistry lab, but many chemistry laboratory manuals are inconsistent on the specific requirements for laboratory footwear. According to the "Safety in the Laboratory" section of the laboratory manual, *Investigating Chemistry in The Laboratory*, the proper shoe to wear in a lab is a non-cloth, non-open toed shoe that is preferably leather because chemicals easily leak through cloth shoes (2). According to the University of California Santa Cruz, due to the danger of spilling corrosive or irritating chemicals on oneself or others; sandals, open-toed shoes, or shoes made of woven material should never be worn into a lab (3). According to Princeton University, perforated shoes, sandals, or cloth sneakers are also not acceptable in labs because they offer no protection from chemicals and broken glass. Instead, chemically resistant boots should be worn to avoid possible exposure to corrosive chemicals or solvents that might penetrate normal footwear. Princeton University also states that leather shoes absorb chemicals and may have to be discarded if they are contaminated with a hazardous material (4).

Many major universities ban open-toed, open-heeled shoes in chemistry laboratories. However, beyond this consensus, disagreement exists as to what type of shoe material is acceptable in lab. According to the United States Department of Labor, the employer must inform the employee of the proper shoe to wear to work, and he must enforce the shoe standard at the workplace. Also, if the employees are in a work environment in which foot injury is possible, they are required to wear protective footwear (5). The United States Department of Labor defines an "environment that requires protective footwear" as an environment that contains electrical hazards or objects that could fall or pierce the sole of the foot and result in injury (5). According to the American Chemical Society (ACS), high-heeled, open toed, cloth, woven leather, any woven material, or sandal shoes should never be worn in the lab. Also, one should never be barefoot in lab. According to the ACS, the proper laboratory shoe should be made of leather or a polymeric leather substitute (6). East Tennessee State University's Mandatory Laboratory Footwear Policy states, "Lab users must wear closed toed shoes made of a non-woven material with non-slip soles" (7). Due to several laboratory accidents that have occurred over the years, agreement on appropriate laboratory footwear amongst different universities is critical.

There have been several laboratory accidents that have occurred in chemistry laboratories. One of the most well-known chemistry laboratory accidents took place at the University of California, Los Angeles (UCLA). On December 29, 2008, Sheharbano "Sheri" Sangii was not wearing a lab coat while she transferred a flammable chemical. During the transfer, she accidently spilled the chemical on herself, which ignited immediately upon contact with the air, and melted her sweater into her skin. She died eighteen days later. Ms. Sangii's professor, Patrick Harran, faced criminal charges for not properly ensuring Ms. Sangii's safety in the laboratory. The University was also sued for not having properly trained Ms. Sangii in laboratory safety nor ensured that she was wearing the appropriate personal protective equipment (PPE). After her death, UCLA quickly installed strict corrective safety measures in the laboratories (8). Another less known chemistry laboratory accident took place at Kansas State University. A College of Veterinary Medicine faculty member carrying a nine pound bottle of 70% nitric acid bumped the doorway of a second-floor lab in Mosier Hall and spilled approximately two and a half liters of acid. Some of it splashed onto her feet and soaked through her shoes leaving her with minor burns. She and five other people who had been within the vicinity of the spill and had inhaled the nitric acid fumes were rushed to the hospital (9). In Colorado, accident files were retrieved from 13 institutions within the state and analyzed (10). Institutions were defined as technical schools, state and local community colleges, private colleges, universities, and medical schools (10). From the data obtained from the accident files it was determined that 574 accidents had occurred between the years 1966 and 1984. The accidents were broken down into 48 accident characteristics and analyzed. The highest number of accidents occurred in entry-level chemistry laboratory courses, and the second highest number of accidents occurred in organic chemistry laboratory courses (10). Seventy-two percent of the accident victims were undergraduate students, and when reagents were involved in the accident, 49% of the reagents were acids and 39% were unidentified chemicals. Twelve individuals had accidents involving their lower limbs (42% right side, 33% left side, 3% both sides). According to the data analyzed, if the accident involved acid, it was a result of an eruption, explosion, or splatter while transferring the acid from one container to another container (10). The possibility of an accident should be considered when deciding on a protective footwear policy to include in the list of personal protective equipment (PPE) a student should wear into lab.

Over a period of six weeks, the thesis experiment compared the initial and final weights of three common shoe materials (leather, canvas cotton, and polyester) before and after they were submerged in different molarities of HCl and NaOH. On week seven, material samples were prepared for an LC-MS analysis to determine different molecules present in the samples. **We hypothesized, that because of its thin fibers and synthetic nature, the polyester would break down more in the hydrochloric acid and the sodium hydroxide. Also, literature references suggested that strong bases can break down polyester fabrics. Cotton has thicker fibers, so we hypothesized that it would break down more slowly and partially, being more vulnerable to acidic solutions, based on our literature review. Finally, we hypothesized that leather, made of animal hide, would not break down much at all due to its thickness and durability, and also it has been extensively treated with strong chemicals to confer stability.**

Chemical Stability of Materials

Three materials were chosen to be evaluated in this research project: canvas cotton, polyester and leather. According to the article “Natural and Synthetic Fibers,” when cotton is exposed to high humidity, water, salts, bases, or concentrated solutions of certain acids it swells. The swelling is caused by the absorption of highly hydrated ions. Cotton can also be damaged by hot diluted acids and cold concentrated acids due to the hydro-cellulose within its fibers. Cotton completely dissolves in a solution of cupramonium hydroxide and cupriethylene diamine, and the degradation of cotton usually occurs due to oxidation, hydrolysis, or both working together (11). Cotton is a fiber composed of cellulose, and has a specific gravity of 1.52 g/cm^3. It resists disintegration in strong alkalis, such as sodium hydroxide, but readily dissolves in strong acids (12). Evidence also exists that glycerol may naturally occur in cotton fibers. After suberin and cutin monomers were analyzed from cotton using gas chromatography-mass spectrometry (GC-MS), traces of glycerol derivatives were detected in the small polar molecules left in the aqueous phase (13).

Polyester has excellent resistance to most substances. It is resistant to acids, oxidizers, most solvents, hydrocarbon fuels, oils and lubricants, but it deteriorates in strong alkalis such as concentrated solutions of sodium hydroxide (lye or caustic soda), calcium hydroxide (lime, mortar), ammonia, trisodium phosphate or sodium carbonate (washing soda, soda ash) (14). Polyester has a chemical composition consisting of polyethylene terephthalate (PET), and it has a specific gravity of 1.38 g/cm^3(12).

Leather, made of animal skins, is composed of many bundles of interwoven collagen protein fibers that move in relation to one another when skin is alive, but upon death, the fibers shrivel and stick together (15). Tanning is a useful process because it permanently separates the dead skin fibers by chemical treatment, and lubricates them so that they can move in relation to one another producing leather. Tanning is completed in eight steps and involves several different chemicals. The skin is first exposed to an alkali solution (sodium or calcium hydroxide) and reducing agent (sodium sulfide) in order to de-hair the skin. Next, the skin is immersed in a solution of alkali and sodium sulfide to make it more porous (15). Then it is treated with enzymes, acid salts and carbon dioxide at pH 9-10 (15). The next step is to pickle the skin by treating it with salt and sulfuric acid at a pH 3.0-3.5 in order to preserve it. Once the pickling is complete, chromium ions are added to the skin to keep it porous (15). Then the skin is neutralized by treating it with a mild alkali and dyed by attaching various compounds onto the chromium in the skin. Once the skin is dyed, it is treated with reactive oils that attach themselves to the fibrous structure of the skin and improve its suppleness and flexibility. Finally, the leather is dried, and a finish, composed of dyes bound to an organic or protein medium, is added to the leather to ensure it has an even color and texture (15). The leather's exposure to the acid, base, and salt chemicals confers the durability of the material, and leads us to hypothesize that it would not disintegrate in strong acids or bases.

Acid and Base

The acid used in this research project was hydrochloric acid (HCl), and the base used was sodium hydroxide (NaOH). HCl, a colorless or slightly yellow fuming liquid or gas with a characteristic pungent odor has a molecular weight of 36.47g/mol (16). Based on the concentration of the solution, exposure to HCl can result in severe burns on the skin and mucous membranes, which may cause ulcers and scarring (16). Frequent exposure to HCl can also result in blindness, dermatitis, respiratory tract diseases, dental decay, digestive diseases, and possibly death (16).

The white odorless base NaOH has a pH of 14, a melting point of 16°C, and an initial boiling point of 128 °C (17). It is used by many chemical and pharmaceutical industries for the production of dyes for fabric, plastics, intermediates, and sodium salts. It is also used for the production of water glass, soaps and other detergent raw materials, such as sodium phosphates and sodium silicates. Food industries use it for the purification of fats and oils, removal of fatty impurities, and industrial treatment of waste waters. NaOH can be harmful to the environment due to its ability to increase the pH value of water, however its harmful effect is not long term. NaOH reacts with water to produce heat, and it violently reacts with acid. It is also corrosive for metals like aluminum, tin, lead and zinc (17). Occasionally, metals react with NaOH and create flammable explosive hydrogen gas. The gas then reacts with ammonium salts to create ammonia, which becomes a fire danger. Inhaling NaOH can cause chemical pneumonitis, pulmonary edema, and chemical burns to the upper respiratory tract (17). The burns can result in coughing, breathing difficulty, and possible coma. Skin contact with NaOH causes burns and deep, penetrating ulcers. In milder cases, the skin may break out into a rash and turn pale blue, or it may become inflamed (17). NaOH can also cause chemical conjunctivitis, corneal damage, tissue damage to the digestive tract and esophagus, digestive tract tissue burns, and shock (17). NaOH can react with carbon dioxide in the air and form sodium carbonate, so in order to keep this from happening, NaOH must be stored in sealed containers in a cool, well-ventilated area. (17).

LC-MS Analysis

LC-MS stands for liquid chromatography and mass spectrometry, and it is used to determine molecular weights, molecular identity, and molecular structure. Liquid chromatography is a separation technique often used in chemistry fields, because it can separate many different types of organic compounds, such as small-molecule drug metabolites, peptides and proteins. Traditional liquid chromatography detectors include refractive index, electrochemical, fluorescence, and ultraviolet-visible (UV-Vis) detectors (18). Some traditional LC detectors generate two dimensional data, while others generate three dimensional data. Two dimensional data is data represented by signal strength as a function of time, whereas, three dimensional data includes signal strength, but it also includes spectral data for each point in time (18). Many detectors exist for liquid chromatography, but mass spectrometer detectors are more sensitive and specific than the other detectors, and the nature of the data generated from a MS detector is different and quite useful. MS detectors ionize molecules and identify the ions according to their mass-to-charge (m/z) ratios (18). Like other LC detectors, they too can generate three dimensional data and mass spectral data, but unlike other LC detectors, they can also determine molecular weight, structure, identity, quantity, and purity of a sample (18). They are also much more sensitive than other LC detectors because they can analyze chromophore lacking compounds and identify components in unresolved chromatographic peaks (18). The data gained from an MS detector is also very unique and incredibly useful due to the capability of the MS detector to generate a mass spectrum, select a specific ion from the spectrum, fragment the ion, and generate another mass spectrum from the ion. This process can be repeated until a complex molecule's structure is completely determined (18).

Survey Results

In order to determine the type of shoe material most commonly worn by undergraduate chemistry students into chemistry labs, a survey was conducted of all the undergraduate students eighteen years old or older who were enrolled in an undergraduate chemistry laboratory course during the Fall 2014 semester at East Tennessee State University. According to the number of available seats open for undergraduate chemistry laboratory courses during the Fall 2014 semester, approximately 1,133 students were enrolled in an undergraduate chemistry laboratory course at ETSU, and of these students, 53 attempted the survey and 50 completed it. The survey was created through SurveyMonkey © and distributed to the students through an email sent to their ETSU Goldmail accounts. According to the IRB approval standards, the email required the students to be eighteen years old or older in order to participate in the survey. The email distributed to the students is shown below.

Greetings!

My name is Sarah Beth Pelley, and I am a chemistry major working on my senior honors thesis. My thesis is entitled "Investigation of the Chemical Capacity of Common Shoe Materials in Undergraduate Laboratories." My thesis will test different shoe materials that are commonly worn into undergraduate chemistry laboratories in order to determine the most durable material to wear into a chemical laboratory. My thesis is a research project that includes a survey. I need your input to make my thesis possible. Please complete the short survey attached to this email. Thank you for your participation!

1. Participation in this survey is completely voluntary.

2. You must be 18 years old or older to participate.

If you have any questions or concerns about this survey please contact me at pelley@goldmail.etsu.edu.

The survey asked the students several questions with different multiple choice answers. The questions and possible answers of the survey are listed below.

1. Are you 18 years old or older?
    - Yes

- No

2. What type of shoe material do you regularly wear into your chemistry laboratory?
    - Leather (Example: Sperry)
    - Canvas Cotton (Example: TOMS Canvas Classics)
    - Polyester (Example: Nike)
    - Other

3. On a scale of 1 to 5, how well do you believe the “closed-toed and closed-heeled” laboratory standard protects your feet from harsh chemicals?
    - 1. It doesn’t protect my feet at all
    - 2. It protects my feet very little
    - 3. Sometimes it protects my feet, and sometimes it doesn’t protect my feet
    - 4. It protects my feet most of the time
    - 5. It completely protects my feet

4. What chemistry laboratory are you enrolled in for the Fall 2014 semester?
    - Gen. Chem. Lab I (CHEM 1111)
    - Gen. Chem. Lab II (CHEM 1121)
    - Organic Chem. Lab I (CHEM 2011)

- Organic Chem. Lab II (CHEM 2021)
- Quant. Analysis Lab (CHEM 2221)
- Intro. Integ. Lab (CHEM 3611)
- Adv. Integ. Lab- Dynamics (CHEM 4611)
- Adv. Integ. Lab-Analytical Tech (CHEM 4631)

5. What year of college are you currently in?
   - Freshman (1st year)
   - Sophomore (2nd year)
   - Junior (3rd year)
   - Senior (4th year)
   - Greater (5th year and over)

The results of the survey were quite interesting and the collected data is shown below.

