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# PURSUE

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**UNDERGRADUATE RESEARCH JOURNAL**



## Aim and Scope

The scholarly journal, “PURSUE”, provides undergraduates an avenue to publish their original research articles in the following areas: (but not limited to) psychology, sociology, biology, chemistry, physics, engineering, computer science, mathematics, humanities, agriculture, architecture, business, and education.

The original research articles included in this journal are peer-reviewed and selected by the journal’s Editorial Board. The review process allows undergraduate researchers to receive feedback from notable scientists in their field of study and teach them about the publication process. Publishing their work will not only inform the scientific community but impact the greater society.

The journal is housed at Prairie View A&M University, a Historically Black University, and is available to all young scientists conducting research. This journal also serves as a means for faculty to extend knowledge beyond the classroom and encourage other students to conduct quality research. All undergraduate research is produced in conjunction with a faculty mentor and is peer reviewed. The journal is open to undergraduates from all Colleges and Universities.

# **PURSUE**

## **Undergraduate Research Journal**

### Contents

#### **Editors' Notes**

Felecia M. Nave, Ph.D.

#### **05 An Analysis of the 2015 Nigerian Presidential Election**

Uduak-Obong I. Ekanem, Ole J. Forsberg

#### **21 Reflection, Calibration and Achievement In Introductory Calculus**

Taylor Kline and Rebecca Dibbs

#### **37 Outcomes of Advancing Women Faculty in Engineering and Technology at Historically Black Colleges and Universities: A Retrospective Analysis of ADVANCE-PAID Participants**

Brittani Turner, Angel Lyles-Grayer, Rochelle L. Williams, and Felecia M. Nave

#### **50 The Effects of a Selective and Non-Selective Organic Herbicides on Amaranthus speciesi**

Chonique S. Long, Nayel N. Novelo, Hanna Derecho and Yolander Youngblood

#### **63 Evaluating alternative crosslinking agents in poly(vinyl alcohol) hydrogels membranes**

Naomi Deneke, Sarah Dohadwala, Quincy Moore, Felecia Nave, Audie Thompson

#### **82 About the Authors**

#### **Acknowledgements**

Inside Back Cover

#### **Editorial Board**



May 2018

# FROM THE Executive Editor

Dear Readers,

I am pleased to share with you the 2nd edition of the scholarly journal PURSUE. This journal provides student research scientists with an avenue to publish their original research. From psychology, sociology, and humanities to agriculture, biology, chemistry, engineering and beyond, this journal is designed to contribute to the broader knowledge research and impact the greater society.

I congratulate our undergraduate writers who contributed to this edition of PURSUE, and I express my sincere gratitude to the faculty mentors who supported these students in their research journeys. As educators, it is our duty to give students opportunities to showcase their knowledge, and this journal does just that, by extending their comprehension beyond the classroom and out into the world.

It is my wish that through PURSUE, each reader will be inspired to undertake a lifelong journey of exploration and discovery. Enjoy it to the fullest.

Felecia M. Nave  
Executive Editor, PURSUE  
Director, Faculty Development Initiative  
Professor, Chemical Engineering  
Prairie View A&M University





# An Analysis of the 2015 Nigerian Presidential Election

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## Abstract

Much time has gone into analyzing the 2015 Nigerian Presidential election, a veritable repeat of the 2011 election between incumbent Goodluck Jonathan and retired General Muhammadu Buhari. Previous elections in Nigeria have been fraught with violence and charges of electoral fraud. While the Nigerian electoral commission worked hard to ensure that these elections were fair, violence and charges of fraud materialized.

Electoral forensics applies statistical techniques to elections, frequently testing for evidence of fraud or of unfairness. Using binomial regression, we tested the official results from the 2015 Nigerian Presidential election for evidence of differential invalidation. Differential invalidation involves invalidating ballots based on whom they are cast.

The results do not strictly indicate evidence of this type of electoral unfairness. The marginal p-values (0.1420 and 0.0346) only suggest that there may be a problem. Furthermore, the invalidation process in Ebonyi state appears to be completely different from that in other states. This leads one to wonder why that difference exists.

**Keywords:** Nigeria, Electoral Forensics, Elections, Binomial Regression

## Introduction

Democratic elections are becoming an international norm. Governments gain legitimacy from such elections as they are receiving their power directly from the people. However, not all elections are democratic, nor are all elections that claim to be democratic actually democratic. In many countries, election unfairness is the rule, violence on election day is frequent, and claims of fraud are numerous (Goodwin-Gill, 2006).

To combat these problems—or to detect them—organizations monitor the election as it happens. Such groups include the Organization for Security and Cooperation in Europe (OSCE), the African Union, and the National Democratic Institute (NDI). The organizations can either send foreign monitors to the state holding the election or empower locals to do this.

However, as Josef Stain apparently stated, “It’s not the people who vote that count. It’s the people who count the votes (Bazhanov, 2002).” More is needed than just watching that people are allowed to freely vote. Ensuring that the votes are equitably counted is also required for the election to be fair. Thus, in addition to election monitoring, there needs to be an analysis of the election outcomes designed to detect unfairness in the counting of the ballots. The field of electoral forensics applies statistical methods to election research questions to evaluate evidence of fairness. Regression is one of the most powerful tools to detect differential invalidation; meaning invalidation rates of ballots can be analyzed by subgroup to identify differences. These subgroups could be based on ethnicity, disability, or voting preference. Because of the structure of the data collected in this study, binomial regression is used to detect differential invalidation. Binomial regression is a statistical technique in which the response variable is the sum of “successes” (invalidated votes) over a given number of trials (total votes cast).

## Nigerian Background

Nigeria gained its independence from the United Kingdom on October 1, 1960, and transitioned to its first republic three years later. For the three-year life of the First Republic, Sir Abubakar

Tafawa Balewa was the Prime Minister and Nnamdi Azikiwe was the President. The coup d'état of 1963, which ended the First Republic, became the leitmotif of Nigeria. Between independence and 1999, power alternated between the military and the people, between autocracy and democracy. However, the last military government of General Abdulsalami Abubakar in 1999 ensured the effective transition to a democratic government. This government marked the beginning of a government of the people selected through an electoral voting process conducted by the Independent National Electoral Commission (Suberu, 2007).

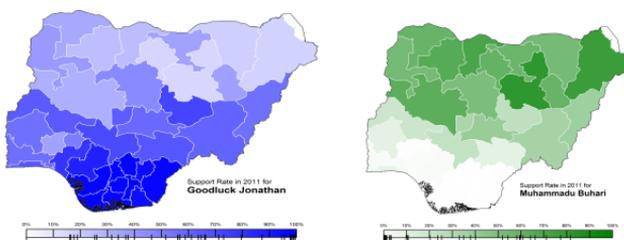
Nigeria conducts political elections every four years. The elections are held in three levels: National Assembly elections (federal legislature), Presidential elections (federal executive), and gubernatorial and state assembly elections (state executive and legislature respectively).

The President is elected directly by voters to a four-year term, with a two-term limit (Tar & Zack-Williams, 2007). While the electoral system is a single-member plurality system, there are adjustments made to ensure that the President has support throughout Nigeria. According to Article 134(2) of the Nigerian Constitution, a candidate can win the presidential election only if they win a majority of the votes cast and “not less than one-quarter of the votes cast at the election each of at least two-thirds of all the States in the Federation and the Federal Capital Territory, Abuja.” This requirement ensures that the President has some support in both the North and the South of the country, which helps to avoid another civil war in Nigeria like the Biafra war of 1967–1970.

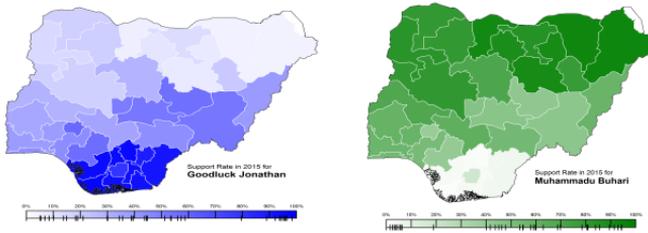
Since the beginning of the Fourth Republic in 1999, the Presidency has been held by the People's Democratic Party (PDP). As such, it is the most influential party in the country (Tar & Zack-Williams, 2007). The most recent election of 2015 came down to two candidates: the incumbent President Goodluck Jonathan, a southerner and flag bearer of the PDP, and retired General Muhammadu Buhari, a northerner and flag bearer of the All Progressive Congress (APC) party. The APC Party was formed from

the merger of four opposition parties and some members of the PDP who had defected to the APC party (Lewis & Kew, 2015).

The 2015 election was special for many reasons. First, it was a repeat battle between the incumbent President and the retired General. The 2011 Presidential election, which pitted these candidates against each other saw Goodluck Jonathan win, 59% to 32% (Animashaun, 2015). Second, this election was a test of the incumbent President's ability to retain the support of the people who had lost patience with government graft scandals, high unemployment, and the Boko Haram insurgency in the north-eastern part of the country ("How Nigeria's presidential election works," 2015). These issues had arisen during the administration of the incumbent President and formed the basis of General Buhari's campaign and slogan — "Change" ("How Nigeria's presidential election works," 2015). Finally, the candidates were from two different parts of the country: Jonathan is from the south and Buhari is from the north. As such, there is an expected level of support from their native regions. For example, the states in the south, east, and parts of the west tend to support a southern candidate, while the northern states tend to support a northerner. Figures 1 and 2 illustrate the distribution of votes in the 2011 and 2015 elections between the candidates across Nigeria. In the midst of this, however, are swing votes coming from highly-populated states.



**Figure 1.** Support levels for Jonathan and Buhari in the 2011 Nigerian Presidential election. Maps created by authors from data available from the Center for Electoral Forensics (*Election dataset for Nigeria*, 2017).



**Figure 2.** Support levels for Jonathan and Buhari in the 2015 Nigerian Presidential election. Maps created by authors from data available from Independent National Electoral Commission (2015 Presidential Election, 2015).

For these reasons, various news stations predicted the Presidency would be won by Buhari. However, there has been long-standing concerns about the vote rigging, violence, and electoral fraud that has characterized Nigeria's elections (Lewis & Kew, 2015). For example, in the 2011 election, the NDI heavily criticized the elections (*Final Report on the 2011 Nigerian General Elections*, 2012). Apart from marked violence, the NDI report cited irregularities, including underage voting, vote buying, stealing of ballot boxes, and lack of secrecy in voting. Due in part to this report, INEC worked hard to enact various measures to curb the concerns on elections in the future. INEC spent more than \$40,000,000 on ensuring that the elections would be free and fair (Whitehead & Saater, 2015). As a result of these changes, the INEC Chairman, Attahiru Jega, stated, "the [2015] elections were reasonably free and fair", and he "attributed the success of the elections to sacrifices made by INEC officials" (Adibe, 2015).

Even with this praise of the 2015 elections, election-day violence was present. Boko Haram, a known terrorist organization, attacked several voting centers in the North, killing at least 39 (Whitehead & Saater, 2015). Given the long-standing reputation of Nigeria's electoral politics, additional doubts remain on the credibility of the election. The goal of this study is to analyze this presidential election for evidence that the INEC fell short of its goal.

### **Analysis Background**

While the rules and regulations for elections differ from country to country, several similarities exist across those countries claiming to be democratic—primarily, that all adults are free to vote for whomever they decide, the candidates and parties have equal access to the media, and each person’s vote counts the same, regardless of characteristics of that person or of that vote (Omotola, 2010). Of these expectations, the first two measure the level of freedom in the election and the last measures its fairness. In this sense, an election is fair if each vote has the same probability of being counted, independent of candidates, party, or demographics. During the counting process, a vote considered valid by the appropriate election authority is counted, while one that is invalidated is not. Should one group of people have a significantly higher invalidation rate than others, then the election could be biased.

For instance, if ballots cast by blind people have a higher probability of being rejected, the election may be biased against blind people. Similarly, if ballots cast in favor of a specific candidate have a higher invalidation rate than those cast for the opponent, then the election may be unfair against the supporters of the first candidate. However, if ballots are systematically invalidated according to candidate choice, then there will be a relationship between the proportion of ballots invalidated and the proportion of ballots cast for that candidate. If that relationship is negative, then the invalidation helped the candidate (fewer invalidated ballots in the states supporting the candidate). If the relationship is positive, then the invalidation helped the opponent.

With regression, the relationship between the invalidation rate and candidate support can be examined to test for such a correlation. Should a statistically significant dependence between the two variables be detected, then there would be evidence of differential invalidation—unfairness—in that election.

## Materials and Methods

### Election Data

Unfortunately, as we are not privy to the actual ballots and the invalidation decision for each, we must rely on publically available aggregated vote counts. Official election results were obtained directly from the Nigerian electoral commission (INEC) website. For the 2015 Nigerian election, at the state level, the number of cast ballots, invalidated ballots, and ballots cast for each candidate are provided (*2015 Presidential Election*, 2015). Thus, we worked not with individual ballots, but with vote counts and proportions.

### Regression Methods

Regression is a set of methods that can be used to test independence between numeric variables. While Ordinary Least Squares (OLS) is a traditional way to estimate that dependence; other methods can take advantage of the inherent structure of the data to better leverage the information. The dependent variable is a count (votes declared invalid) with the number of trials known (votes cast in the state). Thus, the dependent variable is a random variable distributed as something akin to the binomial distribution.

All calculations were performed using the R statistical environment, v 3.3.1 ("The R Project for Statistical Computing," 2016). Mapping was done using R and shapefiles from GADM (Hijmans et al., 2015). R packages used are lawstat (Gastwirth et al., 2017) and sp (Pebesma & Bivand, 2005).

There are four requirements for a random variable to follow a binomial distribution. One limitation of this study was that only three of the requirements are guaranteed to be met by the election data available; those include 1) known number of trials (number of votes cast), 2) each trial results in one of two outcomes (invalidated ballot or not), and 3) a constant success rate (under the null hypothesis, the invalidation rate is independent of the candidate support rate).