Q1: Are you 18 years old or older?

Answered: 53 Skipped: 0

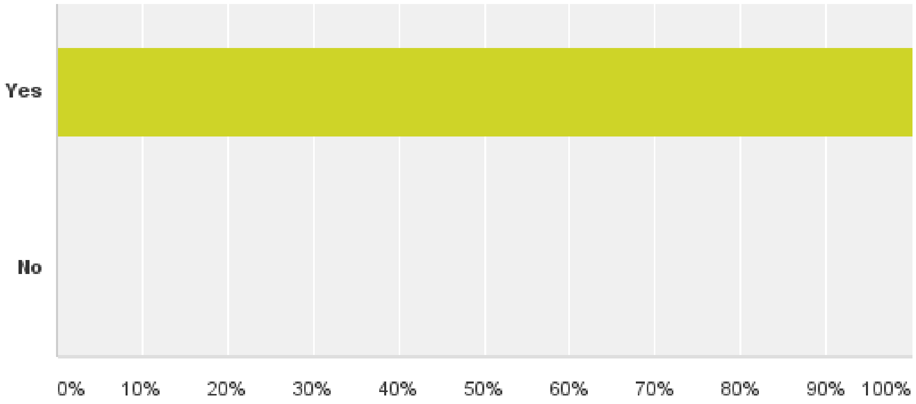
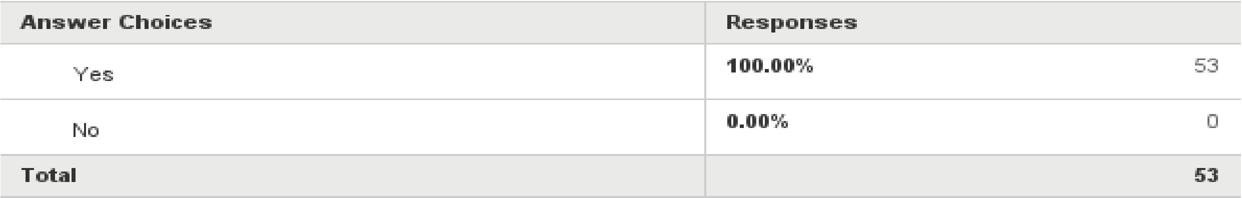


Figure 1. Answers to Question 1 (Survey Monkey)

Table 2. Answers to Question 1 (Survey Monkey)



Answer Choices	Responses	
Yes	**100.00%**	53
No	**0.00%**	0
Total		**53**

Q2: What type of shoe material do you regularly wear into your chemistry laboratory?

Answered: 50   Skipped: 3

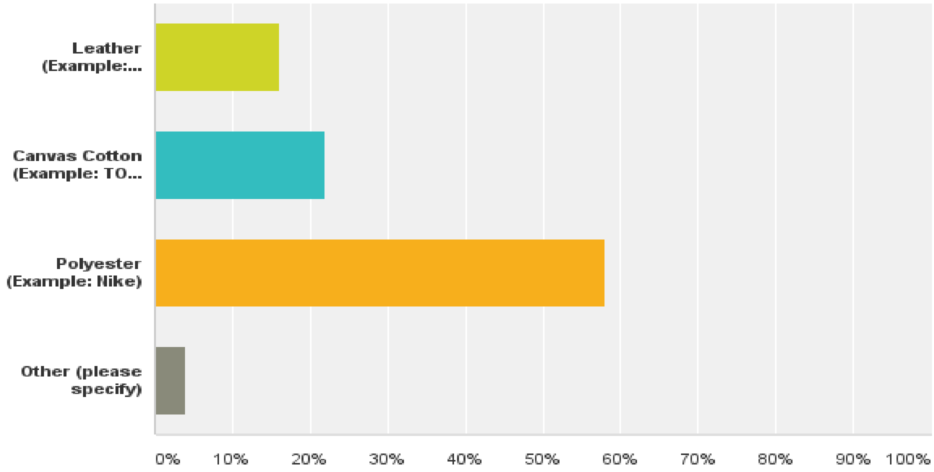


Figure 2. Answers to Question 2 (Survey Monkey)

Table 3. Answers to Question 2 (Survey Monkey)

Answer Choices	Responses	
Leather (Example: Sperry)	**16.00%**	8
Canvas Cotton (Example: TOMS Canvas Classics)	**22.00%**	11
Polyester (Example: Nike)	**58.00%**	29
Other (please specify)	**4.00%**	2
Total		**50**

Q3: On a scale of 1 to 5, how well do you believe the "closed-toed and closed-heeled" laboratory standard protects your feet from harsh chemicals?

Answered: 50 Skipped: 3

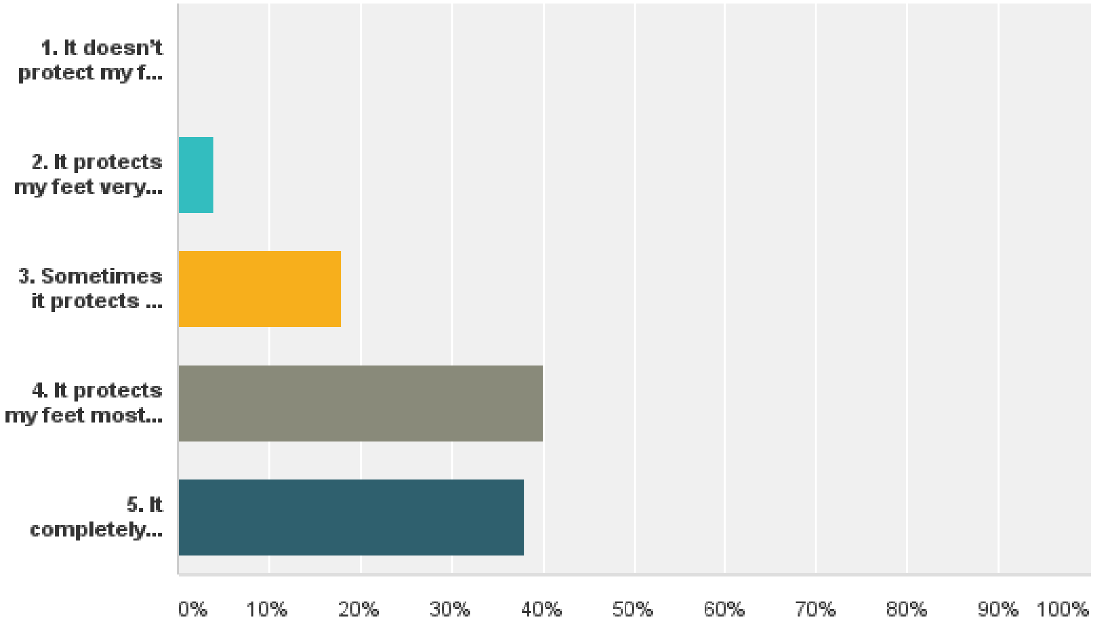
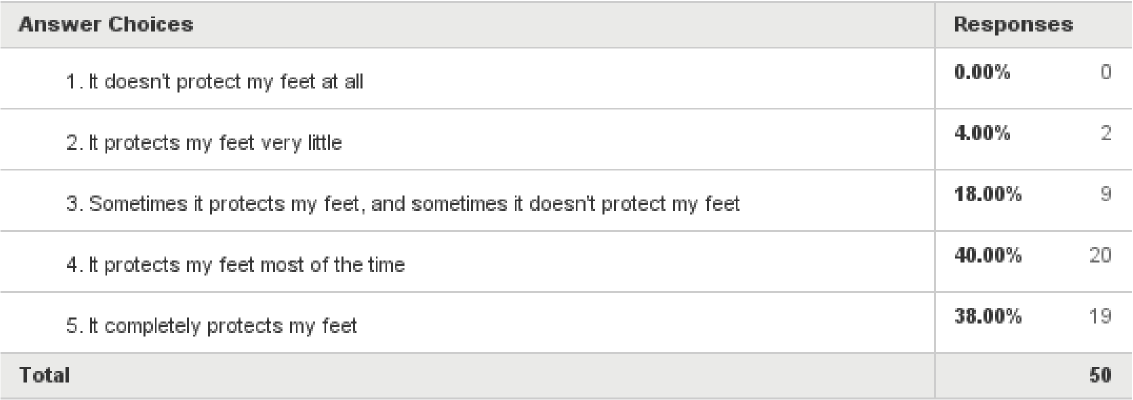


Figure 3. Answers to Question 3 (Survey Monkey)

Table 4. Answers to Question 3 (Survey Monkey)



Answer Choices	Responses	
1. It doesn't protect my feet at all	**0.00%**	0
2. It protects my feet very little	**4.00%**	2
3. Sometimes it protects my feet, and sometimes it doesn't protect my feet	**18.00%**	9
4. It protects my feet most of the time	**40.00%**	20
5. It completely protects my feet	**38.00%**	19
Total		**50**

Q4: What chemistry laboratory are you enrolled in for the Fall 2014 semester?

Answered: 50 Skipped: 3

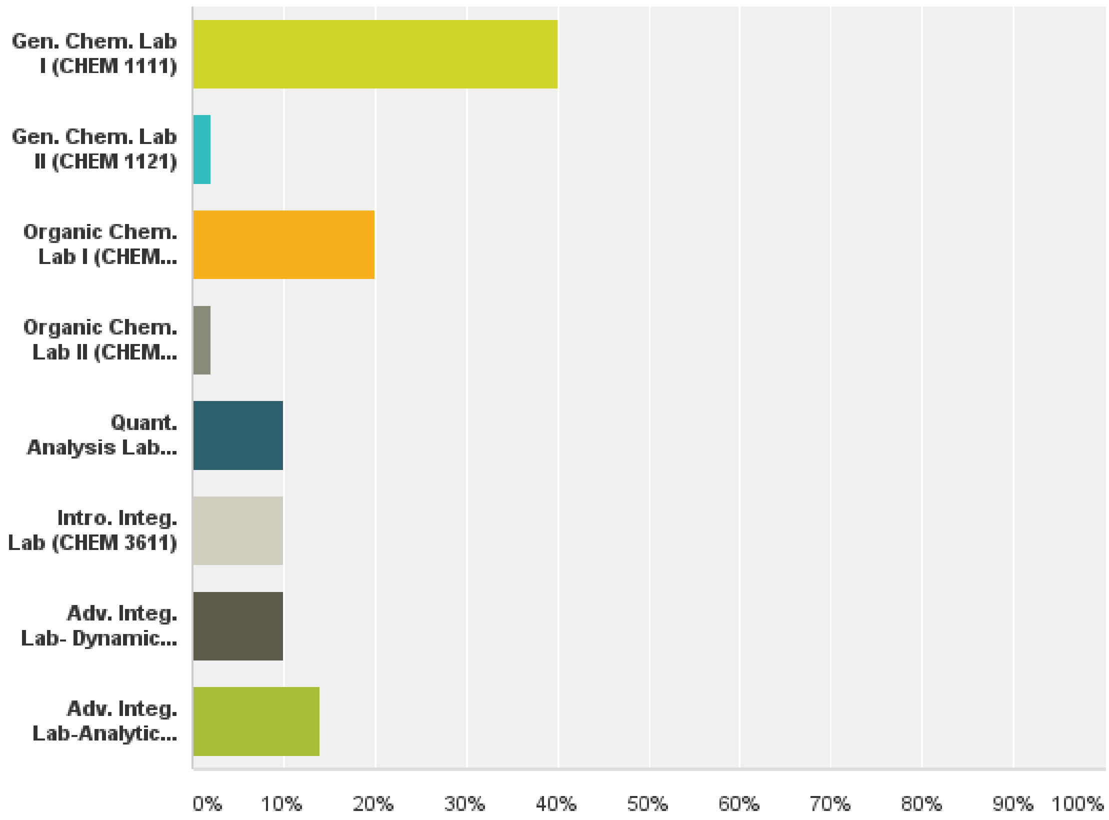
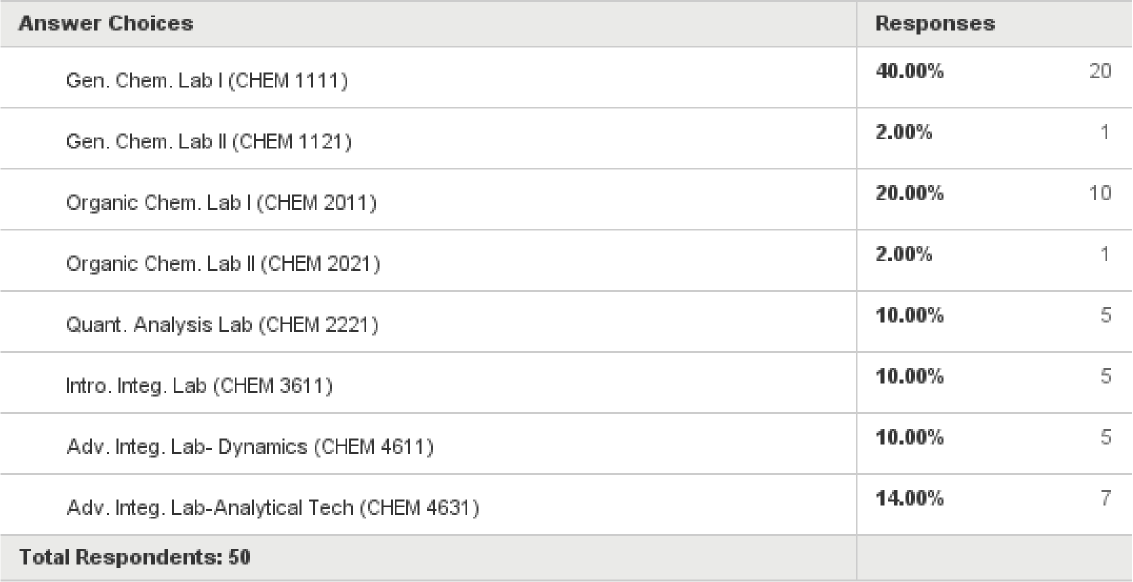


Figure 4. Answers to Question 4 (Survey Monkey)

Table 5. Answers to Question 4 (Survey Monkey)



Answer Choices	Responses	
Gen. Chem. Lab I (CHEM 1111)	**40.00%**	20
Gen. Chem. Lab II (CHEM 1121)	**2.00%**	1
Organic Chem. Lab I (CHEM 2011)	**20.00%**	10
Organic Chem. Lab II (CHEM 2021)	**2.00%**	1
Quant. Analysis Lab (CHEM 2221)	**10.00%**	5
Intro. Integ. Lab (CHEM 3611)	**10.00%**	5
Adv. Integ. Lab- Dynamics (CHEM 4611)	**10.00%**	5
Adv. Integ. Lab-Analytical Tech (CHEM 4631)	**14.00%**	7
Total Respondents: 50		

Q5: What year of college are you currently in?