The final requirement is that the votes are independent. Extant research suggests that this assumption is frequently violated with election data (Lesthaeghe & Neidert, 2009; Mebane & Sekhon, 2004). The effect of violating this requirement, however, is that the assumed dispersion is greater than unity.

Recall that the binomial distribution is a member of the (one-parameter) exponential class, because it can be written in that general form (Bickel & Doksum, 2007; McCullagh & Nelder, 1989):

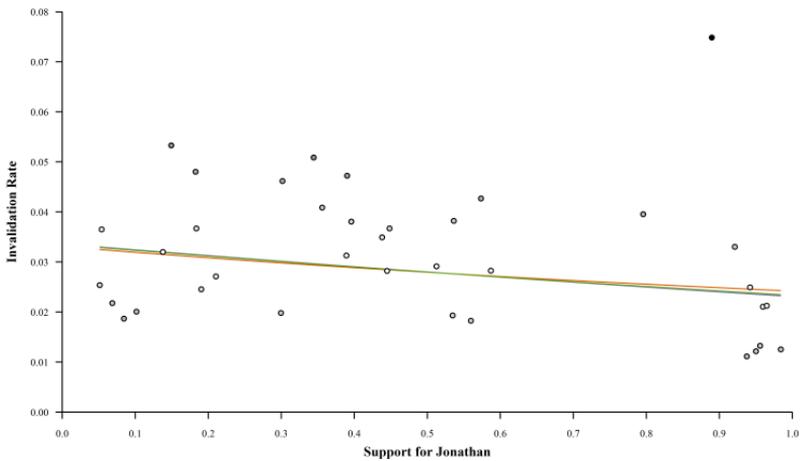
$$\begin{aligned} f(y; \phi) &= \exp \left[ \frac{x\phi - b(\phi)}{a(\phi)} + c(y, \phi) \right] \\ &= \exp \left[ \frac{x \logit(\pi) - \log(1 - \pi)}{1} + 0 \right] \end{aligned}$$

For binomial data,  $a(\phi)=1$ . For overdispersed binomial data,  $a(\phi)>1$ .

From a practical standpoint, this difference only requires that the model be fit using maximum *quasi*-likelihood estimation (Wedderburn, 1974) instead of the maximum likelihood estimation initially proposed by McCullagh and Wedderburn (1972).

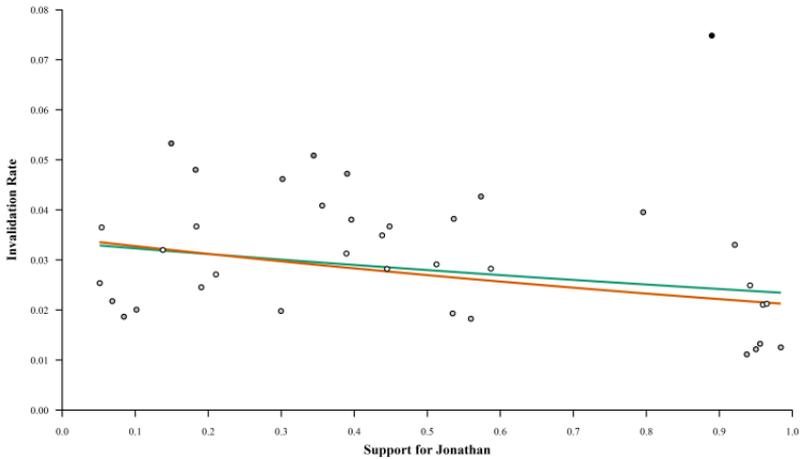
## Results

The canonical link for the binomial distribution is the logit function. However, to increase the veracity of the model, we used five link functions and checked that the predictions were similar. Those five functions are the logit, probit, cauchit, log-log, and complementary log-log. Figure 3 is a scatter plot of the data with the five prediction curves provided. Note that the five models make essentially the same predictions. The complementary outcomes strongly support the contention that the models are appropriate for this data.



**Figure 3.** Prediction curves for the five models with the observed invalidation rates and level of support for Jonathan. The outlier (marked by a solid black dot in the upper right) is Ebonyi state.

Note that in each of the models, the Ebonyi state is an outlier. Reading through the election-day reports from Ebonyi did not turn up any explanation for its high invalidation rate. Because of lack of data (Nigeria apparently did not measure invalidations at the state level in 2011), we are unable to determine if this is a feature of Ebonyi or if it is random noise. Frequently, outliers have little effect on determinations of statistical significance and such is the case here. The substantive conclusions are unchanged whether Ebonyi is included or not.



**Figure 4.** Prediction curves for the logit models. The top line (green) is the model including Ebonyi state. The bottom line (brown) excludes it.

As all link functions produced the same conclusions with respect to evidence of electoral unfairness, the following results are based on the model using the logit link (Figure 4). Recall that the null hypothesis is that there is no statistical relationship between the invalidation rate and the candidate support rate. Should systematic problems exist in either the electoral system or the particular election, such that ballots are invalidated in large part based on who they were cast for, then the null hypothesis is not true.

**Table 1:** Regression tables for the logit model with and without including Ebonyi state. Note that excluding it allows us to conclude a statistically significant relationship between invalidation rate and candidate support rate; excluding it does not.

|                           |                             | Estimate | Std Err | t-value | p-value   |
|---------------------------|-----------------------------|----------|---------|---------|-----------|
| <i>With<br/>Ebonyi</i>    | <b>Constant</b>             | -3.3615  | 0.1281  | -26.242 | << 0.0001 |
|                           | <b>Jonathan<br/>Support</b> | -0.3725  | 0.2477  | -1.504  | 0.1420    |
| <i>Without<br/>Ebonyi</i> | <b>Constant</b>             | -3.3343  | 0.1139  | -29.287 | << 0.0001 |
|                           | <b>Jonathan<br/>Support</b> | -0.5017  | 0.2279  | -2.201  | 0.0346    |

Because the p-value (0.1420) of the model that includes Ebonyi state is greater than the usual  $\alpha = 0.05$ , we cannot reject the null hypothesis (Table 1). That is, significant evidence of differential invalidation does not exist here. Note, that the model, which excludes Ebonyi state, *does* suggest differential invalidation (p-value = 0.0346). Because of the number of models examined, however, a Bonferroni adjustment suggests that this low p-value may be a result of the inherent downward bias of p-values calculated for multiple tests (Westfall, Johnson, & Utts, 1997). As such, we are not prepared to conclude that there is evidence of systematic differential invalidation based on this data and this model.

## Discussion and Conclusions

Nigeria's elections typically involve irregularities ranging from vote buying to ballot box stuffing (*Final Report on the 2011 Nigerian General Elections*, 2012). Through actions, such as improved security, put in place by the INEC, the 2015 elections were called "free and fair" by the chairman of the INEC (Adibe, 2015). However, because of the history of elections in Nigeria, doubts remain about the fairness of this election. We tested the 2015 election for unfairness using regression tests of the invalidation rate against the candidate support level, testing for invalidation as a function of support for Goodluck Jonathan.

The INEC website provided us with sufficient data. The proportion of invalidated votes and level of candidate support were calculated for each state. The invalidation rates ranged from a low of 1.17% (Akwa-Ibom) to a high of 7.49% (Ebonyi). In Akwa-Ibom, Jonathan won with 93.7% of the vote; Jonathan was a native of this region and winning this state was no surprise. The highest invalidation rate was in Ebonyi state, also won by Jonathan with 88.9%. Ebonyi state is governed by candidates from Jonathan's PDP party; thus, the results in that state are also not surprising.

We used binomial regression, a type of generalized linear model, to estimate the relationship between the invalidation rate and the support for Jonathan in each of Nigeria's 36 states and the Federal Capital Territory. The model suggests that the invalidation rate in Ebonyi state is significantly higher than in other states that Jonathan won. However, the model including Ebonyi and the model excluding it produced the same substantive conclusion. The election results do not give significant evidence of differential invalidation.

#### *Limitations of this Research*

The results of this study do not indicate a statistical relationship between the independent and dependent variable for the incumbent candidate. This, of course, does not prove that the election was fair. Statistical techniques rely on the data for their power. For this set of data, the sample size is rather small at 37. Thus, had we election results at the local government area (LGA) level, of which there are 774, our tests would be much more powerful for detecting differential invalidation.

Second, this test only examined one aspect of fairness in the election, that of counting—or not counting—the ballots. There are other aspects of fairness, such as the nine listed by Goodwin-Gill that “guarantee universal and equal suffrage to adult citizens” to “ensure that candidates who obtain the necessary number of votes required by law are duly installed in office” (Goodwin-Gill, 2006). Those nine are, and we quote,

- *hold elections at reasonable intervals, as established by law;*

- *permit all seats in at least one chamber of the national legislature to be freely contested in a popular vote;*
- *guarantee universal and equal suffrage to adult citizens;*
- *ensure that votes are cast by secret ballot or by equivalent free voting procedure, and that they are counted and reported honestly with the official results made public;*
- *respect the right of citizens to seek political or public office, individually or as representatives of political parties or organizations, without discrimination;*
- *respect the right of individuals and groups to establish, in full freedom, their own political parties or other political organizations and provide such political parties or other organizations with the necessary legal guarantees to enable them to compete with each other on a basis of equal treatment before the law and by the authorities;*
- *ensure that law and public policy work to permit political campaigning to be conducted in a fair and free atmosphere in which neither administrative action, violence nor intimidation bars the parties and the candidates from freely presenting their views and qualifications, or prevents the voters from learning and discussing them or from casting their vote free of fear of retribution;*
- *provide that no legal or administrative obstacle stands in the way of unimpeded access to the media on a non-discriminatory basis for all political groupings and individuals wishing to participate in the electoral process;*
- *ensure that candidates who obtain the necessary number of votes required by law are duly installed in office and are permitted to remain in office until their term expires or is otherwise brought to any end in a manner that is regulated by law in conformity with democratic parliamentary and constitutional procedures (Goodwin-Gill, 2006).*

The statistical methods of this paper arguably test none of these. Thus, the election could have been unfair based on these other requirements of fairness.

Despite these two limitations, electoral forensics was contributed by this study and the results serve as an excellent complement to election observing. Whereas election observers can only declare unfairness if they witness it firsthand, electoral forensics can be used to objectively detect it from afar. In this election, however, we did not detect a significant level of differential invalidation.

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# Reflection, Calibration and Achievement In Introductory Calculus

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## Abstract

Studies indicate that calculus acts as a filter for students entering into a STEM discipline, pushing some students to leave their field of study due to failure or disheartenment after going through the course. Since the demand for STEM-trained graduates grows, it is imperative to determine how to prevent more students from switching away from their STEM disciplines. The purpose of this project was to investigate whether the concept of calibration could increase student performance in introductory calculus in order to retain as many students as possible. This was a quasi-experimental study, conducted in calculus I and II courses, imploring CLEAR calculus, which is a nontraditional class style. Results indicated that in calculus I, with data from the first four classroom exams, the calibration could predict performance on the final exam. With calculus II, however, GPA was the only contributing factor to the final exam score. This implies that calibration may be more important in the first course, though further investigation is needed.

**Keywords:** achievement, ANOVA, calculus, calibration, undergraduate

## Introduction

Proteins are very important for the wellness of humans and are the The large numbers of students who switch away from their STEM major during or after taking introductory calculus is a trend that many mathematics researchers are trying to fix. Solving this current issue is important because throughout the United States, and even globally, there is a shortage in the number of workers in the STEM

disciplines (Ellis, Fosdick, & Rasmussen, 2016). One curriculum that shows promise is CLEAR Calculus, an instruction processes developing formalization of calculus concepts that can then be applied to any challenging problem (Oehrtmann, 2008); the course's lab work combined with formative assessment show promise in retaining students; however, we do not understand why this structure helps students stay in STEM past the first semester of calculus (Patterson, 2016). One possible way CLEAR calculus may help students is to improve students' calibration in assessments within the class, which makes students more likely to seek early help. Calibration is based on how accurately a person's self-efficacy matches their execution in a subject area, or what a person expects to achieve versus how they do in terms of a grade (Lin & Zabucky, 1998).

Studying student's calibration was important because of the potential it has to be translated into other classroom settings. Even if the CLEAR Calculus structure was not being used in the curriculum, the calibration aspect could still apply. A quasi-experimental study was designed to test the null hypothesis:

$H_0$ : There is no relationship between student calibration and performance in introductory calculus.

## **Literature Review**

After reviewing the literature, there were five relevant themes to this research project. They were: why calculus matters, retaining STEM intending students, minority student switchers, curriculum aspects and CLEAR calculus, and calibration. These themes are discussed in order in this section.

### **Why Calculus Matters**

Calculus is the gatekeeper class for STEM majors throughout the world, and students' beliefs that they cannot learn this material is a major obstacle to addressing the shortage of STEM workers (Chiu & Klassen, 2010). It is especially important to teach calculus

effectively to STEM majors not intending to enter mathematics, such as those entering fields of engineering, physics, and STEM education, since the non-mathematics STEM majors are the overwhelming majority in college mathematics courses and the needs of the other STEM departments must be met for all of undergraduate STEM programs to be effective (Rasmussen, Marrongelle, & Borba, 2014). However, there has been little research on how non-traditional calculus curriculums and pedagogy helps these groups of students (Ellis, Kelton, & Rasmussen, 2014), and more work is needed in linking calculus research to theoretical results (Rasmussen et al., 2014).

### **Retaining STEM Intending Students**

Due to the rising shortage of STEM workers globally, researchers are examining the trends for students who remain in their STEM field versus those who switch away from STEM major. One such trend is that calculus tends to turn people away from their originally intended discipline, and this has remained especially true for women (Ellis et al., 2016). Those students who do switch away from calculus have reported that a failed connection between student and teacher is to blame, and, as a result, the student is less likely to seek help on challenging topics (Patterson, 2016). Within the classroom, structured lesson plans that included presenting specific example problems throughout the teaching of new material, having extra material for students to use for practice, holding whole class discussions about topics, asking for students to explain processes and thinking behind problem completion on tests and exams, and lecture-styled instruction helped to contributed to success of retaining STEM intending students (Ellis et al., 2014).

### **Minority Student Switchers**

One goal researchers, employers and educators have is to more thoroughly diversify the STEM career field. The shortage of women is likely due to a lack of confidence rather than a lack of ability, and it is believed that by increasing the number of females who stay in their STEM discipline, the number of women in the workforce would nearly double (Ellis et al., 2016). Certain trends among minority students, including the levels of motivation, preparation

before entering college with class difficulty and standardized testing, levels of support at home and from the family, and financial support, are believed to be contributing factors to student failure in mathematics (Lane, 2016).

### **Curriculum Aspects and CLEAR Calculus**

There are many discussions and arguments on how a calculus course should be structured and taught. One key dispute is the difference in content at the secondary level versus that at the collegiate level, as well as how much focus should be placed on different concepts to benefit other STEM fields (Rasmussen et al., 2014). Other issues include the lack of consistently challenging example problems, the progressive nature of the subject, and the translation of the problem-solving skills learned in calculus into other disciplines (Treisman, 1992). A common theme expressed with difficulties in subject matter comes back to the conceptual understanding of limits in calculus, which is a key, basic topic throughout the course (Oehrtmann, 2008). CLEAR Calculus is a suggested curriculum structure to help to solidify understanding within introductory calculus. This structure allows for students to develop a framework of procedures and steps to transfer to all calculus concepts, creating recognition of identifiable similarities throughout the curriculum of the course (Oehrtmann, 2008). The process includes labs and formative assessment to gauge where students are in understanding the material so that the instructor is aware of what needs to be clarified (Patterson, 2016). It has been found that students who do participate in the formative assessments tend to benefit more and score higher overall (Dibbs, 2015).

### **Calibration**

Self-efficacy and the term calibration go hand-in-hand in educational performance. A student's personal measure of self-efficacy can determine how much effort and time each student will put into their academic achievement (Schunk & Pajares, 2009). Calibration is a metacognitive skill, and it is based on how accurately a student's self-efficacy matches their execution in a subject area, or what they expected to achieve versus how they actually scored (Lin & Zabrocky, 1998). A person with a more positive sense of calibration

tends to express more determination and accomplishment, whereas the opposite tends to be true for those with negative calibration assessments (Chiu & Klassen, 2010).

Although calibration is an important skill for students to master, it is rarely formally taught in mathematics classrooms. However, teachers can help students improve their calibration in several ways. Students should receive feedback on their work to evaluate their personal calibration, in order to ensure that students are learning as much as they possibly can (Lin & Zabrucky, 1998). Students assess their calibration based on their experiences, or lack of experience (Schunk & Pajares, 2009). A student's overconfidence could potentially hinder his or her progress with subject material because they will have an incorrect grasp on key concepts and as a result, will not seek help or tutoring for troubling topics (Champion, 2009). Underestimation of calibration could be equally as destructive to one's achievement because a student may not be motivated to strive to their fullest potential (Chiu & Klassen, 2010).

## **Methods**

The purpose of this study was to investigate the relationship between calibration and final exam performance within a calculus course with the CLEAR Calculus structure.

### **Study Design**

This was a quasi-experimental study. The topic for this study was how a student's sense of calibration could relate to their achieved final exam score. This could not be a true experimental study because students were not randomly assigned to the class. Also, it was unethical to withhold an educational treatment that was more effective in order to have a control.

### **Study Population & Sample**

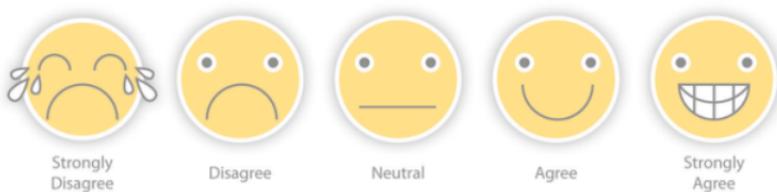
The sample population used in this study consisted of students enrolled in the Fall 2016 calculus I and II courses at a single institution. There were 40 participants total, 20 in Calculus I and 20 in Calculus II, that participated in this study. The students who were entering into the course came from disciplines such as industrial and

construction engineering, computer sciences, physics, and mathematics. The setting of this study was conducted at a rural research university in the southern United States.

The class had the main professor, as well as a TA for Friday classes. The class was held five days a week, on a Monday, Tuesday, Wednesday, Thursday, and Friday schedule. CLEAR Calculus was structured a specific way each week: Monday's were lecture days; Tuesday's were when the lab was held, followed by another lecture day on Wednesday's; Thursday's consisted of lecture, lab and discussion, and Friday's were spent reinforcing the material learned throughout the week. For the purpose of this study, demographic details were collected from the students on the consent forms. Students were asked for their ethnicity, native language, and gender specifications. Based on the data received, each of these were coded with an integer: 0 for male, 1 for female; 0 for English, 1 for Spanish, 2 for Urdu; 0 for Caucasian, 1 for Hispanic, 2 for African American, 3 for Asian, and 4 for Native American. Data collected also included: GPA, actual test scores on the four unit exams and the final, and students' predicted test scores on the four unit exams and the final.

Calibration data was gathered on each test, based on responses on the emoji scale indicated by the students. Students circled an emoji face correlating to their feeling of confidence after completing each problem (see Figure 1).

**Figure 1.** Affective Likert Scale



### Statistical Analysis

Once the data was collected, the emoji scale was converted into numerical values. Then, the test scores and the emoji scores were translated into a calibration score using the methods outlined in Champion (2009). The analysis was conducted using a linear regression. First, the data was checked for a linear relationship by checking to see if there were any significant interaction terms (like calibration\*gender) or if there were any terms with a higher R squared value when the data were squared. None were found. Next, the Shapiro-Wilk test was used to check normality; this test revealed that the data was sufficiently normal to do a linear regression.

The initial model that was investigated was:

$$\text{Score} = a_1(\text{calibration}) + a_2(\text{ethnicity}) + a_3(\text{gender}) \\ + a_4(\text{native language}) + a_5(\text{GPA})$$

The initial model was analyzed using each of the demographics and the emoji scores for the four tests as the independent variables, and the final exam graded score as the dependent variable.

### Reliability

A Cronbach Alpha statistic to determine the reliability of the instrument was used. Ideally, the Chronbach Alpha would result in at least 0.6, preferably closer to 0.8 (Gall, Gall, & Borg, 2014; Thorndike & Thorndike-Christ, 2013).

### Validity

There are several ways to measure validity. Those used in this quantitative study included external validity, internal validity, content validity, and face validity. Each are described in more detail below.

*External Validity.* The external validity element that this study had was ecological validity. That is, population validity from random sampling does not exist since a classroom setting cannot truly be sampled randomly, but by studying intact classrooms, the environment where treatments naturally occur was preserved (Gall et al., 2014; Thorndike & Thorndike-Christ, 2013).

*Internal Validity.* For correlational studies, with less direct interaction with participants, the concern was confounding variables (i.e. native language, gender, first generation college students, and student prior knowledge) (Gall et al., 2014; Thorndike & Thorndike-Christ, 2013). The sample was described previously in such a way in order to disclose any potential issues with confounding variables, and they were all included in the initial model.

*Content Validity and Face Validity.* All tests in the class were constructed using a test blueprint matrix weighted by classroom time spend on each objective. To address face validity, other university staff members including a mathematics education and a mathematician who taught the relevant courses more than ten times examined and approved the instruments being used.

## Results

The demographics of the two calculus classes are detailed in Table 1.

**Table 1.** Summary of participant demographics

|                        | Calculus I | Calculus II |
|------------------------|------------|-------------|
| <b>Gender</b>          |            |             |
| Female                 | 4          | 6           |
| Male                   | 16         | 14          |
| <b>Native Language</b> |            |             |
| English                | 15         | 18          |
| Spanish                | 5          | 1           |
| Urdu                   |            | 1           |
| <b>Ethnicity</b>       |            |             |
| Caucasian              | 8          | 10          |
| Latino/Latina          | 8          | 4           |
| African American       | 3          | 4           |
| Asian                  | 1          |             |
| Native American        |            | 1           |

### Calculus I

An initial model for the Calculus I class was created by using SPSS to perform a linear regression model on the initial model. Table 2 provides the results of the initial model.

**Table 2.** Initial model summary

| Model | R    | R Square | Adjusted R Square | Std. Error of the Estimate | <i>p</i> |
|-------|------|----------|-------------------|----------------------------|----------|
| 1     | .797 | .635     | .370              | 20.19384                   | .000     |

This model was determined to hold significance, because the *p* value was below the significance level of 0.05. Next, an ANOVA test was performed with the data. Table 3 summarizes the results of ANOVA test.

**Table 3.** Initial ANOVA test

| Model |            | Sum of Squares | df | Mean Square | F     | Sig. |
|-------|------------|----------------|----|-------------|-------|------|
| 1     | Regression | 7808.098       | 8  | 976.012     | 2.393 | .090 |
|       | Residual   | 4485.702       | 11 | 407.791     |       |      |
|       | Total      | 12293          | 19 |             |       |      |

Although a *p*-value of 0.090 was not significant, the coefficients were analyzed for any correlations to the final exam score. Table 4 summarizes the coefficients from the initial model.

**Table 4.** Initial model coefficients

| Model              | Unstandardized Coefficients |            | Standardized Coefficients |       |      |
|--------------------|-----------------------------|------------|---------------------------|-------|------|
|                    | B                           | Std. Error | Beta                      | t     | Sig. |
| 1 (Constant)       | 7.069                       | 38.283     |                           | .185  | .857 |
| Gender             | 5.329                       | 13.689     | .086                      | .389  | .705 |
| Native Language    | 6.304                       | 11.753     | .110                      | .536  | .602 |
| Ethnicity          | 3.088                       | 6.586      | .106                      | .469  | .648 |
| GPA                | 4.418                       | 13.100     | .116                      | .337  | .742 |
| Test 1 Emoji Score | -.023                       | .347       | -.022                     | -.067 | .947 |
| Test 2 Emoji Score | .053                        | .391       | .048                      | .135  | .895 |
| Test 3 Emoji Score | .095                        | .306       | .098                      | .311  | .761 |
| Test 4 Emoji Score | .508                        | .398       | .626                      | 1.274 | .229 |

With a significance of .229, Test 4 seemed to be accounting the most for the final exam score. Each of the demographics, except for GPA, were removed from the model, as well as all emoji scores besides test four. Table 5 summarizes the final model summary.

**Table 5.** Final model summary

| Model | R    | R Square | Adjusted R Square | Std. Error of the Estimate | <i>p</i> |
|-------|------|----------|-------------------|----------------------------|----------|
| 1     | .782 | .661     | .566              | 16.76544                   | .002     |

The value of the adjusted R Square increased from the initial model, representing a stronger correlation with the variables, test four emoji score and GPA. Table 6 summarizes the ANOVA test for the final model.

**Table 6.** Final ANOVA test

|   | Model      | Sum of Squares | df | Mean Square | F      | Sig. |
|---|------------|----------------|----|-------------|--------|------|
| 1 | Regression | 7515.443       | 2  | 3757.721    | 13.369 | .000 |
|   | Residual   | 4778.357       | 17 | 281.080     |        |      |
|   | Total      | 12293.800      | 19 |             |        |      |

The coefficients for the final model are summarized in Table 7. GPA and calibration score on Test 4 contribute approximately equally to the adjusted R-squared value. While the coefficient on GPA was not significant, its large contribution to the explanation of variance warranted retaining this variable in the model. There was not a significant correlation between the GPA and test 4 calibration score ( $p = .36$ ).

**Table 7.** Final model coefficients

| Model |            | Unstandardized Coefficients |            | Standardized Coefficients |       | Sig. |
|-------|------------|-----------------------------|------------|---------------------------|-------|------|
|       |            | B                           | Std. Error | Beta                      | t     |      |
| 1     | (Constant) | 16.161                      | 20.466     |                           | .790  | .441 |
|       | GPA        | 4.052                       | 8.814      | .107                      | .460  | .652 |
|       | Test 4     | .566                        | .188       | .698                      | 3.011 | .008 |

Once all of the other independent variables were removed, Test 4 proved to be statistically significant at  $p=.008$ . The model for this Calculus I course is below. It used GPA and Test 4 calibration to predict the final exam score.

$$\begin{aligned} \text{Final Exam} &= 16.161 + 4.025(\text{GPA}) \\ &+ 0.566(\text{Test 4 Calibration}) \end{aligned}$$

## Calculus II

As with the data in Calculus I, an initial model summary for Calculus II was generated by using the same initial model as Calculus I.

**Table 8.** Initial model summary

| Model | R    | R Square | Adjusted R Square | Std. Error of the Estimate | <i>p</i> |
|-------|------|----------|-------------------|----------------------------|----------|
| 1     | .558 | .312     | -.189             | 22.00622                   | .556     |

The initial model summary for Calculus II showed a low R Square value at 0.312, indicating that there was no significance in the variables. Table 9 summarizes the ANOVA test for the initial model.

**Table 9.** Initial ANOVA test

| Model |            | Sum of Squares | df | Mean Square | F    | Sig. |
|-------|------------|----------------|----|-------------|------|------|
| 1     | Regression | 2413.540       | 8  | 301.693     | .623 | .744 |
|       | Residual   | 5327.010       | 11 | 484.274     |      |      |
|       | Total      | 7740.550       | 19 |             |      |      |

The initial model resulted in a p-value of 0.744, suggesting that there was no correlation between calibration and final exam score. Table 10 summarizes the coefficients for the variables from the initial model.