Answered: 50 Skipped: 3

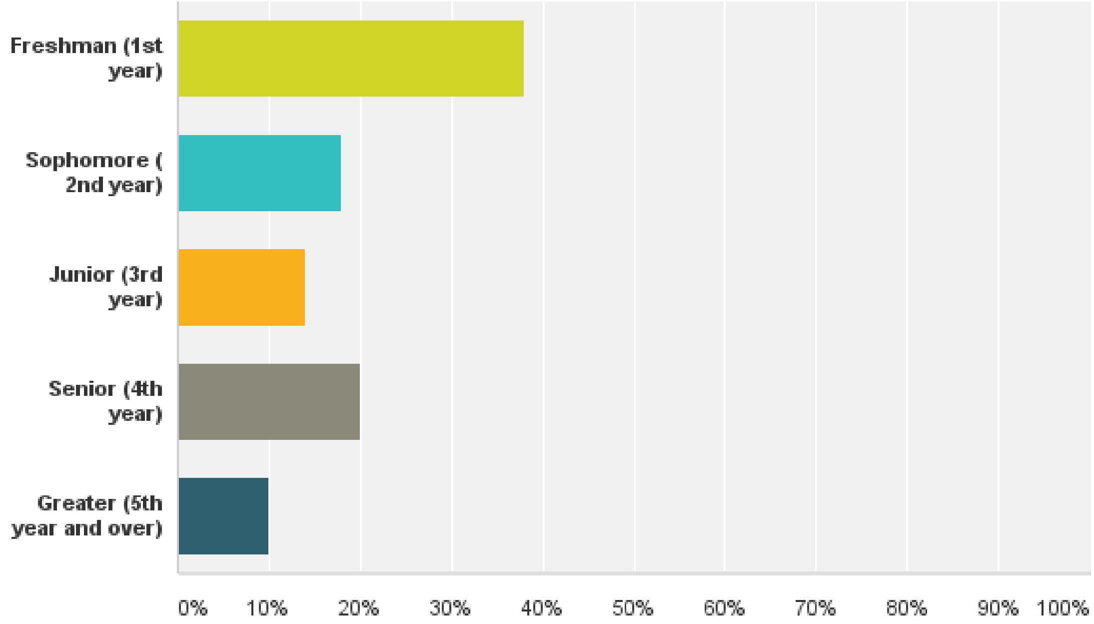


Figure 5. Answers to Question 5 (Survey Monkey)

Table 6. Answers to Question 5 (Survey Monkey)

Answer Choices	Responses	
Freshman (1st year)	**38.00%**	19
Sophomore (2nd year)	**18.00%**	9
Junior (3rd year)	**14.00%**	7
Senior (4th year)	**20.00%**	10
Greater (5th year and over)	**10.00%**	5
Total		**50**

The survey asked the students to mark all the answers that applied to them, so a few of the students may have been enrolled in more than one lab, therefore, the totals may not add up to exactly 100%. According to the data gained, 58% of the students wore polyester shoe materials into lab, 22% wore cotton materials into lab, and 16% wore leather materials into lab. Seventy-eight percent of ETSU undergraduate students enrolled in an undergraduate chemistry laboratory course believed the footwear policy sufficiently protected their feet. Sixty percent of the students who took the survey were enrolled in General Chemistry I or Organic Chemistry I, and the remaining 48% were mainly enrolled in the advanced chemistry laboratories. Fifty-eight percent of the students who took the survey were freshmen and seniors.

Methods

Chemicals, Materials and Equipment

Several chemicals and materials were used in this experiment. The materials used were leather, canvas cotton, and polyester purchased from Hobby Lobby. The leather used was Natural Leather Rounder Shapes (Brand: Silver Creek Leather) from Hobby Lobby. The leather came pre-cut in circular shapes with a width of 3 ¼ in. and a thickness of 1/8 in. There were three circular shapes in one package and forty-five pieces of leather were used in the experiment, so fifteen packages were bought in total. The cotton used was CAN-White Duck Cloth Canvas from Hobby Lobby. The fabric width was 60 in., and 2 yds. of the material was purchased. Circular shapes were then cut from the cotton. The diameters of the cut materials are discussed in the 'Procedure' section of this paper. The polyester used was LNG-Ivory Polyester Lining Fabric from Hobby Lobby. The fabric width was 44 in., and 2 yds. of the material was bought. Five chemicals were used in this experiment along with deionized water from the tap. The chemicals were hydrochloric acid, sodium hydroxide, methanol, acetonitrile, and formic acid. One 1 liter bottle of Pfaltz and Bauer Inc. Hydrochloric Acid 6M was purchased from Fisher Scientific for the experiment. One bottle of 500 grams of Strem Chemical, Inc. Sodium Hydroxide Pellets (97% ACS grade) was purchased from Fisher Scientific for the experiment. The methanol, acetonitrile, formic acid, and LC-MS analysis water were purchased from VWR International and were LCMS grade. The equipment used in this experiment were fifteen 400mL beakers, one 1000 mL graduated cylinder, one 100mL graduated cylinder, four 1 L PYREX © round screw cap storage bottles, four magnetic stirring rods, one analytical balance, one magnetic stirrer, nine 500mL square media bottles, nine 250mL beakers, nine syringes, nine 0.22µm syringe filters, twenty-seven 1mL amber auto-sampler vials, one Shimadzu HPLC (high performance liquid chromatography) system with IT-TOF (ion-trap time-of-flight) mass spectrometer, operating in positive electrospray mode (+ESI), and one Thermo Hypersil-Keystone Aquasil C18 equipped with a 150m x 4.6 mm column with a 5.0 µm particle size.

Procedure

Over a span of six weeks, six trials were performed on the materials. In the first three trials, hydrochloric acid (HCl), a strong acid, was used and in the last three trials, sodium hydroxide (NaOH), a strong base, was used. The molarity concentrations for the hydrochloric acid used in the first three trials were made by using a 6 M HCl solution. To determine how much 6 M HCl should be diluted into one liter of de-ionized water for each molarity concentration, the dilution formula $M_1V_1 = M_2V_2$ was used and solved for V_1 in milliliters. The value of V_1 was then subtracted from 1000 mL to determine the amount of de-ionized water that should be added and mixed with the acid for each molarity concentration. The acid and de-ionized water were measured out using 1000 mL and 100mL graduated cylinders. The acid was added to the water in 1 L PYREX © round screw cap storage bottles. A magnetic stirring rod was placed in the bottom of the bottles and the bottles were each separately placed on a magnetic stirrer until the water and acid were well mixed. The mixtures were then added to the appropriate beakers containing the appropriate materials in the grid. Four individual liters, each with a differing molarity concentration of HCl, were made in total. Half of the four liters, 500mL of each liter, were used for trial one and the last 500 mL of each liter were used for trial two. In order to complete trial three, four more 500 mL batches of hydrochloric acid solution were made for each molarity concentration. The volume of hydrochloric acid and the volume of water for each molarity concentration was halved in order to make the 500 mL volumes instead of the full

1 L volumes. Below is a table of the calculations preformed to achieve the different molarity concentrations for the acid.

Table 7. HCl Molarity Solutions

HCl Molarity Solutions						
1st and 2nd Trials	**Volume of 6M HCl in 1 L Solution**	**Volume of Water in 1 L Solution**		3rd Trial	**Volume of 6M HCl in 500 mL Solution**	**Volume of Water in 500 mL Solution**
	$M_1V_1 = M_2V_2$ $V_1 = \frac{M_2xV_2}{M_1}$	1000mL - V_1			V_1/ 2	1000mL – (V_1/ 2)
2.0 M	333 mL	667 mL		2.0 M	167 mL	333mL
1.0 M	166 mL	834 mL		1.0 M	83 mL	417 mL
0.5 M	83 mL	917 mL		0.5 M	42 mL	458 mL
0.1 M	16 mL	983 mL		0.1 M	8 mL	492 mL

In order to make the molarity concentrations of the sodium hydroxide used in the last three trials, a 500g bottle of NaOH was used and one liter of solution was made for each molarity concentration for trials one and two and 500 milliliters of solution was made for each molarity concentration for trial three. The molarity desired was multiplied by the molecular weight of NaOH to give grams per liter of NaOH. Then, the actual sodium hydroxide pellets were carefully measured out on an analytical balance as close as possible to the grams per liter of NaOH. An equivalent equation was then set up and the volume of de-ionized water needed for each molarity concentration was solved. The de-ionized water and sodium hydroxide for each molarity concentration were added to 1 L PYREX © round screw cap storage bottles. A magnetic stirring rod was placed in the bottom of each bottle and the bottles were each separately placed on a magnetic stirrer until the base was completely dissolved in the water. The mixtures were then added to the appropriate beakers containing the appropriate materials in the grid. Four individual liters, each with a differing molarity concentration of NaOH, were made in total. Half of the four liters, 500mL of each liter, were used for trial one and the last 500 mL of each liter were used for trial two. In order to complete trial three, four more 500 mL batches of sodium hydroxide solution were made, each one with a different molarity concentration. The grams per liter of NaOH were halved to give half as many sodium pellets and half as much water as would be used for the full liter. Below is a table of the calculations preformed to achieve the different molarity concentrations for the base.

Table 8. NaOH Molarity Solutions

NaOH Molarity Solutions			
1st and 2nd Trials	**NaOH Calculated** $\frac{\text{Desired mols}}{1\text{L}} \text{x} \frac{40.0\text{g}}{1.0\text{mol}} \text{x} \frac{1\text{L}}{1000\text{mL}}$	**Amount of NaOH in 1 L Solution (Weighed)**	**Volume of Water in 1 L Solution** $\frac{1\text{L x Weighed NaOH (g)}}{\text{NaOH Calculated}}$
2.0 M	0.08 g/mL	79.52g	994mL
1.0 M	0.04 g/mL	39.86g	997mL
0.5 M	0.02 g/mL	19.93g	997mL
0.1 M	0.004 g/mL	4.03g	1008mL
Molecular Weight NaOH = 40.0g/1.0mol			

Table 9. NaOH Molarity Solutions

NaOH Molarity Solutions			
3rd Trial	**NaOH Calculated** $\frac{1}{2}(\frac{\text{Desired mols}}{1\text{L}} \text{x} \frac{40.0\text{g}}{1.0\text{mol}} \text{x} \frac{1\text{L}}{1000\text{mL}})$	**Amount of NaOH in 1 L Solution (Weighed)**	**Volume of Water in 1 L Solution** $\frac{1\text{L x Weighed NaOH (g)}}{\text{NaOH Calculated}}$
2.0 M	0.04 g/mL	40.10g	501mL
1.0 M	0.02 g/mL	20.49g	512mL
0.5 M	0.01 g/mL	10.24g	512mL
0.1 M	0.002 g/mL	2.13g	533mL
Molecular Weight NaOH = 40.0g/1.0mol			

Once the solutions were made, the materials were cut and labeled. The leather material was pre-cut into forty-five circular pieces each with an 8.0cm diameter and numbered using a black Sharpie © pen. The cotton and polyester materials were both cut into forty-five circular pieces each with an 8.2cm diameter and numbered using a black Sharpie © pen or regular ink pen. The initial weights of all the materials were recorded on an analytical balance. On a lab bench, a grid was assembled to keep up with the material and molarity of the solution I was using at the time. Fifteen 400 mL beakers were used in each grid. Five pieces of each material were used, and four different molarities of the solution plus a control of de-ionized water was used per grid. Each numbered piece of material was chosen at random and placed in a beaker and the correct molarity of the solution was added to the beaker until the material was completely submerged in the solution. The material pieces sat submerged in the solution for a total of three hours before they were removed, rinsed with deionized water, and laid out to dry on paper towels or latex gloves for seven days. The materials were then re-weighed to see if there was any difference in the resulting weight and initial weight. Six trials were done over a span of six weeks. In the first three trials the solution used was hydrochloric acid (HCl), a strong acid, and in the last three trials the solution used was sodium hydroxide (NaOH), a strong base. On the fourth trial, the paper towels stuck to the leather material. The stuck paper towels were removed as best as possible without damaging the leather, and the final weights were taken. To prevent paper towels from sticking to the leather in future trials, the leather was laid out on latex gloves to dry, which sufficiently prevented any further sticking.