**Table 10. Initial model coefficients**

| Model              | Unstandardized Coefficients |            | Standardized Coefficients |       |      |
|--------------------|-----------------------------|------------|---------------------------|-------|------|
|                    | B                           | Std. Error | Beta                      | t     | Sig. |
| 1 (Constant)       | -46.438                     | 56.304     |                           | -.825 | .427 |
| Gender             | .143                        | 16.298     | .003                      | .009  | .993 |
| Native Language    | 1.744                       | 17.333     | .042                      | .101  | .922 |
| Ethnicity          | .609                        | 5.141      | .036                      | .119  | .908 |
| GPA                | 14.680                      | 11.430     | .397                      | 1.284 | .225 |
| Test 1 Emoji Score | .846                        | .708       | .444                      | 1.195 | .257 |
| Test 2 Emoji Score | -.109                       | .267       | -.117                     | -.408 | .691 |
| Test 3 Emoji Score | -.117                       | .305       | -.122                     | -.383 | .709 |
| Test 4 Emoji Score | .171                        | .450       | .122                      | .381  | .711 |

After initially analyzing the data, none of the predictors significantly accounted for the final exam score. From there, the demographics alone were run with the final exam score, each test calibration score with the final exam score, and GPA with each individual test calibration score with the final exam score. None of the analyses were showing significance for the calculus II final exam score. Throughout the process, GPA had the strongest correlation to the final exam score. Therefore, no model was derived from the collected data in relating student calibration to final exam test score.

## Discussion

The question being asked throughout this study was: Is there a relationship between a student's calibration and their performance on the final exam, and can a model be used to predict the final exam

grade based on calibration? For Calculus I, a model was created based on the data. For Calculus II, the result was inconclusive since no significance could be found among the variables.

A model involving a student's GPA and their Test 4 calibration was found to predict that student's final exam grade. With the Calculus I class, calibration from Test 1 had no influence in the final exam score whereas Test 4 had the largest influence. Over the course of the semester, calibration accuracy should increase, and therefore, result in a student applying more time and effort to the outcome of their grade on the final exam (Schunk & Pajares, 2009). This could account for Test 4 mattering the most in the model ( $p = .008$ ). If students have good calibration, pinpointing problem areas would increase efficiency in preparation for tests (Schunk & Pajares, 2009). An unexpected finding that came out of this study was how Test 4 contributed the most to predicting the final exam score. In this Calculus I course, Test 4 covered mostly integral problems, whereas the final exam was mostly comprised of in-depth derivative problems.

There are limitations to this study. One such limitation is the small sample size. Since a convenience sample was used, limited access to participants was the result. Also, while there was diversity in native language and ethnicity among participants, but there was a lack of diversity in gender as very few female students were enrolled. Lastly, the time span of this study may have been a limiting factor, since the study was only conducted over the course of a single semester. Future studies should tracking students who took Calculus I and II with the professor using the CLEAR Calculus curriculum to see how their calibration assessments compared to those in the same Calculus II class who did not have CLEAR Calculus in Calculus I. The results of this study showed that a model for predicting final exam scores of students with the use of calibration could be created for the Calculus I class, but not the Calculus II class. These findings are important for students as well as for teachers. By having a better grasp on their calibration, students can train themselves to prepare for tests accordingly (Schunk & Pajares, 2009). When teachers understand student calibration within their classes, feedback can be

given to students to help them reach a more accurate grasp on their personal calibration (Lin & Zabrocky, 1998). This idea can go beyond calculus and be applied to other subject areas.

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# **Outcomes of Advancing Women Faculty in Engineering and Technology at Historically Black Colleges and Universities: A Retrospective Analysis of ADVANCE-PAID Participants**

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## **Abstract**

Keeping up with their historic missions, historically black colleges and universities (HBCUs) have selected and granted progressively more number of degrees to women over the past few decades. From 1993 to 2006, the number of black women undergrads receiving degrees at HBCUs increased by 24%. For example, in 2002, 75% of black women awarded doctorates in Biology had baccalaureate origins from HBCUs. In 2006, data revealed that there were 900 black women faculty in STEM disciplines at HBCUs and 2,810 black women faculty at non-HBCUs. They accounted for 22% and 2% of the faculty at HBCUs and non-HBCUs.

The ADVANCE-PAID project, Advancing Women Faculty in Engineering and Technology at HBCUs, was a collaboration between Prairie View A&M University and Texas A&M University that received National Science Foundation funding from September 2009 through August 2014. The project implemented four key activities: (1) annual workshops; (2) seed grants to support research efforts; (3) professional career coaching; and (4) weekly writing groups. Forty-seven women faculty from 14 HBCUs attended at least one annual workshop, 13 from 9 HBCUs received seed grants,

11 from 9 HBCUs participated in career coaching, and between 4 and 6 women attended weekly writing groups.

A retrospective analysis was conducted to determine the long-term impacts of the four activities. One-on-one, semi-structured interviews were conducted with past participants to determine the impact activities had on their careers. Current faculty rank and position of each participant were compared to the rank and position held when they initially participated in ADVANCE-PAID activities. Surveys were sent to participants to assess how this project impacted their success, informed their decision or interest in pursuing administration, and how the various activities helped them overall. Group averages were compared by activity or combination of activities.

**Keywords and phrases:** NSF ADVANCE-PAID, career coaching, seed grants, writing groups, women faculty in engineering and technology, women of color in STEM

## Introduction

During the 1800s, many Historically Black Colleges and Universities (HBCUs) were established during a time when practically all colleges and universities only granted admission to men. However, due to their unique missions, HBCUs were open and available to black women (Martinez Aleman, A. and Renn, K., 2002). Between 1975 and 1991, HBCUs were exceedingly high in serving as the baccalaureate origin of black women PhDs, and today, HBCUs account for nearly 30% of all black students who are science and engineering doctorate recipients (National Science Foundation (NSF), 2017).

Reports, such as the 1976 Double Bind Report, provided critical information and, in many cases, layouts for increasing representation of black women especially in the science, technology, engineering and math (STEM) disciplines (Mack, K., Rankins, C. & Winston, C., 2009). Black women continue to remain underrepresented in the STEM (science, technology, engineering, and mathematics) disciplines. They comprise less than 2% of the tenured/tenure-track

faculty positions in STEM at U.S institutions of higher education, and a recent study reports that they are disproportionately concentrated at HBCUs (Mack, K., Rankins, C. & Winston, C., 2009). HBCUs have also been shown to serve as the undergraduate institution of choice for black women who attain doctoral degrees in STEM at a higher rate than black students who attended predominately white institutions (PWI). Thus, black HBCU students are more likely to pursue a postgraduate education in STEM than their counter parts at PWIs.

### ***Why there's a Need for more Black Women Faculty at HBCUs***

HBCUs have selected and granted progressively more degrees to women over late decades. From 1993 to 2006, the number of black women undergrads receiving degrees at HBCUs increased by 24% (NSF, 2009). For example, in 2002, 75% of black women awarded doctorates in Biology had bachelor's origins from HBCUs. In 2006, data revealed that there were 900 black women faculty in STEM disciplines at HBCUs and 2,810 black women faculty at non-HBCUs. They accounted for 22% and 2% of the faculties at HBCUs and non-HBCUs. This suggests that, on average, there are 10 black women STEM faculty per HBCU and 1 black woman STEM faculty per non-HBCU (NSF, 2009).

Black women faculty play an important role in educating, mentoring, and serving as role models for female undergraduates. Studies suggest that one of the greatest influences of academic success in STEM disciplines is access to same-gender role models, especially for women of color (Bettinger, E. & Long, B., 2005; Perna, L. W., 2009). It has also been shown that same-gender and same-race mentoring yields better career outcomes for minority students (O'Neill, R., 2002). Because of HBCUs like Spelman College and Howard University, black women were able to work in academic leadership roles before the congressional approval of Title VII. Title VII, The Civil Rights Act of 1964, prohibited discrimination based on sex and race in employment. Although, the number of women in institutional leadership roles at HBCUs did not outnumber men at that time, their presence was still significant. As a result of their success, they attracted young African American girls and college

students to academic careers by motivating them toward the opportunities to become leaders within their institution. This plays a powerful role in educating and training the next generation.

According to the National Science Foundation's 2017 report on Women, Minorities, Persons with Disabilities, women represent 25.7% of all tenured positions in STEM at 4-year degrees and universities, while women faculty of color in STEM represent only 2.3% (NSF, 2017). To increase these numbers, NSF has focused on several programs. One effort, the ADVANCE program, concentrates on increasing the participation and advancement of women in academic science and engineering careers. In order to increase the number of tenured women faculty of color in STEM, Prairie View A&M and Texas A&M University collaborated on a project that helped women faculty in engineering and technology at HBCUs advance through the faculty ranks and into administrative positions.

#### ***About the ADVANCE-PAID Project***

The ADVANCE-PAID Project, *Advancing Women Faculty in Engineering and Technology at Historically Black Colleges and Universities*, received National Science Foundation funding from September 2009 through August 2014. The overall goal of the project was to generate a blue-print regarding effective strategies and promising practices for women faculty to use to successfully advance through their careers in colleges or schools of engineering and technology at HBCUs. Achievements of this goal was to be supported through implementation of four specific objectives:

- Develop a continuum of activities that will assist women faculty in their professional development and growth while retaining them within the academic and administrative ranks at HBCUs in Colleges or Schools of Engineering and Technology.
- Explore the perspectives of stakeholders (Deans, Department Chairs/Heads, etc.).
- Establish communication and networking method and systems for life-long engagement.

- Publicize best practices to HBCU engineering administrators.

This project implemented four key activities, mainly targeting women of color faculty in HBCU Colleges or Schools of Engineering and Technology: (1) Annual Workshops, which were held in Houston, Texas; (2) Seed Grants to support research efforts; (3) Career Coaching; and (4) Weekly Writing Groups.

### *Annual Workshop*

The project activity that involved the greatest number of women faculty from HBCU Colleges or Schools of Engineering and Technology was the annual workshop. This project had a total of 47 women to attend from 14 different HBCUs. The annual workshop had six learning objectives that they implemented: (1) Negotiating and Assertive Skills; (2) Making the Right Connections; (3) Defining Research Agenda & Writing and Securing Grants, Building Collaborations; (4) Promotions and Advancement & Time Management; (5) Writing Research Articles & Strategies for Getting Articles Published; (6) Establishing an Engineering Research Institute, Building Collaborations. Participants were able to:

- Acquire the skills needed to become more assertive
- Identify and establish academic and professional growth needs
- Develop skills to approach individuals and small groups with confidence
- Identify characteristics of potential collaborators
- Deliver a concise message about themselves, their line of research, and organizations
- Identify potential funding agencies
- Discuss and identify budgets, administrative procedures, partnerships, and collaborators
- Identify the principles of good time management
- Identify and learn strategies to overcome isolation in their work environment
- Identify their research agenda

- Understand the expectations/requirements related to tenure/promotion for their department/college
- Identify mentors
- Improve research and develop skills to foster collaborations with various institutions

### *Weekly Writing Groups*

For 15 weeks, the writing group assisted ADVANCE-PAID participants in their endeavors to write grant proposal narratives and produce published papers. In the midst of those 15 weeks, the weekly writing groups provided motivation as well as inspiration, while being able to network amongst other individuals from other institution. Participating in the writing groups gave participants the opportunity to read what others had wrote, ask questions, and engage in conversations while sharing ideas. During the groups, participants were also provided constructive criticism. They had the opportunity to share their writings and receive feedback. By having a group of other writers look at their pieces, participants received honest opinions and advice, in result participants were able to see where each other's strengths and weaknesses lay. Weekly writing groups also offered guidance when having writers' block and support. The meetings began in July 2012 and continued through the end of October 2012. Each week, participation ranged from 4 to 6 women faculty with an average of 5 women in attendance. By the end of the semester, participation decreased until there were no participants.

### *Seed Grants*

In addition to the writing groups, the ADVANCE-PAID project provided women engineering and technology faculty at HBCUs opportunities to apply for seed Grant funding. The seed grant was an opportunity designed to promote research activities and provide grant-writing mentoring. From 2011 to 2013, thirteen individual grants and one collaborative grant, totaling \$150,000 (individual=\$10,000; collaborative=\$20,000) were awarded to women faculty from nine HBCUs. The goals of the grants were to advance innovative research projects, which were essential to getting external funding, making presentations, and/or submitting paper for publication.

### *Career Coaching*

This project also provided the opportunity for eleven women engineering and technology faculty at HBCUs to receive career coaching. The career coaching activity was focused on career planning and career mapping. A professional coach worked closely with the participants in order to help the participant determine where they would like their careers to go. The coaches constructed solutions and strategies that would be most beneficial in their journey to success. Career coaches also helped participants of the ADVANCE-PAID with their time-management skills, by teaching them how to balance being a mom and/or partner, while still trying to reach that goal of tenured positions and advancement into administration. They also gave participants the confidence that they need, as well as assurance to know that they are not alone in their endeavors to become a full professor.

## **Methods**

This research project was two-fold. First, an internet search was done to determine the current faculty rank of past ADVANCE-PAID participants. This was used to compare their advancement in rank since participating in ADVANCE-PAID activities. Then, semi-structured, one-on-one interviews were conducted with three past ADVANCE-PAID participants. Interviews were held via WebEx. Interviews were recorded for accuracy in transcribing and were conducted over a one-week time period.

### *Interview Questions*

1. What activities did you participate in during the ADVANCE-PAID PROJECT?
2. What impact, if any, has participating in “\_\_\_” had on your career?
3. Have you been promoted or changed job titles since participating in ADVANCE-PAID? Do you believe the activities in the ADVANCE-PAID project aided you in your promotion?
4. How have you used the information (or skills) gained in your day-to-day life?