Once the six trials were completed, the material samples that had been subjected to the strongest concentration of acid and base (2.0M) underwent a methanol extraction to prepare them for LC-MS analysis in order to determine if the chemical composition of the material samples differed between the acid and base treated samples. Methanol was chosen as an extraction solvent based on previous data published in the journal *Analytical Chemistry*, which indicated that methanol was highly suitable for metabolomic GC-MS application, compared to ethanol, acetonitrile, acetone, and chloroform (19). All nine of the control material samples from the acid trials were used as the controls in the methanol extraction. The three leather control materials from the three acid trials were combined into one 500mL square media bottle, and the same procedure was done for the polyester and cotton controls used in the acid trials. All three of the cotton material samples used in the 2.0M sodium hydroxide trials were combined into one 500mL square media bottle. All three of the polyester samples used in the 2.0M sodium hydroxide trials were combined into one 500mL square media bottle, and all three of the leather samples used in the 2.0M sodium hydroxide trials were combined into one 500mL square media bottle. The same procedure was done with the 2.0M acid materials. 500mL of methanol was added to all the leather samples, 450mL of methanol was added to all the cotton samples, and 300mL of methanol was added to all the polyester samples. The samples sat in the methanol until the following week. Once the material samples had set for a week, three 1mL vial samples of each media bottle were taken. A small amount of the methanol solution from each bottle was poured out into 250mL beakers. Then, using a different syringe for each beaker of solution, 3mL of solution was extracted from each beaker and filtered through a 0.22µm syringe filter into three separate 1mL amber auto-sampler vials. All of the vials were numbered and labeled according to the sample material and as either a base, acid, or control. All the vials were placed into the freezer and stored at -10°C until LC-MS analysis began. In order to complete the LC-MS analysis, a 10µL aliquot of each vial sample was injected by a Shimadzu HPLC (high performance liquid chromatography) system with IT-TOF (ion-trap time-of-flight) mass spectrometer, operating in positive electrospray mode (+ESI) into a Thermo Hypersil-Keystone Aquasil C18 equipped with a 150m x 4.6 mm column with a 5.0 µm particle size. The column oven was set at 40 °C. The flow rate through the column was 0.400 ml/min, and the mobile phase consisted of A (70% water/30% acetonitrile with 0.1% formic acid) and B (0.1% formic acid in acetonitrile). The gradient program held 5% B for one minute, then ramped five minutes, then held 95% B over twelve minutes, and finally held at 95% B for one minute. Masses were acquired from m/z 200 to 2000 with a 10msec dwell time. One injection of each sample was analyzed, allowing for a three minute equilibration of the column between samples. The data was exported to XCMS Online © and acid-treated versus base-treated materials for each fabric were compared (20).

Data

All six grids and the initial and resulting weights of the materials used in each grid are listed below.

Table 10. Sample 1 (HCl)

Sample 1 (HCl) Date: 10/03/14			
Material	**Number**	**Result Weight (g)**	**Initial Weight (g)**
cotton	2	1.6849	1.6708
cotton	6	1.6515	1.6489
cotton	3	1.6712	1.6666
cotton	4	1.6618	1.6570
cotton	7	1.6465	1.6415
polyester	1	0.2362	0.2363
polyester	4	0.2443	0.2442
polyester	5	0.2478	0.2472
polyester	3	0.2436	0.2437
polyester	6	0.2474	0.2475
leather	38	12.2389	13.2283
leather	24	13.8534	14.4828
leather	41	13.0238	13.9818
leather	45	12.4262	13.2781
leather	40	12.6864	13.3996

Table 11. Grid 1 (HCl)

Grid 1 (HCl) Date: 10/03/14					
	Control	**0.1M**	**0.5M**	**1.0M**	**2.0M**
Cotton Number	▪ 2*	(stiff but flexible) 3	(stiff but flexible) 4	(stiff but flexible) 6	(stiff but flexible) 7*
Polyester Number	▪1*	▪2	▪3	▪5	▪6*
Leather Number	▪41*	(stiff but flexible, slightly darker brown) 38	(stiff but flexible, slightly darker brown) 45	(stiff but flexible, slightly darker brown) 24	(stiff but flexible, slightly darker brown) 40*
*Used in methanol extraction. ( ) Change in Physical Appearance after experiment. ▪ No change in physical appearance after experiment.					

Table 12. Sample 2 (HCl)

Sample 2 (HCl) Date: 10/08/14			
Material	**Number**	**Result Weight (g)**	**Initial Weight (g)**
cotton	45	1.6170	1.6150
cotton	43	1.6384	1.6351
cotton	42	1.5875	1.5863
cotton	44	1.6416	1.6390
cotton	1	1.6180	1.6121
polyester	31	0.2358	0.2359
polyester	25	0.2371	0.2368
polyester	23	0.2365	0.2365
polyester	28	0.2380	0.2356
polyester	29	0.2422	0.2430
leather	39	12.8968	13.8291
leather	19	10.9312	10.8213
leather	44	12.9653	13.7137
leather	18	12.9991	13.6655
leather	17	14.1878	14.6483

Table 13. Grid 2 (HCl)

<table>
<tr><th colspan="6">Grid 2 (HCl)
Date: 10/08/14</th></tr>
<tr><th></th><th>Control</th><th>0.1M</th><th>0.5M</th><th>1.0M</th><th>2.0M</th></tr>
<tr><td>Cotton Number</td><td>▪45*</td><td>(stiff but flexible) 44</td><td>(stiff but flexible) 1</td><td>(stiff but flexible) 43</td><td>(stiff but flexible) 42*</td></tr>
<tr><td>Polyester Number</td><td>▪31*</td><td>▪28</td><td>▪29</td><td>▪23</td><td>▪25*</td></tr>
<tr><td>Leather Number</td><td>▪39*</td><td>(stiff but flexible, slightly darker brown) 19</td><td>(stiff but flexible, slightly darker brown) 44</td><td>(stiff but flexible, slightly darker brown) 18</td><td>(stiff but flexible, slightly darker brown) 17*</td></tr>
<tr><td colspan="6">*Used in methanol extraction.
( ) Change in Physical Appearance after experiment.
▪ No change in physical appearance after experiment.</td></tr>
</table>

Table 14. Sample 3 (HCl)

<table>
<tr><th colspan="4">Sample 3 (HCl)
Date: 10/17/14</th></tr>
<tr><th>Material</th><th>Number</th><th>Result Weight (g)</th><th>Initial Weight (g)</th></tr>
<tr><td>cotton</td><td>8</td><td>1.5857</td><td>1.6023</td></tr>
<tr><td>cotton</td><td>10</td><td>1.5623</td><td>1.5795</td></tr>
<tr><td>cotton</td><td>9</td><td>1.557</td><td>1.5737</td></tr>
<tr><td>cotton</td><td>5</td><td>1.6576</td><td>1.6675</td></tr>
<tr><td>cotton</td><td>11</td><td>1.6022</td><td>1.6194</td></tr>
<tr><td>polyester</td><td>39</td><td>0.2367</td><td>0.2371</td></tr>
<tr><td>polyester</td><td>22</td><td>0.2421</td><td>0.2424</td></tr>
<tr><td>polyester</td><td>43</td><td>0.2409</td><td>0.2413</td></tr>
<tr><td>polyester</td><td>38</td><td>0.2387</td><td>0.2391</td></tr>
<tr><td>polyester</td><td>40</td><td>0.2396</td><td>0.2401</td></tr>
<tr><td>leather</td><td>16</td><td>13.1023</td><td>14.0366</td></tr>
<tr><td>leather</td><td>13</td><td>12.8716</td><td>14.0752</td></tr>
<tr><td>leather</td><td>15</td><td>11.3996</td><td>12.7780</td></tr>
<tr><td>leather</td><td>14</td><td>12.3258</td><td>13.7503</td></tr>
<tr><td>leather</td><td>12</td><td>11.6732</td><td>11.6335</td></tr>
</table>

Table 15. Grid 3 (HCl)

Grid 3 (HCl)
Date: 10/17/14

	Control	**0.1M**	**0.5M**	**1.0M**	**2.0M**
Cotton Number	▪5*	(stiff but flexible) 8	(stiff but flexible) 9	(stiff but flexible) 10	(stiff but flexible) 11*
Polyester Number	▪22*	▪43	▪40	▪39	▪38*
Leather Number	▪16*	(stiff but flexible, slightly darker brown) 15	(stiff but flexible, slightly darker brown) 14	(stiff but flexible, slightly darker brown) 13	(stiff but flexible, slightly darker brown) 12*

*Used in methanol extraction.
( ) Change in Physical Appearance after experiment.
▪ No change in physical appearance after experiment.

Table 16. Sample 4 (NaOH)

Sample 4 (NaOH)
Date: 10/24/14

Material	**Number**	**Result Weight (g)**	**Initial Weight (g)**
cotton	37	1.5933	1.6029
cotton	39	1.6041	1.6249
cotton	40	1.6525	1.6725
cotton	38	1.6390	1.6567
cotton	41	1.5654	1.5834
polyester	41	0.2396	0.2400
polyester	30	0.2388	0.2391
polyester	44	0.2410	0.2414
polyester	32	0.2402	0.2404
polyester	45	0.2400	0.2403
leather	43	12.3463	13.5731
leather	42	10.3898	13.5081
leather	36	11.8106	12.2742
leather	35	12.1198	13.7247
leather	34	13.8849	14.2048

Table 17. Grid 4 (NaOH)

Grid 4 (NaOH) **Date: 10/24/14**					
	Control	**0.1M**	**0.5M**	**1.0M**	**2.0M**
Cotton Number	▪ 41	(stiff but flexible) 40	(stiff but flexible) 39	(fringed edges, stiff, flexible) 38	(fringed edges, stiff, flexible) 37*
Polyester Number	▪ 44	▪ 45	▪ 41	▪ 30	▪ 32*
Leather Number	▪ 43	(slightly darker brown, stiff, flexible) 42	(dark brown, stiff, slightly flexible) 36	(Deep dark brown, stiff, inflexible) 35	(Black, stiff, inflexible) 34*
*Used in methanol extraction. ( ) Change in Physical Appearance after experiment. ▪ No change in physical appearance after experiment.					

Table 18. Sample 5 (NaOH)

Sample 5 (NaOH) **Date: 10/31/14**			
Material	**Number**	**Result Weight (g)**	**Initial Weight (g)**
cotton	35	1.6157	1.6212
cotton	36	1.6292	1.6414
cotton	32	1.5770	1.5673
cotton	33	1.6305	1.6311
cotton	34	1.6367	1.6363
polyester	42	0.2392	0.2394
polyester	34	0.2419	0.2420
polyester	37	0.2403	0.2406
polyester	35	0.2422	0.2424
polyester	36	0.2405	0.2396
leather	33	12.6413	13.9532
leather	32	11.4052	13.1108
leather	31	12.7024	14.9111
leather	30	13.1462	13.8760
leather	29	14.8331	14.8093

Table 19. Grid 5 (NaOH)

Grid 5 (NaOH) Date: 10/31/14					
	Control	**0.1M**	**0.5M**	**1.0M**	**2.0M**
Cotton Number	▪ 36	(stiff but flexible) 35	(stiff but flexible)34	(fringed edges, stiff, flexible) 33	(fringed edges, stiff, flexible) 32*
Polyester Number	▪ 42	▪ 34	▪ 37	▪ 36	▪ 35*
Leather Number	▪ 33	(slightly darker brown, stiff, flexible) 32	(dark brown, stiff, slightly flexible) 31	(Deep dark brown, stiff, inflexible) 30	(Black, stiff, inflexible) 29*
*Used in methanol extraction. ( ) Change in Physical Appearance after experiment. ▪ No change in physical appearance after experiment.					

Table 20. Sample 6 (NaOH)

Sample 6 (NaOH) Date: 11/5/14			
Material	**Number**	**Result Weight (g)**	**Initial Weight (g)**
cotton	14	1.5842	1.6293
cotton	13	1.5659	1.6106
cotton	12	1.5492	1.5933
cotton	31	1.5589	1.6092
cotton	30	1.5644	1.6049
polyester	33	0.2420	0.2429
polyester	27	0.2428	0.2436
polyester	26	0.2339	0.2413
polyester	24	0.2389	0.2397
polyester	14	0.2472	0.2481
leather	11	15.5375	16.5158
leather	10	14.3050	15.4135
leather	9	10.8381	12.5771
leather	28	12.0853	13.8006
leather	27	14.1440	14.3813

Table 21. Grid 6 (NaOH)

Grid 6 (NaOH) **Date: 11/5/14**					
	Control	**0.1M**	**0.5M**	**1.0M**	**2.0M**
Cotton Number	▪ 14	(stiff but flexible) 13	(stiff but flexible) 12	(fringed edges, stiff, flexible) 31	(fringed edges, stiff, flexible) 30*
Polyester Number	▪ 33	▪ 27	▪ 26	▪ 24	▪ 14*
Leather Number	▪ 11	(slightly darker brown, stiff, flexible) 10	(dark brown, stiff, slightly flexible) 9	(Deep dark brown, stiff, inflexible) 28	(Black, stiff, inflexible) 27*
*Used in methanol extraction. ( ) Change in Physical Appearance after experiment. ▪ No change in physical appearance after experiment.					

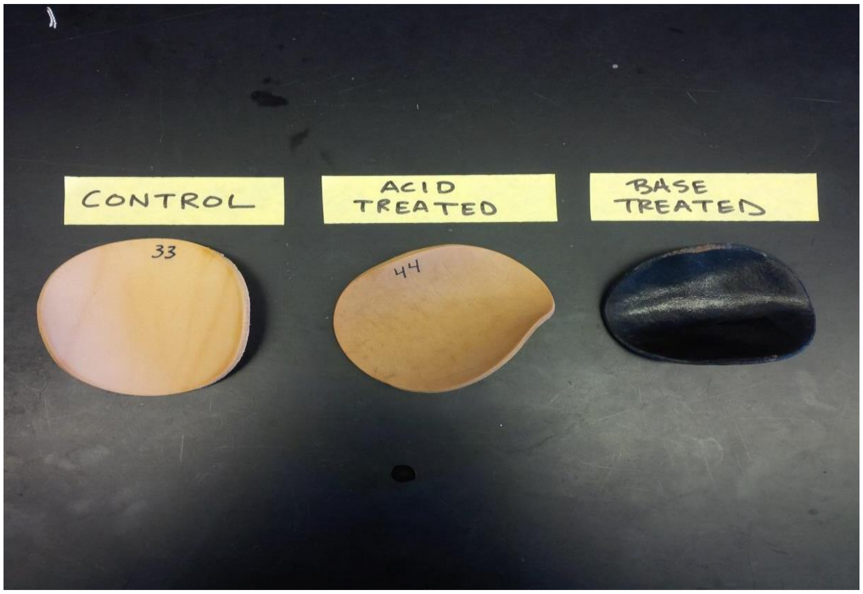


Figure 6. Leather Samples

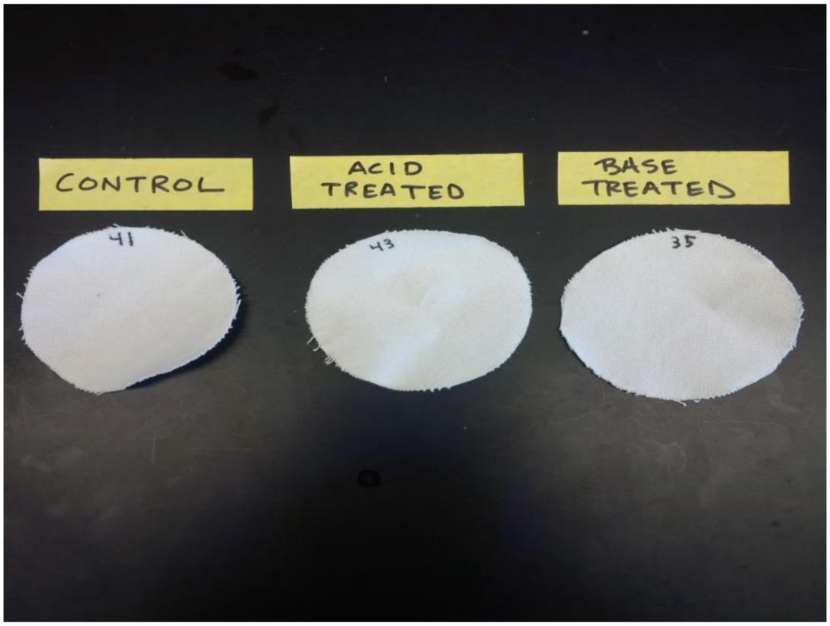


Figure 7. Cotton Samples

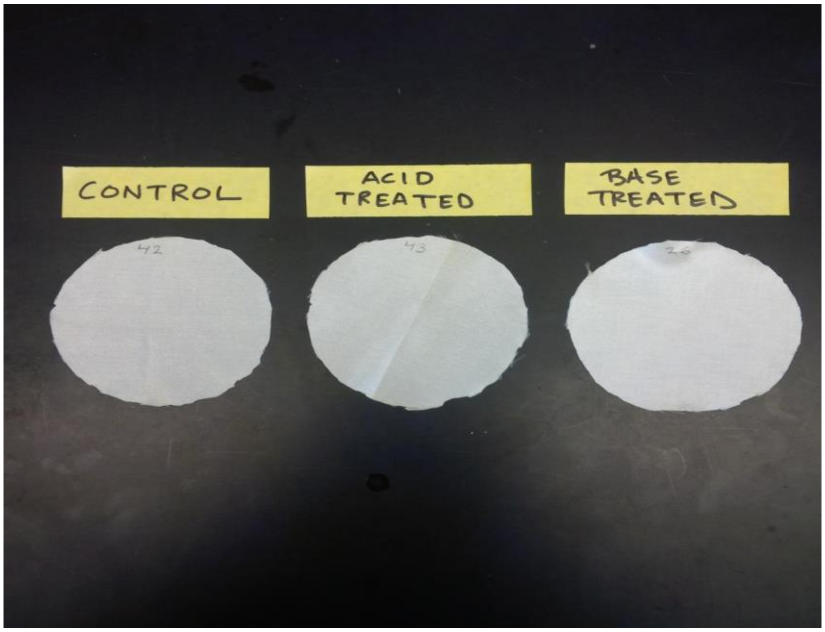


Figure 8. Polyester Samples

Calculations

Masses of each fabric sample were recorded using an analytical balance before and after chemical treatment, following a seven day drying period at room temperature. The mass differences between pre- and post-treated samples were calculated using Microsoft Excel, and the absolute values of those data were imported into GraphPad Prism © (version 5.03; La Jolla, CA). In GraphPad ©, the data was subjected to a one-way analysis of variance (ANOVA) with a 95% confidence interval ($p < 0.05$ indicating a statistically significant difference). Control data for each treatment was compared to each chemical concentration, and all concentrations were compared to each other using a Newman-Keuls Multiple Comparison Test. Additionally, data for each fabric was compared between acid and base treatments using the aforementioned parameters. Finally, data from all four concentrations was pooled for HCl and NaOH treated samples, and compared using a two-tailed t-test ($p< 0.05$). In the LC-MS analysis, the data was exported to XCMS Online © and acid-treated versus base-treated materials for each fabric were compared (20). XCMS Online © is a graphing program that allows users to upload and process their LC/MS data in order to profile the metabolites contained in the data (20). In order to process the data using XCMS Online ©, a user account and job page were created. The datasets were then uploaded to the job page, and the job was defined by selecting a parameter set to correspond to the job. Finally, the job was submitted for processing. After submission of the job, the results, details, datasets, and parameter set used were visible on the "View Results" page of the XCMS Online © software. The results were then discussed and placed in the "Results" section of this paper (20).

Results

Changes in Material Appearance

Grid 1, 2, and 3 were the HCl experiments. In these experiments there was no change in physical appearance of any of the controls or of any of the polyester materials after the materials were removed from the HCl concentrations. However, the cotton and leather materials of the experiments did become stiff, but were still flexible after being removed from the HCl concentrations, and the leather materials were a slightly darker brown color after removal from the acid. Grid 4, 5, and 6 were the NaOH experiments. Once again, there was no change in the physical appearance of the controls or of any of the polyester materials after the materials were removed from the NaOH concentrations, but there were great changes in the leather and cotton materials. The 0.1M and 0.5M cotton materials were stiff, but flexible after being removed from their respective NaOH concentrations. The 1.0M and 2.0M cotton materials were stiff, flexible, and had fringed edges upon removal from their respective NaOH concentrations. The 0.1M leather material was a slightly darker brown color, stiff, and flexible after removal from its respective NaOH concentration, and the 0.5M leather material was a dark brown color, stiff, and slightly flexible after removal from its respective concentration. The 1.0M leather material was a deep dark brown color, stiff, and inflexible after removal, and the 2.0M leather was a black color, stiff, and completely inflexible after removal from its respective concentration. Figure 6 on page 33 shows an example of a leather control material, a leather acid material, and a leather base material after they have been submerged and removed from their respective concentrations. Notice the black color and stiffness of the leather base material. All controls were submerged in deionized water only. Figure 7 on page 34 shows an example of a cotton control material, a cotton acid material, and a cotton base material after they have been submerged and removed from their respective concentrations. It is difficult to see in the figure, but the cotton base material is more fringed around the edges than the other two cotton material pieces in the figure. Figure 8 on page 34 shows an example of a polyester control material, a polyester acid material, and a polyester base material after they have been submerged and removed from their respective concentrations. Notice that there appears to be no significant change in appearance of any of the polyester materials in the figure.

Statistical Results

For the HCl treated samples, no statistically significant difference ($p < 0.05$) in mass change was noted between the control samples and the acid concentrations for any of the fabrics. Furthermore, there was no statistically significant difference found in mass changes between the various concentrations of acid treatment (0.1, 0.5, 1.0, and 2.0M). The ANOVA returned a p-value for cotton samples of 0.9990, indicating a high degree of similarity among the treatment groups. The p-value from the ANOVA for the polyester samples was also high at 0.6870. The leather samples were the most dissimilar, and trended toward a statistically significant difference with a p-value from ANOVA of 0.0788. These data (average and standard error of the mean, SEM), are shown in Figures 9 through 11 below.

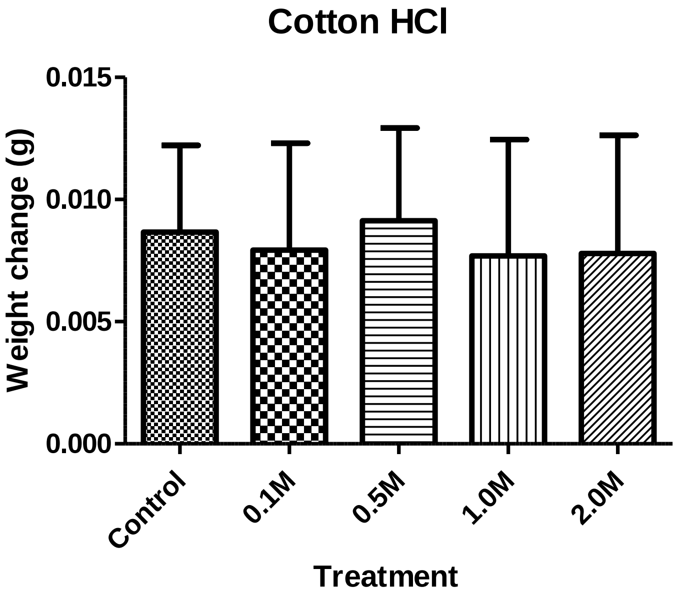


Figure 9. Cotton HCl (Graph Pad Prism ©)

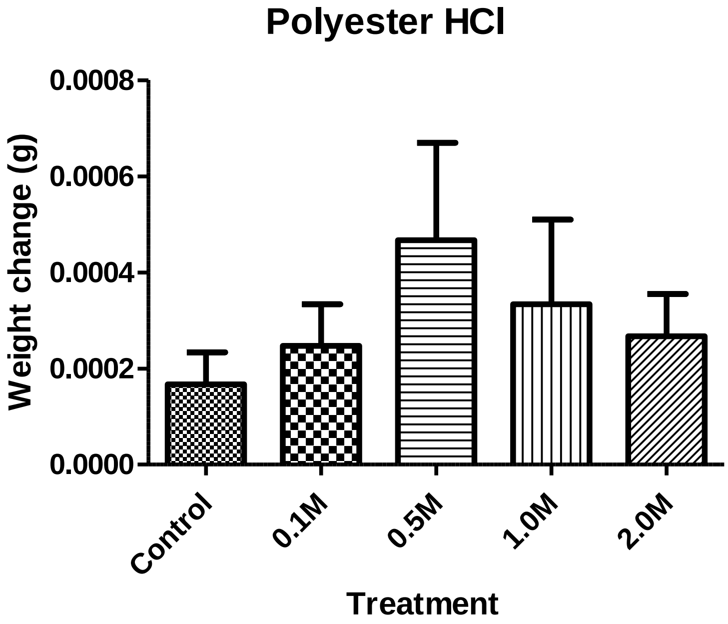


Figure 10. Polyester HCl (Graph Pad Prism ©)

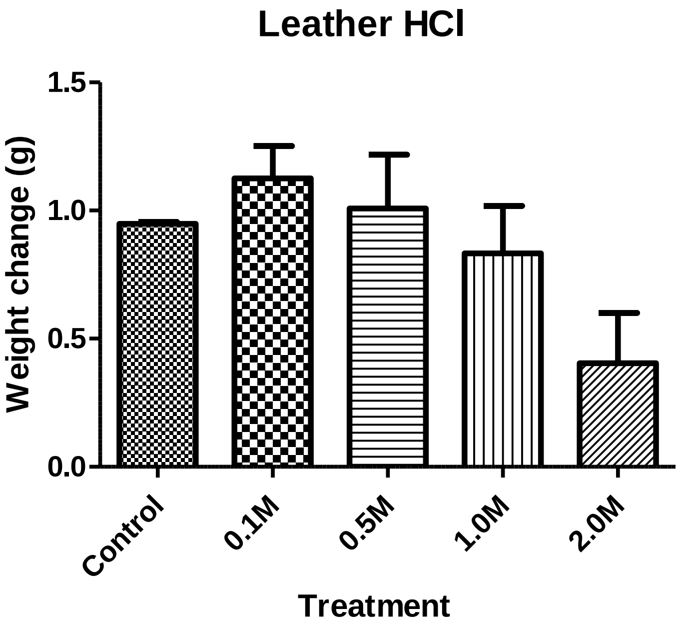


Figure 11. Leather HCl (Graph Pad Prism ©)

For the NaOH treated samples, no statistically significant difference ($p < 0.05$) in mass change was noted between the control samples and the acid concentrations for any of the fabrics. Furthermore, there was no statistically significant difference found in mass changes between the various concentrations of base treatment (0.1, 0.5, 1.0, and 2.0M). The ANOVA returned a p-value for cotton samples and polyester samples of 0.9985 and 0.8675, respectively, indicating a high degree of similarity among the treatment groups. The leather samples were the most dissimilar, and trended toward a statistically significant difference with a p-value from ANOVA of 0.0798. Curiously, the largest documented weight change in the leather was in the 0.1M treated group. Additionally, the 2.0M NaOH treated samples experienced a dramatic color and texture change, but showed little variation in weight between treated and untreated. These data (average and standard error of the mean, SEM), are shown in Figures 12 through 14 below.