5. What other types of program activities do you think we should incorporate to be implement to build on this one?

## Results and Discussion

### *Where are they now?*

During the research phase, an online search utilized websites such as LinkedIn, and their college/department webpages to determine where past ADVANCE-PAID participants are currently working. Researchers discovered that the initial two participants who were Adjuncts are now Assistant Professors and the woman who was the Visiting Assistant Professor is now an Assistant Professor. Additionally, of the original 14 Assistant Professors, nine advanced to Associate Professor, five remained in the same position. Upon further review, this is likely due to the fact there has not been enough time for them to advance. Similarly, 9 of the 19 Associate Professors stayed at same faculty rank, while 10 participants advanced to Full Professor. Of the four who were initially Full Professors, one was promoted to a position in upper administration.

**Table 1.** The number of participants and their initial faculty ranks during and after ADVANCE-PAID activities.

| Faculty Rank     | <b>During</b>  | <b>After ADVANCE-PAID Activities</b> |         |        |      |     |                  |
|------------------|----------------|--------------------------------------|---------|--------|------|-----|------------------|
|                  | No. of Faculty | Adj                                  | Assist. | Assoc. | Full | Ad* | Outside Academia |
| Adj.             | 2              |                                      | 2       |        |      |     |                  |
| Visiting Assist. | 1              |                                      | 1       |        |      |     |                  |
| Assistant        | 15             |                                      | 5       | 9      |      | 1   |                  |
| Associate        | 19             |                                      |         | 9      | 10   | 4   |                  |
| Full             | 8              |                                      |         |        | 8    | 4   |                  |
| Administration   | 3              |                                      |         |        |      | 2   | 1                |

\*Ad= Administration position in addition to faculty rank

### *What do they have to say?*

#### *Annual Workshop*

The project conducted three workshops, where all participants attended at least one of the three workshops. As a result, some of the women felt that the workshops impacted them with respect to

promotion and/or tenure, while others felt that their professional lives were not affected by attendance.

Participants of the annual workshops shared the following statements on how the annual workshop impacted their success:

*“I also benefited greatly from the ADVANCE-PAID conference. I recall attending a session working in administrative positions, the role models really helped me to make a decision to move into an administrative position of which I am now serving as a department head of electrical engineering.”*

*“The workshops were also beneficial to be me, because, for us female who entered the professional workforce as assistant, our colleagues are males. It’s very good for us to get together to exchange ideas, and we can learn what our next step is. It’s a very good opportunity, it happens annually, so I participated in all three. I made several friends and we still have connection. All of us are working towards full professorship.”*

#### *Weekly Writing Groups*

*“I wanted to continue to write beyond the writing group that way we can hold each other accountable”*

Due to busy lives, the weekly writing groups had specific times and dates that the participants had to meet to ensure that they successfully worked on their projects and met their writing goals. However, creating schedules that work for multiple participants created huge barriers resulting in decreased participation.

*“I participated, but I only went 2 or 3. I wasn’t able to finish the last program I signed up for, because it had specific times and I didn’t have time to finish.”*

Overall, the writing groups had very little impact compared to the other activities. Although, the writing group has the potential to positively promote successful writing because of an emphasis on accountability and increased implementation of successful writing strategies, the barriers of creating schedules that work for multiple

participants must be overcome before this kind of group can have a widespread impact.

### *Seed Grants*

Recipients of the ADVANCE-PAID seed grant shared the following information during the semi-structured interview on how the seed grant impacted her success:

*“I was an assistant at that time, the seed was a really great help me. I was able to strengthen my research and then I was able advance through and get my tenured.”– Recipient of individual and collaborating seed grant*

*“I benefit extensively the grant allowed me to build my research and by building my research I was to get promoted to an associate professor.” “In addition the grant allowed me to publish my research.”*

During the interviews, participants made statements how there were limited opportunities for funding for women at HBCUs and how the heavy teaching loads they have limits the time they have for working on proposals for external funding. They also shared examples of how receiving the seed grants had given them credibility with other faculty, especially male faculty members, and opened up opportunities for collaborations, resulting in another impactful success.

### *Career Coaching*

Based on the grant’s final external report by an independent evaluator, the career-coaching participants all agreed that their coaching sessions had been very important and beneficial, and they indicated that having these opportunities would be very important to women faculty who are trying to advance through faculty ranks at HBCUs.

## Conclusion

In conclusion, the ADVANCE-PAID project played a role in advancing women faculty in engineering and technology at HBCUs through the faculty ranks and in administrative positions. Based on the interviews and the final external report, the overall project had a positive impact, both professionally and personally, on the women faculty who participated in project activities.

*“Absolutely, absolutely,” said a recipient of the seed grant and participant of the annual workshop, when asked if she would participate in the activities again.*

*“I think it serves a good purpose. If it could last longer that would be wonderful, 5 years?”* said another participant, who also agreed that they would participate again.

From interviews conducted, it was found that participants still use the knowledge and skills that they’ve gained in their everyday life.

*“Day to day I improve my writing. I know the importance of publishing. I try to reach milestones on a day to day basis,”* said a participant of the writing group when asked how she used the information or skills gained in her everyday life.

Overall, project participants were more knowledgeable and more skilled. Participants were also better equipped to navigate the academic ranks and move into administrative positions and other positions of leadership. They have become more productive with their research efforts, conference presentations, and publications. Participants also have been successful in getting external funding to support their research. They now have a better understanding of the importance of networking and have expanded their own professional networks. In addition, ADVANCE-PAID participants have more confidence in their skills in communicating with other faculty in their departments and with their department heads or supervisors. More importantly, they have created a better work-life balance for themselves. As a whole, the ADVANCE-PAID project played a major role in helping advance women faculty of color in STEM

disciplines. Assessing the long-term impacts will allow the ADVANCE-PAID project to share with others institution of higher education, especially those who desire to increase the number of tenured faculty amongst women of color.

### **Further Studies**

When asked “*What other types of program activities do you think the ADVANCE-PAID should incorporate be implemented to build on this one?*” interviewees had positive comments about the program activities. However, the interviewees shared common responses that more seed grants are needed and more programs that teach aspiring young women of color how to build a career as a STEM female professor and why it is so important for women succeed in these fields. One interviewee suggested that the ADVANCE-PAID should implement more information on how to obtain career coaching outside of the ADVANCE-PAID activities. The interviewee then went on to state how there should have been more information on who to contact or how to obtain career coaching outside of the writing program and if there is any more opportunities to get career coaching through her department.

### **Acknowledgments**

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## The Effects of a Selective and Non-Selective Organic Herbicides on *Amaranthus species*

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### Abstract

The *Amaranthus* species has adaptive abilities that give them competitive advantages and invasive tendencies. Their high seed production, seed viability, quick growth rate, and C4 metabolism have allowed some of the species to become resistant to some types of herbicides, causing soybean, corn, and cotton crop yield losses in North America. For this investigation, different organic herbicide solutions were analyzed to determine their affects on the *Amaranthus species*. Different concentrations of acetic acid, eucalyptus volatile oil, and okanin were combined to test the hypothesis that the unique characteristics of each organic herbicides should safely and effectively deter *Amaranthus* growth, even at low concentrations. The organic herbicide cocktail significantly affected the growth rates and germination percentages of resistant *A. palmeri*, susceptible *A. palmeri*, *A. viridis*, and *A. tricolor*. Spouts died when the solution was applied daily, and seeds did not germinate after application. The solution did not have a large effect on *A. hypochondriacs* and *A. caudatus*, but most of those sprouts' length was diminished, and growth ceased.

### Introduction

*Amaranthus* is a globally diverse species that can be divided into four main classification groups: cultivated, weedy, racial, and landrace (Stallknecht & Schulz-Schaeffer, 1993). Though weedy types are the focus of this research, the cultivated types also have a

chance to become an invasive species due to phylogenetic relationships (Stetter & Schmid, 2017). *Amaranthus* are annual herbaceous plants and are a part of the few dicot C4 plant species. Their C4 metabolism allows them to adapt to a diverse range of environmental conditions making them resilient to change and stress (Ward, Webster, & Steckel, 2013). Due to their high seed production, seed viability, quick growth rate, and C4 metabolism, they have become an invasive species in agriculture around the world. In the southern United States, they have caused up to 50% crop yield loss in cotton, soybeans, and corn (Ward et al., 2013). Though farmers have used herbicides to combat their invasion, *Amaranthus* are becoming resistant to the chemical herbicides including: ALS inhibitors, EPSP inhibitors, photosystem II inhibitors, and PPO inhibitors (Dominguez-Valenzuela et al., 2017; Francischini et al., 2014).

Due to chemical overuse, resistant forms of invasive weeds have evolved. Acetic acid, eucalyptus volatile oil, and okanin are alternative herbicides that have been proposed for their unique properties. Acetic acid in a 5-10% concentration strips away the plant's epicuticle protective wax, thereby removing the plant's ability to retain moisture. Small plants are affected by the low acetic acid concentration; however, larger *Amaranthus* plants require higher concentrations (Smith-Fiola & Schulz-Schaeffer, 2017). Even though acetic acid will convert into the water in the soil, the long-term buildup of acetic acid is unknown. Also, due to acetic acid's conversion into water, the alternative not affect the roots, thereby granting an opportunity for the weeds to recover. Eucalyptus oil's monoterpenes have been shown to inhibit growth and induce oxidative stress in *Amaranthus*'s roots (Singh, Batish, Kaur, Arora, & Kohli, 2006). Eucalyptus oil's allelopathic potential, which is naturally occurring, can destroy chlorophyll and reduce seed germination of *Amaranthus viridis* (Kaur, Singh, Batish, & Kohli, 2011). Okanin is a natural pentachalcone in the *Asteraceae* family that selectively inhibits phosphoenolpyruvate carboxylase (PEPC), an essential enzyme in the C4 photosynthetic pathway to allow the plant to store carbon, produce energy, seed germination, CO<sub>2</sub> fixation, cell expansion, and to tolerate abiotic stress (Nguyen et al.,

2016; O'Leary, Park, & Plaxton, 2011). When this compound inhibits the C4 plants photosynthetic pathway, the plant's resilience weakens.

Investigators hypothesize that combining the non-selective acetic acid with the selective eucalyptus oil and okanin will deter *Amaranthus* growth, even at lower concentrations due to a synergistic combination effect.

## Methods

### *Potting*

Field soil obtained from Prairie View A&M's agriculture land was collected in a basin and brought into the Botany greenhouse for use. The soil was placed in 12 plastic pots with dimensions of 11cm X 15cm. The first six pots contained the control set for comparison and the next six planted later contained the sprayed set. The control and sprayed sets both contained around 100 seeds of *Amaranthus* species, two of which (*A. palmeri* susceptible and *A. palmeri* resistant) were obtained from Dr. Youngblood. *A.tricolor*, *A.hypochondriacus*, and *A. caudatus* were obtained online from David's Garden (El Segundo, CA), and *A. viridis* was obtained online from Frozen Seed Capsules (Palm beach, Florida).

Pots were monitored during weekdays and were watered daily with double ionized water with a spray bottle daily to keep the soil moist. Pots were placed under Industrial Glowlight WS, UV light (manufactured in Mexico), 24/7.

An additional pot of *A. Palmeri* was planted with the intent to allow growth to the adult height.

### *Materials*

The material used for the solution was Carolina Methanol (Carolina, Burlington, NC), DMSO 99.7% (Fisher BioReagents, Pittsburgh, PA), BDH Tween 20 (Millipore Sigma, Darmstadt, Germany), Glacial acetic acid (Carolina Biological Supply, Burlington, NC),

Eucalyptus Globulus Oil (DeLaCruz, Los Angeles, CA) The okanin extract was obtained from the plant *B. pilosa*, received from the Mercer Botanic Gardens (22306 Aldine Westfield Rd, Humble, TX).

### *Extraction*

The adult plants of *B. pilosa* were washed then dried in an air dryer for 24 hours and manually ground with a marble pestle and mortar. Once ground into a powder, it was mixed with 70% methanol for ultrasonic extraction with a Q500 Sonicator system with standard ½ diameter probe. It was extracted for 30 minutes at 40hz and 500W with 10 seconds rest intervals. The solution was then vacuum filtered to obtain the solvent that would then be placed in a rotary evaporator, Rotavapor R-100 for 2 hours. The remaining solute and oil was vacuumed filtered again to remove remaining residue. The resulting solution was placed in a transparent glass vial.

### *Solution*

In a 500mL beaker, a 200mL solution was made with 5% acetic acid, 0.2% eucalyptus oil, 17.65% of the *B. pilosa* extract, 0.1% of Tween 20, and 2.4% DMSO in DI water. The solution was then placed in an 800mL plastic spray bottle.

### *Application*

The application of the herbicide solutions was started on day 2 of measurements, when growth was seen, with a two-day intermission due to the weekend. Control groups were sprayed daily with deionized water. The experimental groups of the *Amaranthus spp.* were daily sprayed with the solution described above (two full pumps each). The plants were sprayed on Days 0, 1, 2, 4, 5, 6, 7, and 8. Results were measured at the time of application.

Another *A. palmeri* plant that was potted for the intent of growing to adult height, received only deionized water applications until they reached 20cm to test the herbicides application on larger plants. At that time, it was sprayed from top to base of the stem until thoroughly coated with the solution described above.

## Analysis

The measurements and daily log were recorded in a lab notebook. Line graphs were generated via Microsoft Excel® and included the daily height data and the daily average growth data (calculated by averaging the daily formula:  $\frac{y_2 - y_1}{T_2 - T_1}$ ). Germination rates were calculated using formula:  $(\text{Number of germinated seed} \div \text{Total number of seeds}) \times 100$ .

## Results

### Growth Comparison

*A. palmeri* resistant control's average growth rate in an 8-day period is 0.33cm/d. *A. palmeri* resistant sprayed average growth rate in an 8-day period is a negative -0.20, due to there being a two-day gap (Days 2 & 3) in sprays, thereby giving an opportunity for sprouts to grow. Once spray was applied, the sprouts wilted and died, but no other seeds would sprout after.

As seen in *Figure 1*, the growth of control and sprayed growth was similar at the beginning of measurements, but once the solution was applied again, no more growth was seen on Days 5-8. The initial application of the solution did not seem to affect the growth rate of sprayed pot.

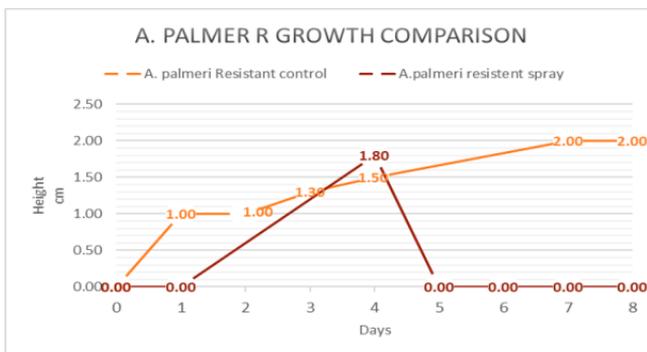


Figure 1: The daily height of control and sprayed set of *A. palmeri* resistant over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate of *A. palmeri* susceptible control is 0.36cm/d, and *Figure 2* shows the gradual development of the control. *A. palmeri* susceptible spray's average growth rate over 8 days was in the negative at -0.13cm/day due to the application of the solution causing the plant's length to decrease and growth to stop. In *Figure 2*, the initial treatment of solution decreased the growth rate when compared to control. The sprayed sprouts length greatly decreased after Day 4 with the continuous daily application.

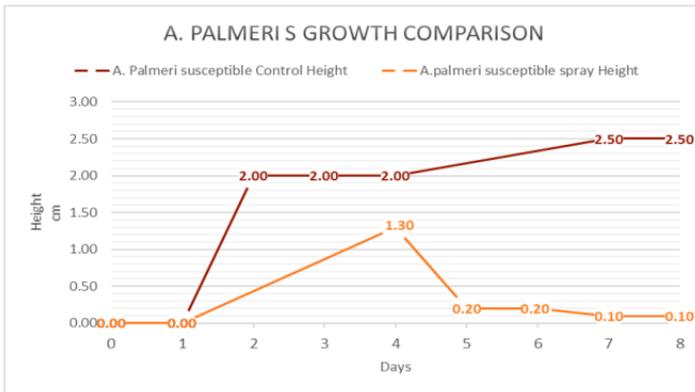


Figure 2: The daily height of control and sprayed *A. palmeri* susceptible over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate for *A. viridis* control is 0.56 cm/day over the 8-day period, while the average growth rate of *A. viridis* sprayed is negative -0.13 cm/day due to sprouts decreasing in length after application of solution on Day four as seen in *Figure 3*. The initial application of the solution on Day 1 did affect the growth rate as compared to control.

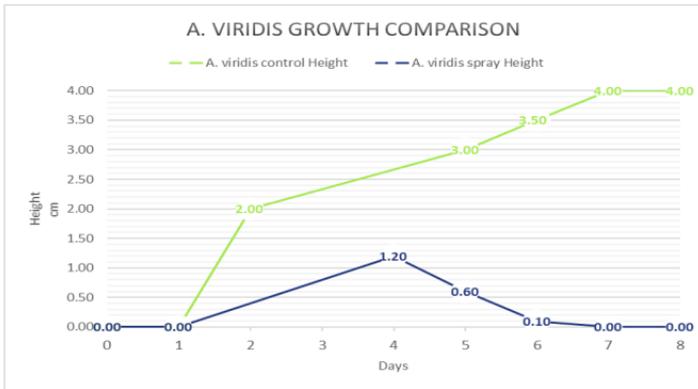


Figure 3: The daily height of control and sprayed *A. viridis* over an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

The average growth rate of *A. hypochondriacus* control in the 8-day period is 0.38 cm/day, while the average growth rate of *A. hypochondriacus* sprayed is -0.07 cm/day due to sprouts decreasing in length, as seen in Figure 4, after Day 4. The initial application of solution did affect growth rate once compared to control.

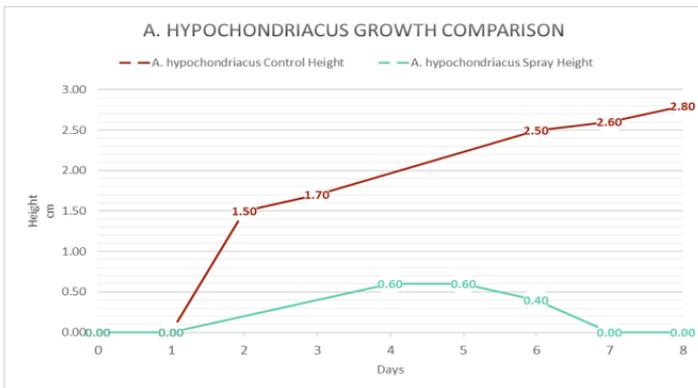


Figure 4: The daily height of control and sprayed pots of *A. hypochondriacus*. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

*A. caudatus* control's average growth rate is 0.34 cm/day, while *A. caudatus* sprayed average growth rate is -0.08 cm/day in the 8-day span due to sprouts length decreasing once the solution was applied.

As seen in *Figure 5*, the growth of sprayed and control have similar increases in growth until Day 4, when the solution was applied to the sprayed pots that cause sprouts to wither, stop seed germination, and cease radicle growth.

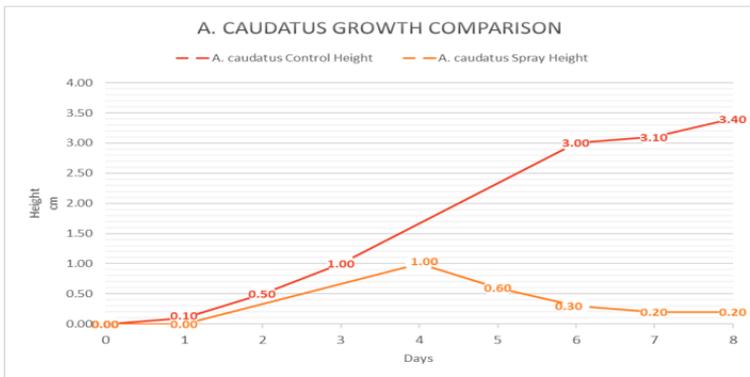


Figure 5: The control and sprayed daily height of *A. caudatus* in an 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

*A. tricolor* control's average growth rate over the eight-day period is 0.46 cm/day, while the average growth rate of sprayed *A. tricolor* is -0.10 cm/day due to sprouts length decreasing after continuous daily application of the solution (*Figure 6*). The initial treatment reduced the growth rate when compared to control. After Day 4, sprayed pot length decreased, and no other seeds germinated with continuous daily spray.

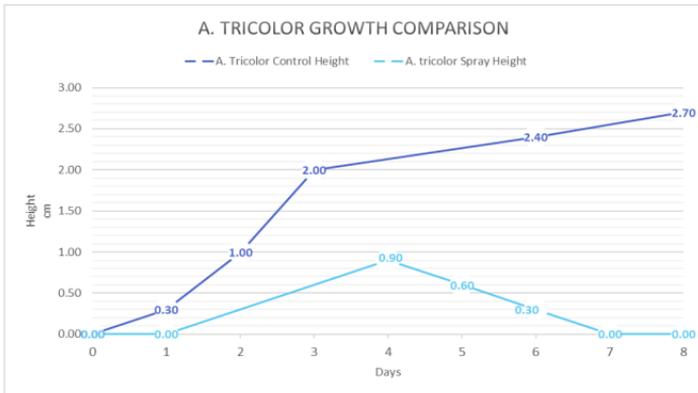


Figure 6: Daily height of control and sprayed *A. tricolor* in the 8-day period. Initial application of solution started on Day 1, then continuous treatment began again on Day 4.

## Germination

The germination percent of the control *Amaranthus spp.*, as seen in *Figure 7*, shows *A. viridis* with a germination percentage of 45%--the highest of the control species. Susceptible *A. palmeri* germination percentage at 19%, is on par with *A. caudatus* and *A. hypochondriacus*. The lowest percentages of control were resistant *A. palmeri* at 7% and *A. tricolor* at 10%.

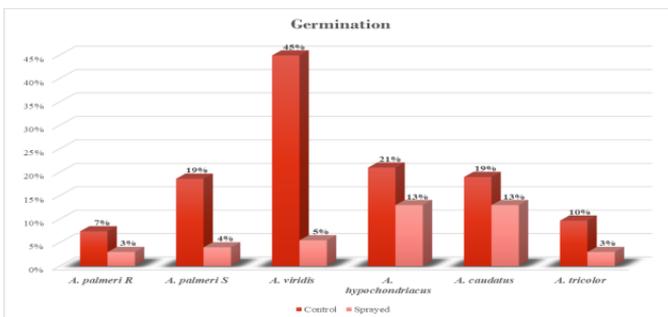


Figure 7: The germination percentage of Control and Sprayed sets of *Amaranthus spp.* in an 8-day period.

For the sprayed pots, the solution affected all species germinations percentages. The largest effects were on *A. viridis* and susceptible *A. palmeri* of 40% and 15% different respectively when compared to control. *A. hypochondriacus* and *A. caudatus* germination percentage is 13%, only a slight decrease from control percentage.

#### *Adult Amaranthus palmeri application*

For the second experiment, the adult plant was fully erect before the solution was applied, but three hours after application the plant shows visible drooping, wilting, and spots of lighter discoloration appear on leaves. Three days after application, most leaves wilted along with parts of the stem.

### **Discussion/Conclusion**

The average growth rate and high germination percentage of the control susceptible *A. palmeri* plants confirms the invasive characteristics previously reported. Resistant *A. palmeri*, on the other hand, has a lower growth rate and a low germination rate; thus, it poses less of a threat to an invasive species as they are less likely to survive, compared to the susceptible species. *A. viridis* high growth rate and high germination rate, which means it is more invasive than *A. palmeri* and should be monitored in agriculture to prevent infestations. *A. hypochondriacus*, mainly used for grain, has a slightly higher growth rate and germination percentage than susceptible *A. palmeri*; therefore, it could also become an invasive species. *A. caudatus*' growth rate and germination percentage are on par with susceptible *A. palmeri*. Though *A. caudatus* are used for their grains, they have a possibility to become just as invasive as susceptible *A. palmeri* in unwanted areas. *A. tricolor*'s germination percentage is low, and their growth rate is high; therefore, they have a medium chance to become an invasive species.

The organic herbicide cocktail significantly affected the growth rates and germination percentages of resistant *A. palmeri*, susceptible *A. palmeri*, *A. viridis*, and *A. tricolor*. Spouts died when the solution was applied daily, and seeds did not germinate after application. The solution did not have a large effect on *A. hypochondriacs* and *A.*

*caudatus*, but most of those sprouts' length was diminished, and growth ceased. Comparing the difference in growth and germination rates of control and sprays, the organic herbicide solution affected *A. viridis* and *A. palmeri* the most, but the less invasive species were not affected to the same extent. A plausible reason for less invasive species being less affected would be that the eucalyptus volatile oil does not have allelopathic potential against *A. hypochondriacus*, and *A. caudatus*.

The organic herbicide solution had a noticeable effect on the larger plant when applied thoroughly. The organic herbicide solution also significantly affected the adult *A. palmeri*, causing it to wilt within 3 hours. After three days, parts of the adult are unaffected, probably due to the solution not being applied evenly. Because okanin is known to cause a lighter discoloration in leaves of *A. retroflexus* (Nguyen et al., 2016), we believe the same effect three hours occurred in this experiment and that the application of the okanin on *A. palmeri* affected the PEPC pathway and caused the plant to be more susceptible to the acetic acid and eucalyptus volatile oil.

While further research is needed, the organic herbicide containing acetic acid, volatile eucalyptus oil, and okanin shows promise. Future experiments should investigate application timing procedures and also should explore starting the application at different stages of growth. Further, experiments should be run on the key crops (corn, soybean, etc) that are harmed by this weed, to see how the herbicide will affect their growth.

Due to the low solute concentration, this solution should not cause a meaningful change or build up in the soil and environment; however, future experiments should analyze the soil.

In conclusion, due to the components availability and low cost, the proposed organic herbicide cocktail could be a cheaper and effective alternative to traditional herbicides.

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## Evaluating alternative crosslinking agents in poly(vinyl alcohol) hydrogels membranes

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### Abstract

Hydrogels are a network of polymer chains with properties that absorb, store and transport solutions. A hydrogel membrane has a permeability that allows influx and excretion. Therefore, it is the ideal material for medicated membranes. This study investigates the crosslinking of poly(vinyl alcohol) (PVA) hydrogel membranes using different agents and explores the usability of the candidate membranes as drug delivery systems. The model protein, bovine albumin serum (BSA), was used to test the stability and controlled drug release rate characteristics of the candidate hydrogel membranes. This investigation also evaluated the stability different crosslinkers for hydrogel membranes. Glutaraldehyde (GA) and an alternative crosslinking method of ultraviolet irradiation with the sensitizer, sodium benzoate (SB), were used to crosslink PVA containing BSA. In GA crosslinked membranes, BSA release diffusion experiments showed 48%, 45%, and 63% recovery of protein at pH 6.5, 7.4 and 8.0, respectively; this confirmed that this system is suited for physiological conditions and controlled release. Although SB has been used for membrane fabrication, our Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA) results indicate that UV(SB)-crosslinked films are not suited for drug delivery, despite the release of BSA.