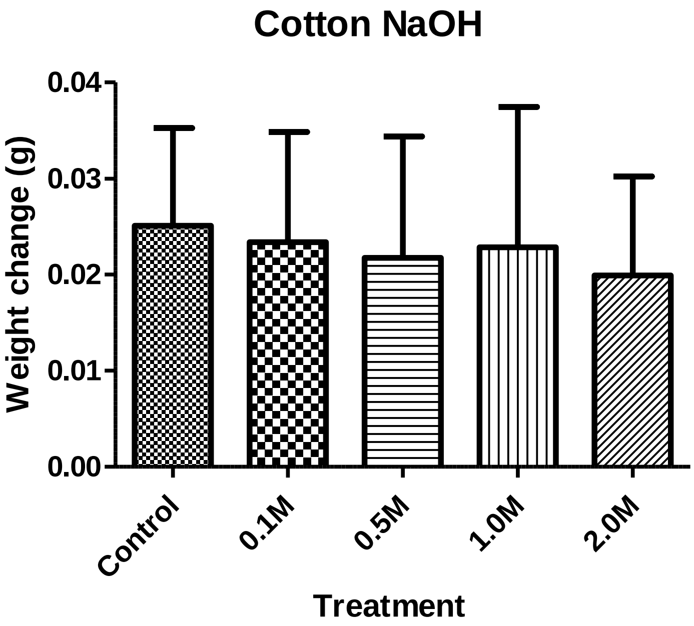


Figure 12. Cotton NaOH (Graph Pad Prism ©)

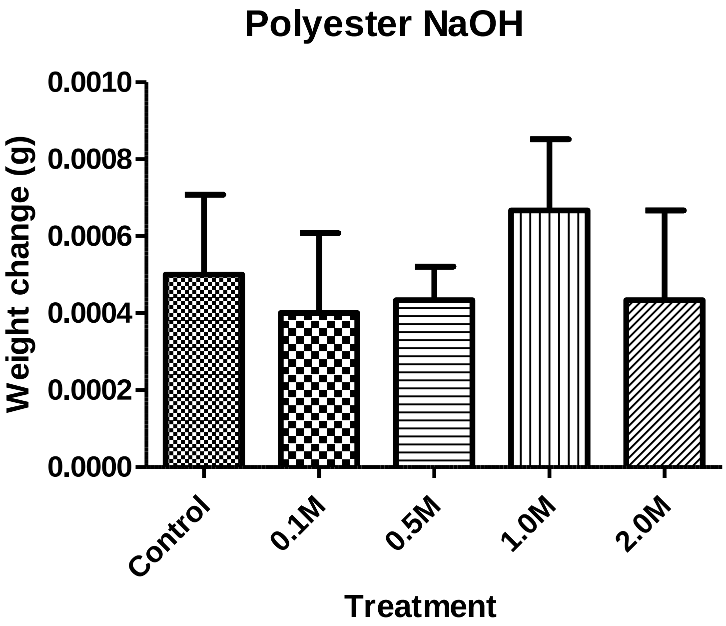


Figure 13. Polyester NaOH (Graph Pad Prism ©)

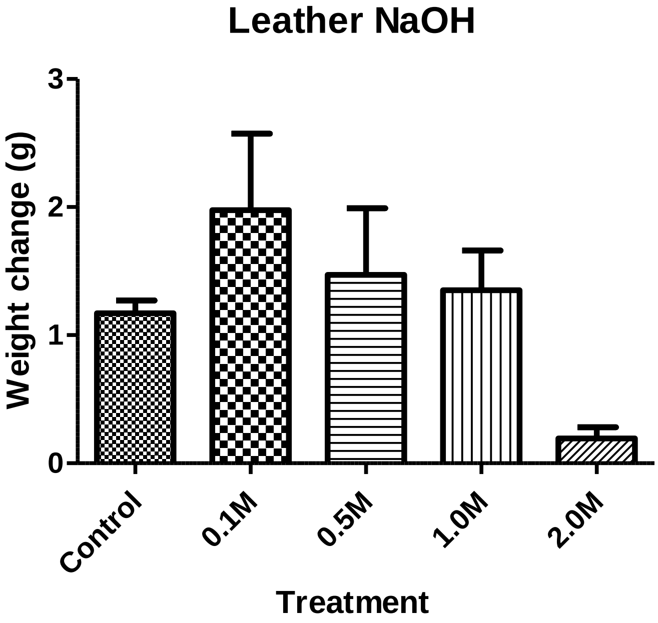


Figure 14. Leather NaOH (Graph Pad Prism ©)

When HCl treated samples were compared to NaOH treated samples, a more dramatic trend was noticed in the numerical weight changes; however, only data in the leather sample groups showed statistically significant difference. For cotton, the NaOH-treated samples had a greater weight change than the HCl-treated ones, but the large standard error weakened the case for statistical significance (P-value = 0.7088). Polyester samples were very similar across treatments (P-value = 0.7330). Finally, there was a statistically significant difference noted in the one-way ANOVA treated leather (P-value = 0.0296), with a specific difference highlighted in the Newman-Keuls post-test. These data (average and standard error of the mean, SEM), are shown in Figures 15 through 17 below.

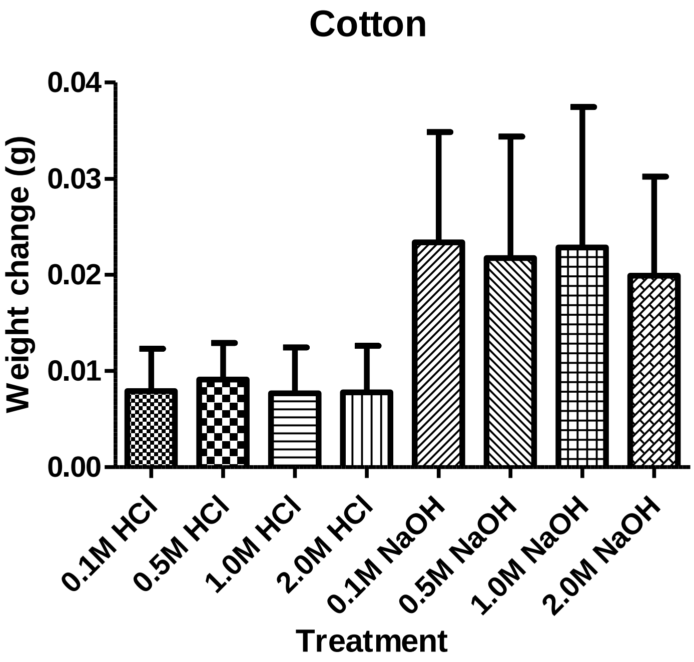


Figure 15. Cotton (Graph Pad Prism ©)

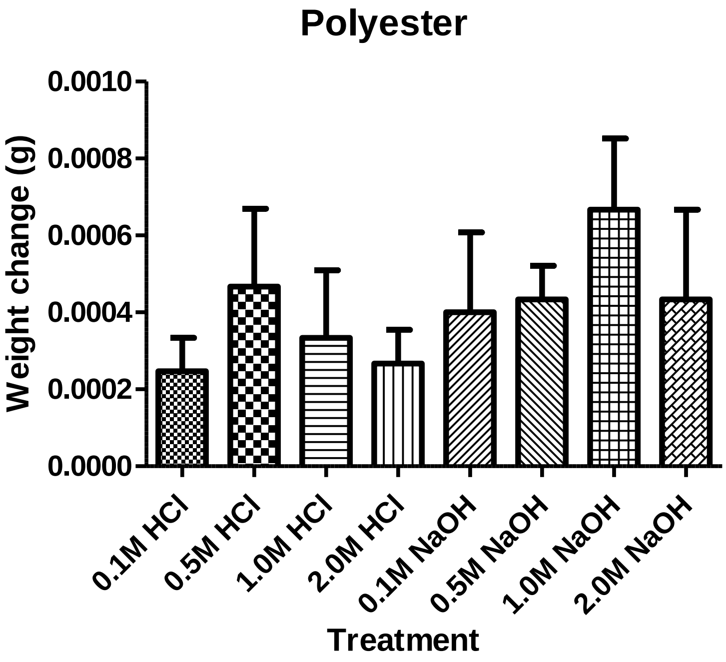


Figure 16. Polyester (Graph Pad Prism ©)

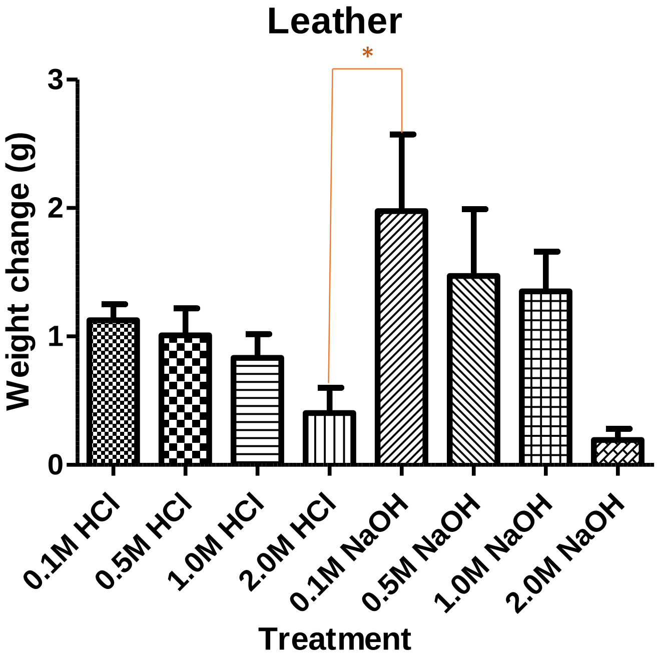


Figure 17. Leather (Graph Pad Prism ©)

Since there were only three replicates in each concentration treatment, the data was pooled to result in n = 12 HCl and n = 12 NaOH-treated samples for each fabric. This data was then subjected to a two-tailed t-test with $p < 0.05$ indicating statistical significance. The cotton samples indicated a statistically significant difference between the chemical treatments, with the NaOH-treated samples showing much more change in weight (P-value = 0.0282). No such difference could be detected for the polyester and leather samples, with P-values of 0.1733 and 0.1801, respectively. The graphical representation of these pooled data is shown in Figures 18 through 20 below.

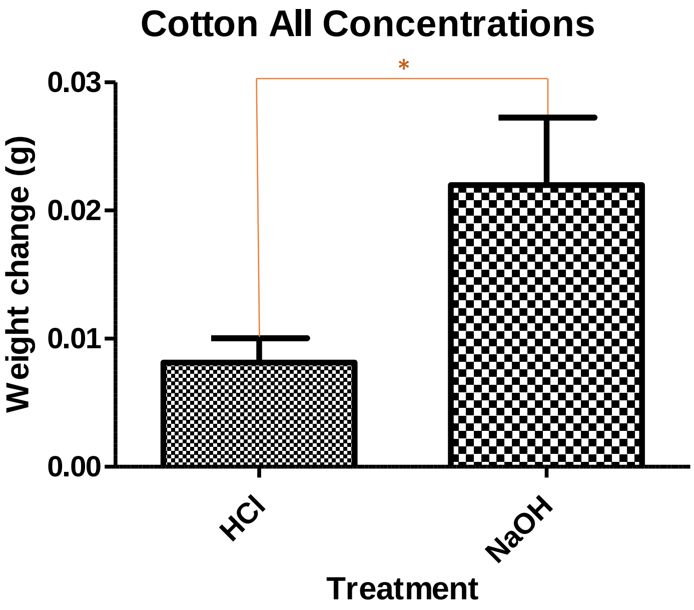


Figure 18. Cotton All Concentrations (Graph Pad Prism ©)

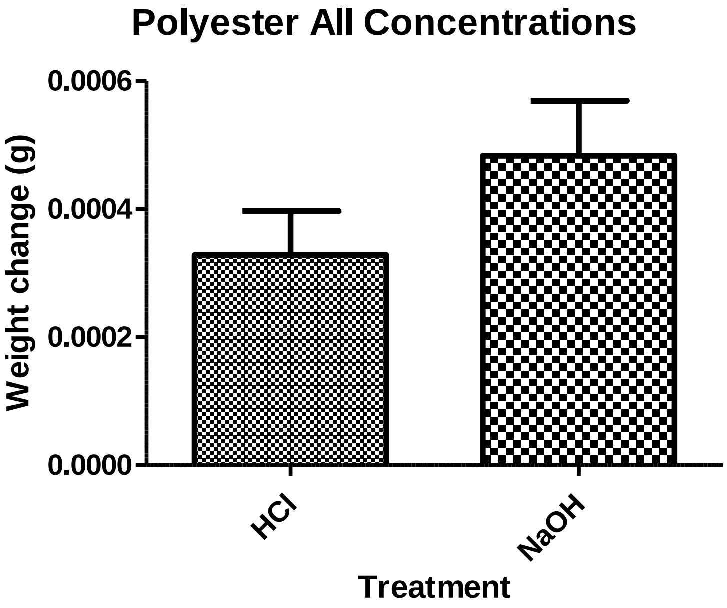


Figure 19. Polyester All Concentrations (Graph Pad Prism ©)

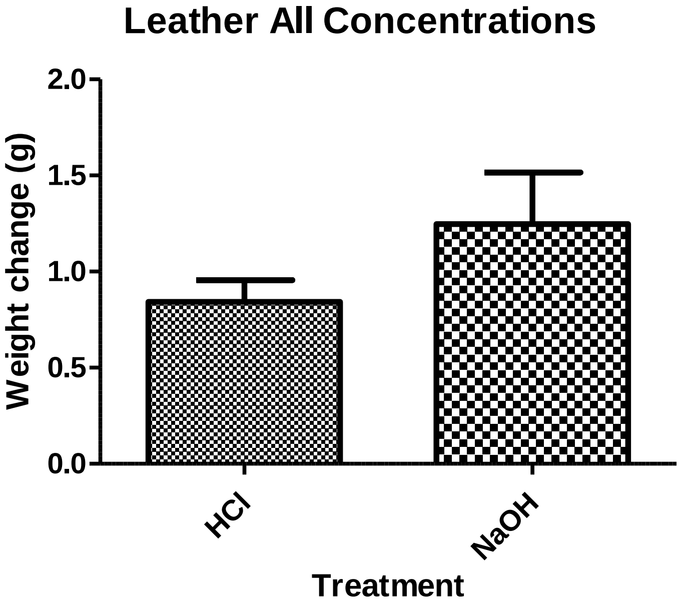


Figure 20. Leather All Concentrations (Graph Pad Prism ©)

Chromatography Results

The figure below shows super-imposed chromatographic runs of acid treated (n = 3) and base treated (n = 3) polyester samples following methanol extraction. There are very few differences obvious in these sample groups. Additionally, there are very few overall peaks in the chromatogram, which tells us that there were not many compounds to be extracted by the methanol for this fabric.

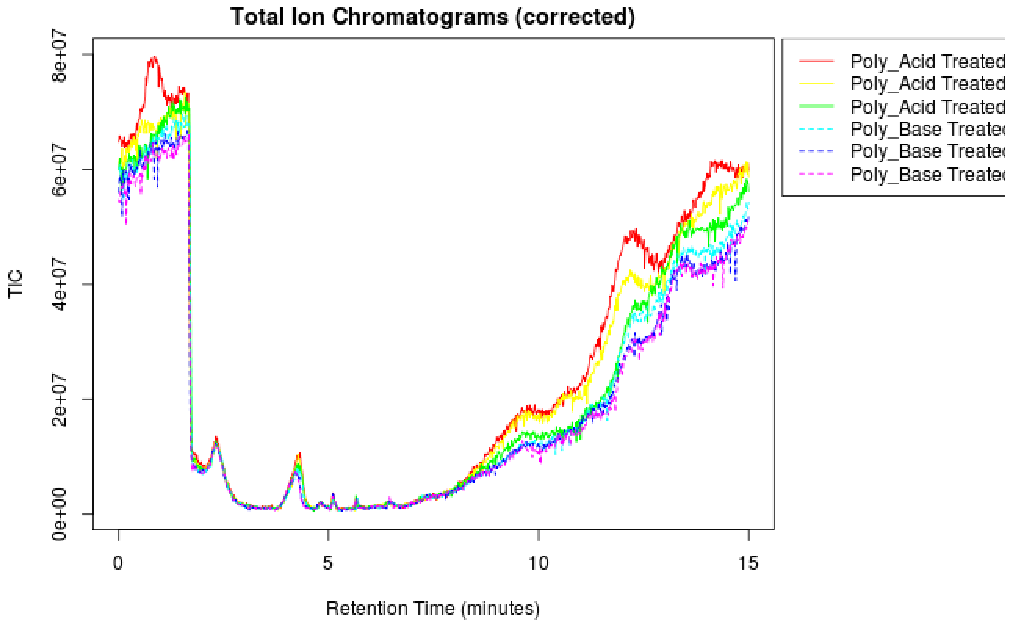


Figure 21. Total Ion Chromatograms (19)

The cloud plot below depicts the differences in presence or concentration of the features (ions) found in the mass spectra associated with the chromatograms. The upper traces are the acid treated samples and the lower traces (inverted) and the base treated samples. Processing by XCMS Online © through the Scripps Center for Metabolomics indicates that there are 64 ions that differ between these two treatment groups in a statistically significant way ($p \leq 0.01$) and have a ≥ 1.5 fold difference (fold change) (20). Red dots on the cloud plot indicate that a particular ion is more abundant in the acid-treated samples, and green dots indicate that a particular ion is more abundant in the base-treated samples. The size and darkness of the dots corresponds to the magnitude of these differences. One feature was tentatively identified by the data base as a ceramide polymer (m/z 672.622 $[M + Na^+]$ ion), which had an ion count 58x higher in the acid treated samples, indicating that if this molecule was indeed present in the polyester fabric, the base treatment was more effective in diminishing it (20).

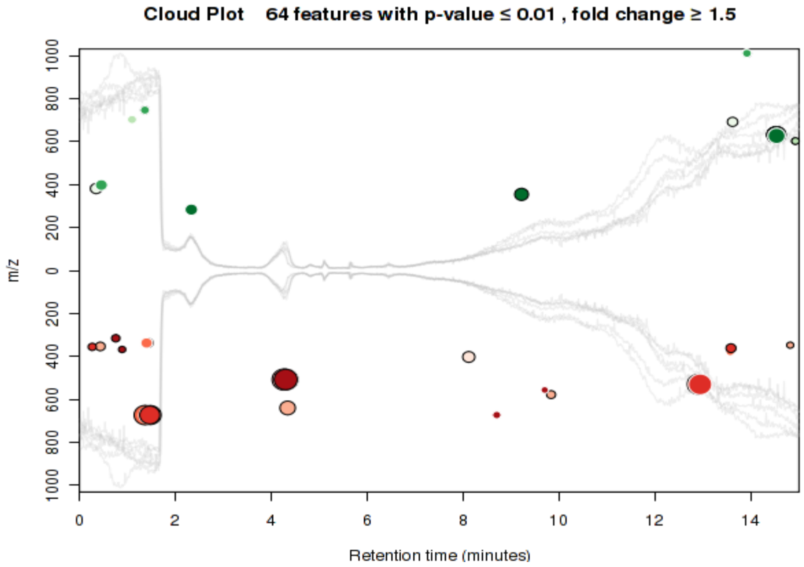


Figure 22. Cloud Plot, 64 Features with p-value ≤ 0.01, Fold Change ≥ 1.5 (19)

The figure below shows super-imposed chromatographic runs of acid treated (n = 3) and base treated (n = 3) cotton samples following methanol extraction. There are a few obvious differences in these sample groups, including larger peaks in the base-treated samples at retention times around 1, 4 and 5 minutes.

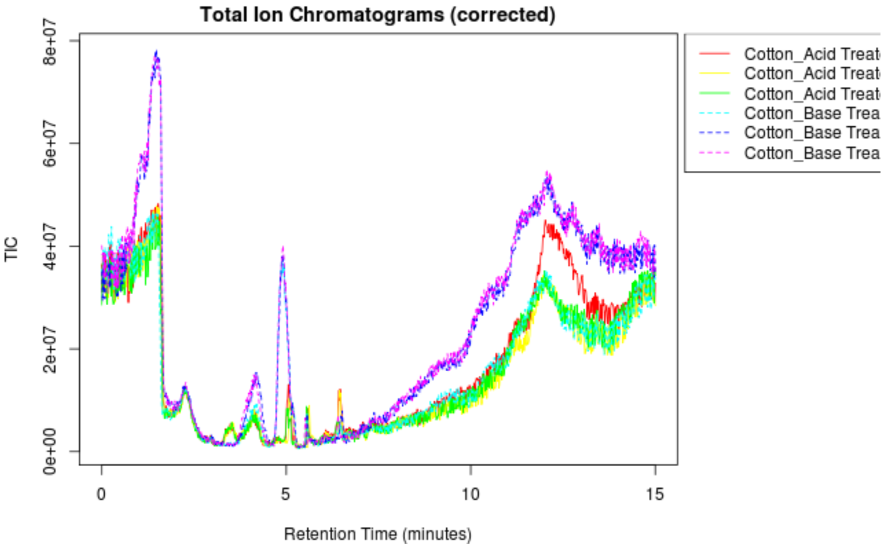


Figure 23. Total Ion Chromatograms (Corrected) (19)

The cloud plot indicates that there are 449 ions that differ between these two treatment groups in a statistically significant way ($p \leq 0.01$) and have a ≥ 1.5 fold difference (fold change). Preliminary identification for the large peak in the base-treated chromatograms points to a mixture of hydrocarbon derived glycerol molecules (retention time 4.90). There is some evidence supporting the presence of glycerol in cotton fibers (13). An example structure is as follows:

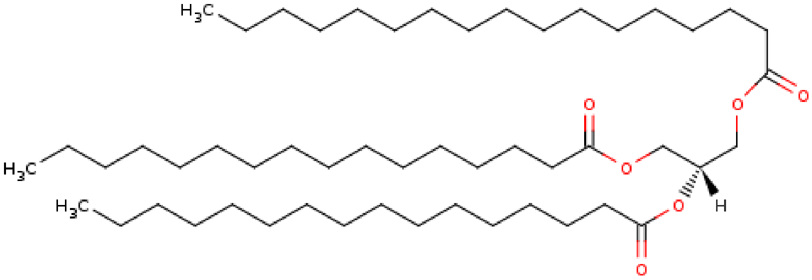


Figure 24. Glycerol (19, Metlin Database)

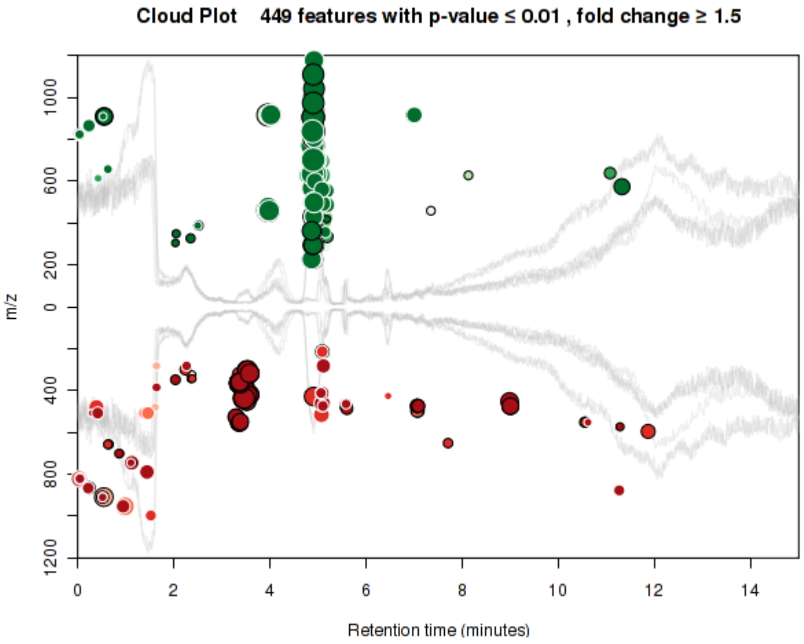


Figure 25. Cloud Plot, 449 Features with p-value ≤ 0.01, Fold Change ≥ 1.5(19)

The total ion chromatograms figure indicates that the leather samples were much more complex than the other fibers in this study. The acid treated samples appear to have several peaks that were higher than the base-treated samples, possibly indicating that these components were destroyed as a result of the NaOH exposure. However, the cloud plot indicates an abundance of difference between the acid- and base treated samples, with over 4000 detectable ions with a statistically significant difference (p-value ≤ 0.01). In order to interpret the results from so many features, the threshold for statistical significance was tightened for the leather samples to p-value ≤ 0.0001, which narrowed down the feature list to 354 compounds. Most of the components with a preliminary identification by XCMS Online © were small peptides (3 – 4 amino acids) (19). This indicates a difference in breakdown of protein infrastructure (collagen) of the leather following chemical treatment with acid versus base.