**Keywords:** poly(vinyl alcohol), hydrogels, sodium benzoate, glutaraldehyde, cross-linking agents, ocular drug delivery

## Introduction

Over 30 years ago, polymeric hydrogels were introduced to the field of drug delivery and had been used ever since (Umesh K. Parida, 2011; Yang & Su, 2011). Hydrogel membrane micromatrices technologies have been developed for a number of applications. They have become very popular for use in treating diseases because their unique properties allow for the slow and sustained release of drugs through a porous surface (Umesh K. Parida, 2011; Yang & Su, 2011). Hydrogels are a hydrophilic polymeric network that can be cross-linked using a physical or chemical process to create a three-dimensional, porous matrix that can absorb aqueous solutions (Kamoun, Chen, Mohy Eldin, & Kenawy, 2015; Kiani & Asempour, 2012; Prabhu, Dubey, Parth, & Ghatge, 2015).

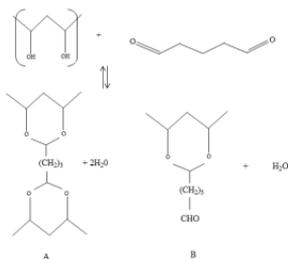
Hydrogels are hydrophilic biomaterials that can be composed of a variety of polymers, allowing for a range of chemical and physical properties (Mishra, Majeed, & Banthia, 2011; Prabhu et al., 2015; Yang & Su, 2011). Poly(vinyl alcohol) (PVA) hydrogels are an ideal material for drug delivery systems, because they are a linear hydrophilic polymer that is nontoxic and biocompatible (Kamoun et al., 2015; Mishra et al., 2011; Umesh K. Parida, 2011). Hydrogel membranes are fabricated by the chemical crosslinking of polymer chains using glutaraldehyde (GA) through intra/intermolecular interaction via hydrogen bonding. Therefore, medications can be incorporated into the porous structure of the membrane (Figueiredo, Alves, & Borges, 2009; Prabhu et al., 2015). The release rate is controlled through the permeability of the matrix (Lin & Anseth, 2009). A PVA hydrogel membrane drug delivery system that is applied topically has the advantage of bypassing the first-pass metabolism (Kamoun et al., 2015; Kiani & Asempour, 2012). Another advantage is the reduction in toxic symptoms or side effects, because the drug can be withdrawn by simply removing the membrane. As the hydrogel membrane allows for the slow and gradual release of the protein drug, this in turn allows the reduction of the overall dosage amount.

Crosslinking is the process that bonds polymer chains together to create a network mesh. The density of the crosslinking can

manipulated through different fabrication techniques (Hu et al., 2011; Kamoun et al., 2015; Sirousazar, Kokabi, & Hassan, 2012) to change the rate of drug release via the various sizes of pores. Thus, the network transiently houses small molecules that will easily diffuse out upon direct contact with a solution (Hsu, Fentzke, & Chauhan, 2013; Sittiwong, Niamlang, Paradee, & Sirivat, 2012). Utilizing different water-soluble polymers and cross linkers have paramount importance when researching the development of drug delivery patches and contact lenses.

Casting techniques are important to the fabrication of hydrogels, and depending on the method, can alter the structure of the pores. Throughout literature, different casting methods (such as freeze-dry method, autoclaving, freestanding solvent casting, UV photo polymerization and spin coater method) have been used (Mishra et al., 2011; Prabhu et al., 2015; Umesh K. Parida, 2011). In this research, PVA hydrogels were created via two different crosslinking methodologies and were utilized to fabricate hydrogel membranes loaded with model proteins to resemble a drug-loaded matrix.

This first methodology used GA to crosslink PVA by abstracting a proton from the hydroxyl group of PVA and forming an acetyl bridge between the two chains as shown in Figure 1 (Figueiredo et al., 2009).



**Figure 1.** Mechanism of the reaction between poly(vinyl alcohol) and glutaraldehyde.

Acidic catalysts increase the speed of the reaction, which makes abstracting the proton more favorable. Despite the wide usage of GA as a crosslinker (Figueiredo et al., 2009; Nave, Luo, & Coleman, 2008), it has been shown to cause respiratory and skin irritation from extended/repeated exposure, even at concentrations as low as 0.05 ppm (Endo, 2006). After membrane fabrication, a rinse is used to remove any remaining or unused GA. Therefore, GA is not a part of the final product.

The ocular environment is an immunologically privilege area which makes is highly sensitive to toxins. To use PVA-GA membranes in ocular environments, it is important to investigate whether the membrane will elicit an immune response. Human Corneal Epithelial cells (HCECs) cells were chosen due to their lack of defense mechanisms to danger. A PVA hydrogel membrane placed in HCEC was used to determine if the membrane alone would elicit an immune response. In other words, would the human cells recognize the membrane as harmful?

Photopolymerization uses a light source to create networks in drug delivery membrane matrices (Baroli, 2006; Mishra et al., 2011). UV polymerization is a viable alternative to GA crosslinking (J. Delville, 2002) and PVA has been shown to crosslink with UV irradiation in the presence of a sensitizer, sodium benzoate (Miranda, Gonçalves, & Amorim, 2001). Sodium benzoate's mechanism involves radical formation, but the specific pathway has not been elucidated. However, possible reaction pathways are proposed by Miranda et al. (Miranda et al., 2001), and the mechanism likely involves the splitting of the benzoate ring (Wittaya, 2012). This research seeks to evaluate whether UV-SB crosslinking is a feasible alternative to GA crosslinking by investigating the protein release behavior of BSA.

## **Methods**

### *Fabrication of GA-crosslinked membranes*

A solution of 12.5% by mass PVA was prepared by mixing 5 g of solid PVA in 40 mL of deionized water, while the water was heated and stirred continuously. 4.0 mg/mL BSA stock was prepared by

adding 1.0 g of crystallized protein to 250 mL sodium phosphate buffer at pH 7.4. To prepare the membranes, 0.960 mL PVA was added to 0.250 mL BSA, and the mixture was left to stir for approximately 5 minutes. Next, 0.218 mL of 10% sulfuric acid was added as a catalyst, 0.036 mL of 10% acetic acid was added as a buffer and 0.036 mL of 50% methanol as a quencher. Then, the cross-linker, 0.108 mL of 1% by mass GA, was added and stirred for 20 seconds. Finally, the solution was poured onto a petri dish, left to set for 15 minutes, and placed on a spin coater with increasing rpm to complete membrane fabrication. Lastly, the membrane was allowed to air dry overnight. The membrane was dislodged using deionized (DI) water.

#### *Fabrication of Ultraviolet (UV) Sodium Benzoate (SB)-crosslinked membranes*

Stock solutions of PVA and BSA were prepared as previously detailed above. The stock of SB was prepared by mixing 0.3 g solid SB in 50 mL of 30 mmol sodium phosphate buffer at pH 7. Three types of UV(SB)-crosslinked membranes were fabricated: (1) membranes lacking protein by mixing 0.750 mL of 12.5% PVA with 0.750 mL of 0.6% SB; (2) membranes with a 0.63 mg/mL concentration of protein by mixing 0.250 mL of BSA stock with 0.625 mL of PVA; and (3) 0.625 mL of SB stock. Membranes with a concentration of 1.26 mg/mL protein in the membrane were prepared by mixing 0.500 mL of protein, cross-linker, and polymer. These solutions were poured into 50 mL beakers or watch glasses (approximate diameter of membranes formed: 3.5 cm) and dried in an oven at 150 °C for approximately 2.5 hours. After drying, they were removed from the glass and placed under UV light at 254 nm for 1.5 hours.

#### *Attenuated Total Reflectance- Fourier Transform Infrared (ATR-FTIR)*

For FTIR analyses, the membranes were dried at 150 °C for approximately 2 hours. The UV(SB) membranes were dry upon formation and were used without additional heating. This technique was used to determine functional groups, such as hydroxyl groups present within the cross-linked membrane matrix.

### *Equilibrium Swelling*

Membranes were left in 20 mmol sodium phosphate buffer at pH 6.5, 7.4, and 8 for 24 hours. After equilibrating in solution, their weight was measured. Then, the samples were placed in an oven at 150 °C for approximately 2 hours to dry. The dry weight was measured, and the equilibrium swelling value was calculated as  $(\text{wet}_{\text{weight}} - \text{dry}_{\text{weight}})/\text{wet}_{\text{weight}}$ . From there, the mesh size and crosslinking density were calculated as described by Matsuyama (Hideto Matsuyama, 1997).

### *Thermogravimetric Analysis (TGA)*

The thermal properties and stability was analyzed through Thermogravimetric Analysis (TGA). Samples were weighed in an analytical balance, and the TGA was tared with the aluminum crucible. The pre-weighed sample was then placed in the crucible, and into the TGA. The TGA analyzes the samples from 30 to 575 °C at a rate of 10 °C/min.

### *Release Experiments*

BSA was used as the model protein to investigate the release profile. BSA is a 65 kD protein that has been used in research for drug delivery models (Censi, Di Martino, Vermonden, & Hennink, 2012; Kamoun et al., 2015). The release of protein was observed in 20 mL buffer solution either with (1) membrane sandwich between diffusion cell with only one side exposed to buffer or (2) membrane submerged in 20 mmol sodium phosphate buffer at pH 6.5, 7.4 and 8.0. The temperature was kept constant at 37 °C using a water bath, and samples were shaken at 83 rpm.

### *Quantitative Measurement of Release in GA membranes*

For the GA-crosslinked membranes, approximately 1 mL samples were taken out to be tested and then returned to the solution. Absorbance was measured at 279 nm to determine the concentration of BSA, and at 399 nm to establish a baseline measurement. The two readings were subtracted for the final absorbance used to calculate the concentration. The percentage of the protein mass recovered in solution was calculated with the Lambert-Beer Law.

### *Qualitative Measurement of Release in UV(SB) Membranes*

In the UV(SB)-crosslinked membranes, absorbance measurements could not be used to calculate concentration due to the interference of sodium benzoate's absorption at 279 nm. Instead, membranes were removed from the buffer solution, and the time removed was noted. Then, the samples were concentrated using 20 mL (Corning) Spin X Concentrator centrifuge tubes, running the centrifuge for 15 minutes at 5000 rcf for three cycles until they were concentrated to approximately 500  $\mu$ L. The concentrating tubes were rinsed with DI water, for 15 minutes at 5000 (rcf) between samples. Then, the concentrated solutions were moved to Eppendorf tubes, and mixed with an equal volume of 10% trichloroacetic acid (TCA) to precipitate the protein; these samples were run in a micro-centrifuge for 25 minutes at 10 rpm. After that cycle, all liquid was removed from the tube, without disturbing the protein pellet, and 400  $\mu$ L of 0.3 M Tris buffer was added to the tube to rinse off the TCA; this mixture was run in the centrifuge for two minutes at 10 rpm. Finally, the Tris buffer was removed from the tube, and 50  $\mu$ L of loading buffer was added. These were spun for approximately 30 seconds at 10 rpm to ensure that the protein pellet was mixed with the buffer. The SDS PAGE gel was run for approximately one hour, at 120V, until the loading buffer reached the bottom of the gel. Afterwards, the gel was stained with comammassie stain for approximately 30 minutes. If necessary, the gel was destained by heating with 5% acetic acid until boiling, then rinsing with DI water thrice. The gel was then photographed using a lightbox.

### *Human Corneal Epithelial Cells*

HCECs were purchased from Invitrogen and maintained in keratinocyte media supplemented with 0.2% v/v bovine pituitary extract (BPE), 1  $\mu$ g/mL recombinant human insulin-like growth factor-I, 0.18  $\mu$ g/mL hydrocortisone, 5  $\mu$ g/mL bovine transferrin and 0.2 ng/ml human epidermal growth factor. HCECs were grown to confluency and were introduced to the hydrogel without protein, for 24 hours in separate experiments. The supernatant was collected to use in an enzyme linked immunosorbent assay (ELISA).

### *ELISA*

HCEC exposed supernatants were analyzed with specific ELISA kits: Interleukin-6 (IL6), Interleukin-8 (IL8), Interferon gamma (IFN- $\gamma$ ), Tumor necrosis factor alpha (TNF- $\alpha$ ), Interleukin-12 (IL-12) P40, Interleukin-12 (IL-12) P70, Interleukin-16 (IL-16) and GRO- $\alpha$  (Ray Biotech). The homogenates were incubated in the ELISA plate at 4 °C overnight, which was followed by a wash according to the manufacturer's protocol. Diluted biotin-conjugated antibodies specific to the cytokine being evaluated were added to each well and allowed to incubate at 4 °C overnight. Wells were washed and incubated at 25 °C for 45 min with diluted horseradish peroxidase-conjugated streptavidin. The wells were washed, and 100  $\mu$ l of TMB One-Step Substrate Reagent was added to each well and allowed to incubate at room temperature for 30 min. The reaction was stopped by a solution and read at 450 nm immediately with the Biotech (Synergy HT) plate reader at 450 nm for ELISA analysis.

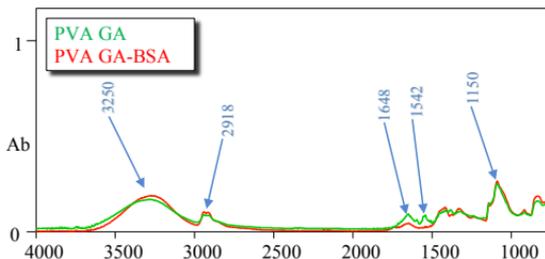
## **Results and Discussion**

### *FTIR Studies*

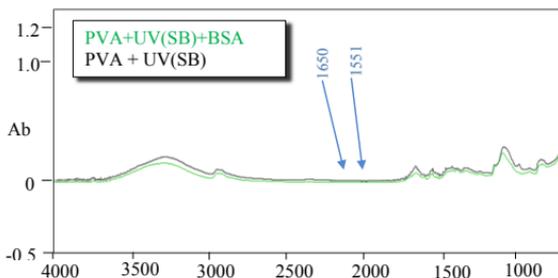
FTIR spectra of the GA-crosslinked membranes in comparison to the spectrum for pure PVA showed a reduced hydroxyl peak at 3330 – 3350 1/cm, shown in Figure 2. Furthermore, the C-H stretch peak at around 2800 1/cm, is reduced in both spectra. FTIR spectra of the UV(SB)-crosslinked membranes also showed a reduced hydroxyl peak. The UV(SB) membranes also showed new peaks at 1551 1/cm (aromatic stretch) and 1650 1/cm (alkenes) as shown in Figure 2a. Spectra of the GA-crosslinked membranes confirm the presence of BSA and crosslinking in the gel. Furthermore, visible peaks at 1648 1/cm and 1542 1/cm are indicative of amide I and amide II peaks in the gels containing protein confirmed protein integration (Figure 2a).