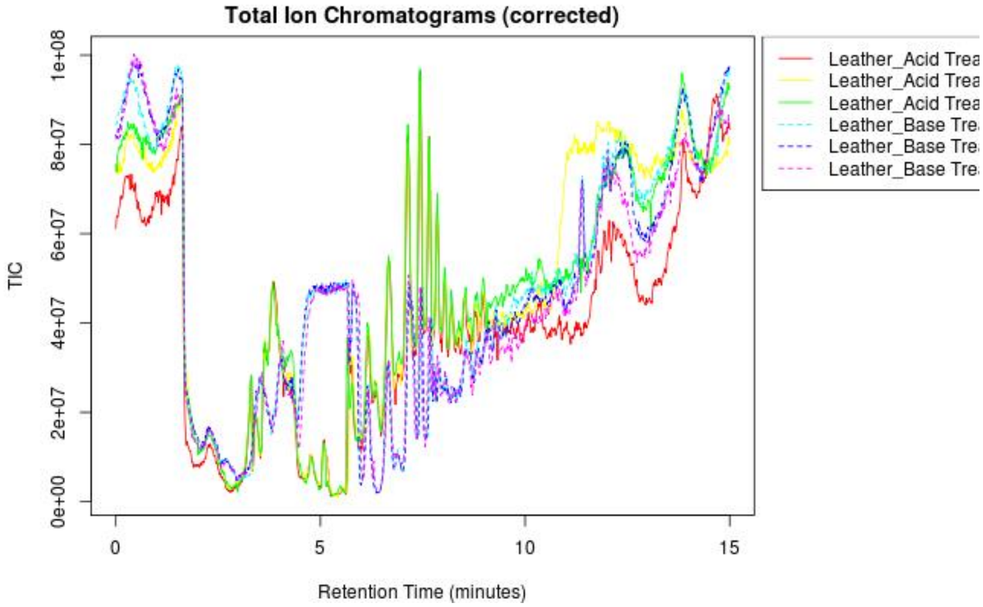


Figure 26. Total Ion Chromatograms (Corrected) (19)

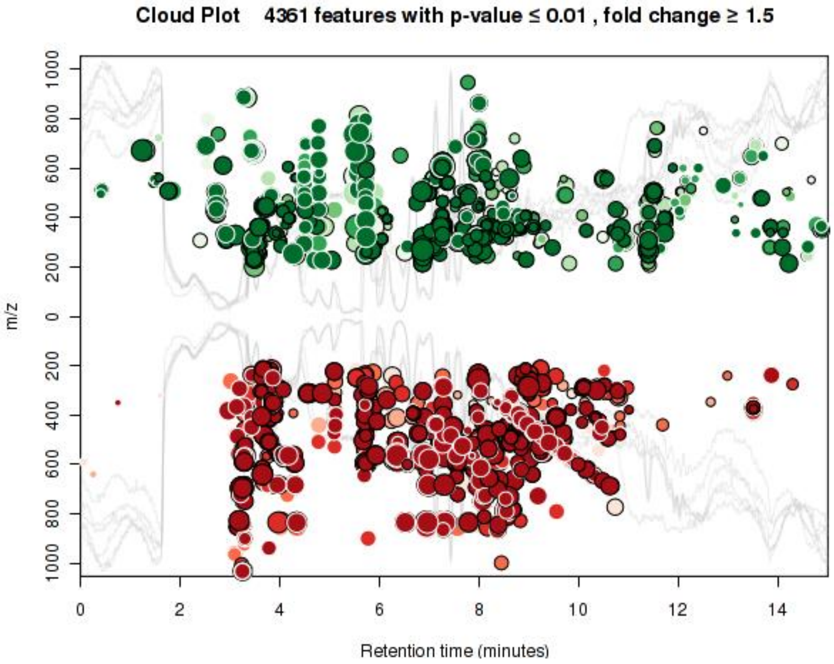


Figure 27. Cloud Plot, 4361 Features with p-value ≤ 0.01, Fold Change ≥ 1.5 (19)

Discussion and Conclusion

Methanol was used as the extraction solvent for the LC-MS experiments conducted in this work, and this choice of solvent was supported by an experiment completed at Umeå University in Sweden entitled “Extraction and GC/MS Analysis of the Human Blood Plasma Metabolome.” The experiment proposed that analysis of the human blood plasma metabolome by gas chromatography-mass spectroscopy (GC/MS) could provide deeper insights into disease mechanisms and diagnosis markers (19). In order to accomplish the experiment, the performance of five organic solvents (methanol, ethanol, acetonitrile, acetone, chloroform), singly and in combination, was investigated to optimize the metabolome extraction. A Partial Least Squares (PLS) analysis revealed that methanol extraction was very efficient and reproducible, which suggested that methanol alone was the best of the tested solvents to use for the extraction of metabolome from blood plasma (19). Because methanol is a good extractor, it was used to extract the compounds that were present in the post-experimental material samples of this thesis experiment so that the samples could be analyzed by LC-MS analysis.

According to the survey results from Table 3, most students (58%) wore polyester into lab, then cotton (22%), then finally leather (16%). According to the ANOVA tests Figures 9 through 11 and Figures 12 through 14, there was no statistically significant difference ($p < 0.05$) in mass change between the control samples and the acid concentrations for any of the fabrics treated with HCl or between the control samples and the base concentrations for any of the fabrics treated with NaOH. There also was no statistically significant difference found in mass changes between the various concentrations of acid treatment and base treatment (0.1, 0.5, 1.0, and 2.0M), but there were however, several interesting discoveries. Curiously, the largest documented weight change in the leather was in the 0.1M NaOH treated group, and additionally, the 2.0M NaOH treated samples experienced a dramatic color and texture change, but showed little variation in weight between treated and untreated samples. Also, when HCl treated samples were compared to NaOH treated samples, a dramatic trend was noticed in the numerical weight changes; however, only data in the cotton sample groups showed statistically significant difference. According to the XCMS © data in Figure 26, the acid treated leather samples appeared to have several peaks that were higher than the base-treated samples, possibly indicating that these components were destroyed as a result of the NaOH exposure (20). However, the cloud plot, Figure 27, indicated an abundance of difference between the acid- and base treated samples, with over 4000 detectable ions with a statistically significant difference (p-value ≤ 0.01) (19). It seemed very odd that the highest molarity (2.0M) of the NaOH treated leather experienced the dramatic color and texture change, but did not experience the greatest weight change or have the highest peaks in the XCMS © data (20). According to literary sources, cotton is resistant to alkalis, such as sodium hydroxide (12), but the cotton samples in the thesis experiment indicated a greater weight change from NaOH treatment compared to HCl treatment. According to Figure 18, the NaOH-treated samples showed a greater change in weight (P-value = 0.0282) than the HCl samples, but no such difference could be detected for the polyester and leather samples (Figures 19 and 20), with P-values of 0.1733 and 0.1801, respectively. Literary evidence that glycerol naturally occurs in cotton fibers (13), was supported by closer examination of the large peak of the base treated chromatograms of cotton (Figure 23). The peak revealed a mixture of hydrocarbon derived glycerol molecules (retention time 4.90) (Figure 24). According to literature, polyester is resistant to most substances, including acids, but deteriorates in strong alkalis such as concentrated sodium hydroxide (14). However, the experimental results demonstrated that polyester withstood the NaOH and HCl solutions better than the leather or cotton materials, which may have been due to a concentration effect (Figure 19). The higher concentrations of solutions did more damage to the leather and cotton materials than they did to the polyester material. Also, polyester’s weight did not change significantly. According to the experiment, several peptides were liberated from the leather in the HCl solutions (Figure 27), but the leather exposed to the NaOH solutions experienced the most color and texture change (Figure 6). **We hypothesized, that because of its thin fibers and synthetic nature, the polyester would break down more in the hydrochloric acid and the sodium hydroxide, while cotton, with thicker fibers, would break down more slowly and partially, being more vulnerable to acidic solutions, based on our literature review, and lastly, the leather, made of animal hide, would not break down much at all due to its thickness and durability.** After the experiment, the hypothesis remained true only for the cotton material. The cotton material partially broke down in the sodium hydroxide solution becoming frilled along its edges (Figure 7), but the polyester withstood the sodium hydroxide and hydrochloric acid better than the leather or the cotton, which was not expected (Figure 8). The leather structurally changed the most, changing texture, color, and weight in the sodium hydroxide, and liberating several peptides in the hydrochloric acid (Figure 6 and Figure 27).

This study has several limitations toward generalizability of the results for the laboratory footwear. In order to strengthen the results of this experiment, a stretch test would be beneficial. One could stretch the control materials and the treated materials to the breaking point and measure how much force was required to break each of the materials exposed to different solutions at different concentrations. The materials that disintegrated the most in the solutions should be the materials that break apart the easiest under force. Conducting such a test would be difficult, however, especially because the leather became so rigid during the chemical treatment. Also, the range of concentrations tested in this study is narrow, and higher concentrations of NaOH and HCl are likely to have more pronounced effects on the fabrics. NaOH and HCl, while common, do not represent the full scope of acids and bases used in undergraduate chemistry labs. Also, the survey could have given broader and more accurate results if more participants took the survey and completed it. Giving incentives for survey completion can increase response rate, but doing so was outside the scope of this project. Finally, we could have cut out pieces of material from actual shoes and test their durability, but this method would have been more expensive, and more difficult to standardize the samples.

The XCMS © software is a powerful tool for overlaying chromatographic and mass spectral data in order to highlight differences between sample groups, but the bulk of the individual ions lacked sufficient data for definitive identification (20). However, if a small subset of ions were identified from this initial screening as important, additional mass spectrometric experiments could help clarify compound identity. An additional limitation of this experiment was the use of a single extraction solvent (methanol), which may not have captured the full range of the polarities of the ions present in the samples.

In conclusion, according to the ANOVA tests, exposure to the different concentrations did not appear to negatively affect the structural integrity of the fabrics, and the NaOH caused more weight change across the materials, but, with the exception of cotton, was not significant. According to the mass spectrometry results, the three fabrics differed in individual complexities as well as in the compounds extracted following acid and base treatments. The polyester fabric yielded very few overall ions, and there was little difference between the acid and base-treated samples. The cotton had more statistically significant features in the base-treated samples, with a possibility that many of these differences were related to a derived glycerol. Finally, the leather yielded an abundance of ions when it was analyzed by LC-MS, implicating several small peptides as the underlying differences between acid- and base-treated samples.

References

(1) Sieloff, A. C.; Shendell, D. G.; Marshall, E. G.; Ohman-Strickland, P. *J. Chem. Health Saf.* **2013**, 20, 17-26.

(2) Collins, D. *Investigating Chemistry in the Laboratory,* 2nd ed.; W. H. Freeman and Company: New York, 2009.

(3) Environmental Health and Safety. http://ehs.ucsc.edu, University of California Santa Cruz, 2012, (accessed Nov. 30, 2013).

(4) Environmental Health and Safety. http://web.princeton.edu/sites/ehs/labsafetymanual/sec6c.htm, Princeton University, 2011, (accessed Nov. 30, 2013).

(5) Occupational Safety and Health Administration. http://www.OSHA.gov, United States Department of Labor, 2009, (accessed Nov. 30, 2013).

(6) Young, J. A. *Safety in Academic Chemistry Laboratories*, 7th ed. American Chemical Society: Washington D.C., 2003; Vol. 1.

(7) Mandatory Lab Safety Rules. http://healthsafety.etsu.edu/static/chemistry, East Tennessee State University Office of Environmental Health and Safety, (accessed Nov. 30, 2013).

(8) Deal Settles Prosecution of UCLA. *Associated Press,* Jun. 2014, http://www.foxnews.com/us/2014/06/20/deal-settles-prosecution-ucla-chemistry-professor-in-lab-fire-death-researcher/, (accessed Aug. 19, 2014).

(9) Rogers, L. Six Sent To Hospital After Nitric Acid Spill. *Wibw*, Jun. 2012, http://www.wibw.com/home/localnews/headlines/Six_Sent_To_Hospital_After Nitric_Acid_Spill_In_K-State_Lab_156476915.html, (accessed Aug. 19, 2014).

(10) Hellmann, M.A.; Savage E.P.; Keefe, T.J. *J. Chem. Educ*. **1986**, 63, A267.

(11) Natural and Synthetic Fibers. *SWICOFIL AG Textile Services*, Sept. 2008, http://www.swicofil.com/products/001cotton.html#Properties, (accessed Jan. 12, 2014).

(12) Chemical & Physical Resistance Data for Fibres. *Lenntech,* http://www.lenntech.com/feedback/feedback_uk.htm?ref_title=Visitors%20information, (accessed Jan. 12, 2014).

(13) Moire, L.; Schmutz, A.; Buchala, A.; Yan B.; Stark R. E.; Ryser U. *Plant Physio*. **1999**, 119, 1137–1146.

(14) Solvent and Chemical Resistance Information. *Carlisle,* rfelektronik.se/manuals/Datasheets/solventguide.pdf, (accessed Jan. 12, 2015).

(15) Mann, B. R.; McMillan, M.M. The Chemistry of the Leather Industry. chemicalprocessesleather.pdf, (accessed Jan. 20, 2015).

(16) Hydrochloric Acid. *Iarc Monograhs,* 54, 189-211. http://monographs.iarc.fr, (accessed Jan. 20, 2015).

(17) Material Safety Data Sheet. *HIP-Petrohemija Pančevo,* **2014**, 4, 1-9.

(18) *Basics of LC-MS Primer;* Agilent Technologies, 2001.

(19) Jiye, A.; Trygg, J.; Gullberg, J.; Johansson, A. I.; Jonsson, P.; Antti, H.; Marklund, S. L.; Moritz T. *Anal. Chem*. 2005, 77, 8086-8094.

(20) *XCMS Online*, version 2.01.05; Scripps: La Jolla, CA, 2015; (accessed Jan. 20, 2015).