For UV(SB)-crosslinked membranes, crosslinking was not seen as dramatically; the characteristic duplet was reduced (Figure 2b). Wittaya (Wittaya, 2012) suggested various pathways of the SB reaction with UV light, and Miranda et al. suggested that ring-opening of the benzoate is probable (Miranda et al., 2001). However, the FTIR spectra obtained from UV(SB) membranes were unable to confirm either pathway – peaks at 1650 suggest that the aromatic

ring did not open up, and peaks at 1551 suggest that the ring was opened during radical formation. Furthermore, absorbance at 220 nm indicated the presence of SB in the release solution, confirming that some of the sensitizer was not consumed in the reaction. Thus, the crosslinking was incomplete, and SB remained intact. More irradiation time has shown the decomposition of the sodium benzoate (Miranda et al., 2001) and more crosslinking may have occurred.



**Figure 2a.** FTIR spectra comparison of poly (vinyl alcohol) membrane (PVA) crosslinked with glutaraldehyde (GA) without protein and poly (vinyl alcohol) (PVA) crosslinked with glutaraldehyde (GA) with protein,BSA membrane.

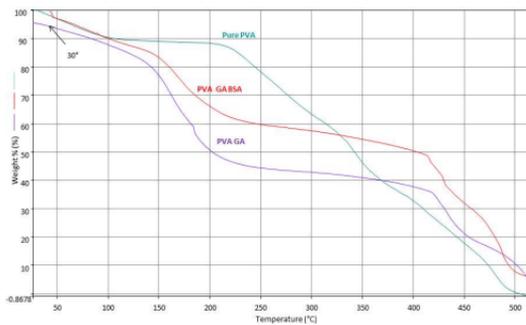


**Figure 2b.** FTIR spectra comparison of poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) as sensitizer without protein and poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) as sensitizer with protein, BSA.

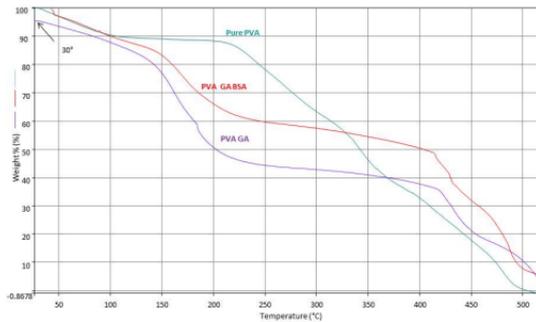
*Thermogravimetric Analysis (TGA)*

TGA results, seen in Figure 3a, of glutaraldehyde (GA)-crosslinked membranes show a gradual change in mass over temperature change, as compared to pure PVA. Results of UV-crosslinked with sensitizer, sodium benzoate (SB), membranes, seen in Figure 3b, show that UV(SB) films have similar rates of mass change as pure PVA. However, TGA curves for UV (SB)-crosslinked membranes indicated rapid thermal degradation under heat. This decreased stability is likely due to the inconsistent and random crosslinking of the polymer found in UV (SB)-crosslinked films.

a)



b)



**Figure 3.** Thermogravimetric analysis results of polyvinyl alcohol chemically crosslinked with glutaraldehyde (GA) (a) and UV crosslinked with sensitizer, sodium benzoate (SB) (b) incorporating model protein, bovine serum albumin.

*Equilibrium Swelling (ES)*

The values in Tables 1 and 2 are for both sets of membranes and reveal the low standard deviations calculated, assuring the consistency in the results. For all pH values, the GA-crosslinked membranes showed an average swelling percentage of 86.5%, while the UV(SB) membranes showed an average of 73.8%. The GA membranes had larger mesh sizes and lower crosslink densities in comparison to the UV(SB) films, indicating that the UV(SB) can load less protein in each pore than the GA gels. Similar values were found among protein concentrations of 0.63 mg/ml and 1.26 mg/ml in the UV(SB) membranes, suggesting that doubling the protein loading does not affect mesh size.

**Table 1.** Equilibrium swelling of poly (vinyl alcohol) (PVA) crosslinked with glutaraldehyde (GA) membranes with or without protein, bovine serum albumin (BSA).

| pH                                 | Swelling    | Mesh Size<br>(Å) |
|------------------------------------|-------------|------------------|
| 12.5 % PVA, 1 % GA, 0.63 mg/mL BSA |             |                  |
| 6.5                                | 86.0±0.001% | 155              |
| 7.4                                | 90.5±0.007% | 155              |
| 8.0                                | 85.7±0.036% | 290              |
| 12.5 % PVA, 1 % GA, No BSA         |             |                  |
| 6.5                                | 87.4±1.60%  | 212              |
| 7.4                                | 89.0±0.21%  | 148              |
| 8.0                                | 82.2±0.03%  | 108              |

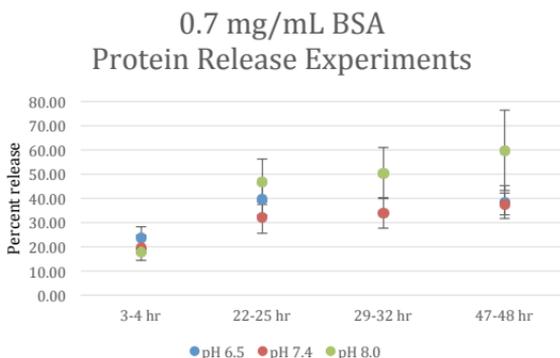
**Table 2.** Equilibrium swelling of poly (vinyl alcohol) (PVA) UV crosslinked with sensitizer, sodium benzoate (SB), membranes with and without protein, bovine serum albumin (BSA).

| pH                                   | Swelling    | Mesh Size (Å) |
|--------------------------------------|-------------|---------------|
| 12.5 % PVA, 0.6 % SB, 0.63 mg/mL BSA |             |               |
| 6.5                                  | 70.9±0.005% | 41            |
| 7.4                                  | 74.0±0.009% | 53            |
| 8.0                                  | 73.1±0.022% | 48            |
| 12.5 % PVA, 0.6 % SB, 1.26 mg/mL BSA |             |               |
| 6.5                                  | 76.8±0.030% | 66            |
| 7.4                                  | 75.3±0.022% | 57            |
| 8.0                                  | 71.7±0.052% | 46            |
| 12.5 % PVA, 0.6 % SB, No BSA         |             |               |
| 6.5                                  | 75.8±0.017% | 59            |
| 7.4                                  | 72.2±0.004% | 45            |
| 8.0                                  | 75.0±0.018% | 56            |

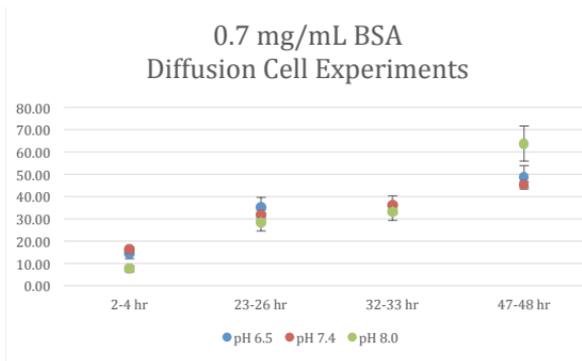
### Release Studies

Release kinetics were evaluated using Higuchi's equation and protein percent release over 48 hours (Siepmann & Peppas, 2011). Protein concentration taken at various time points are used to calculate the amount of protein diffused with only one side of the membrane exposed to buffer or protein release with the membrane submerged in buffer. The BSA release resulted in a curve graph indicating a gradually for both types of experiments as shown in Figures 4 and 5. The diffusion experiments showed averages of 48%, 45%, and 63% percent recovery of protein for GA-crosslinked membranes at pH 6.5, 7.4, and 8.0 respectively, as seen in Figure 4. The release experiments showed slightly lower averages of 36%,

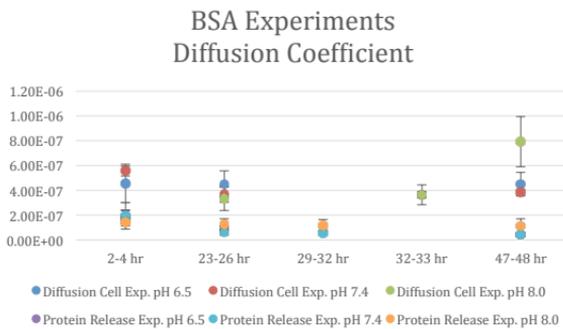
38%, and 60% percent recovery of protein for GA-crosslinked membranes at pH 6.5, 7.4, and 8.0 respectively, as seen in Figure 5. The diffusion coefficients for all pH values demonstrate a decreased rate during the 32 hour period and showed a burst afterwards. The concentration gradient is the driving force of the initial release. As the protein leave unoccupied pores, other protein will occupy these spaces before releasing from the protein. Resulting in a rate increase as the BSA protein moved out seen in Figure 6. The protein release experiments did not show an increase after 32 hours due to both faces being exposed to buffer. Therefore, the rate has decay due to the protein hovering around the surface of the membrane and interrupting the concentration gradient. SDS-PAGE was used to confirm BSA release from the UV(SB) membranes. The release SDS-PAGE, depicted in Figure 7, shows the presence of protein between 40 – 75 kD markers confirming that BSA (65 kD) was detected. Furthermore, the protein samples were taken at various time points over 24 hours, and the gel demonstrates that protein was released (detected) over time. Though UV(SB) films gradually release protein, as confirmed by the gel, they were unstable in the buffer, and the crosslinking appeared to be incomplete as indicated by FTIR. Some membranes became semi-solid during the 24-27 hour experiment.



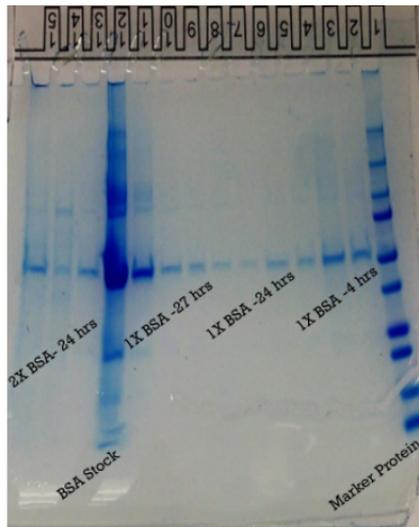
**Figure 4.** Bovine Serum Albumin (BSA) release experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 5:** Bovine Serum Albumin (BSA) diffusion experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 6.** Bovine Serum Albumin (BSA) diffusion coefficient for both release and diffusion experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 7.** Poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) Release SDS-PAGE at various times.

BSA release experiments confirmed the gradual release of the protein over a 48-hour period with GA-crosslinked membranes. The protein release rate demonstrates a plateau due to the migration of protein to the exposed surface of the membrane after the amount at the surface was exhausted. Though UV(SB) films gradually released protein as confirmed by the SDS-PAGE, they were unstable in water, and the crosslinking appeared to be incomplete. Some membranes began to disintegrate while in the buffer, thus, indicating the instability of the UV(SB) membranes for drug delivery at 37°C.

### *Cytokines ELISA*

Cytokine ELISA was utilized to investigate host immune factors that are involved in the ocular environment when the hydrogel is present. The immune response was investigated utilizing specific ELISA assays of cytokines present during a bacterial infection. The corneal homogenates collected at post-exposure to bacteria were analyzed using human inflammatory cytokine arrays. The cytokine difference relative to the media and hydrogel membrane was determined and

listed in Table 3. The results demonstrated no contrasts in the cytokine expression profiles in the corneal homogenates collected from exposure to media alone and hydrogel.

**Table 3.** Cytokine profiles of supernatants from human corneal epithelial cells exposed hydrogel.

| Sample            | INF-gamma   | IL-12 P40   | IL-12 P70   | IL-16       | IL-6        | Gro-alpha    |
|-------------------|-------------|-------------|-------------|-------------|-------------|--------------|
| Control           | 0.055±0.007 | 0.311±0.066 | 0.254±0.068 | 0.436±0.091 | 0.167±0.072 | 0.048±0.0033 |
| Hydrogel membrane | 0.062±0.006 | 0.291±0.056 | 0.231±0.043 | 0.410±0.216 | 0.151±0.059 | 0.046±0.0004 |

Numbers are expressed as Mean ± Standard Error of the Mean (SEM)

## Conclusion

The GA-crosslinked membranes were far more suitable for drug delivery than the UV(SB)-crosslinked membranes, which were unstable. The GA-crosslinked membranes retained their structural integrity in release buffer, while the UV (SB)-crosslinked membranes did not. They degraded in physiological temperatures, showed rapid mass changes in TGA, and the FTIR was unable to confirm crosslinking in the UV(SB) membranes. Overall, though UV(SB) was able to partially crosslink the membranes; it did not do so to the same degree as GA. However, UV(SB) crosslinking may still retain potential in other areas of drug delivery, such as in skin patches with longer irradiation and additional freeze/drying cycles. The results from this study give insight into the minimum presence of cytokines, which are indicators of an innate immune response. Based on these findings, the presence of the hydrogel will not trigger a human immune response that could potentially hinder the action of the drug therapy. This study provides the groundwork for applications of this hydrogel membrane delivery system in various infection models, specifically ocular models.

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