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POSTGRADUATE DENTAL COLLEGE
SOUTHERN REGION OFFICE
2787 WINFIELD SCOTT ROAD, SUITE 220
JBSA FORT SAM HOUSTON, TEXAS 78234-7510
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Name of Candidate: CPT Michael Rice
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[redacted]

11 MAY 18

William J. Greenwood, COL, DC
DEAN, FORT HOOD AEGD-2yr RESIDENCY
Committee Chairperson

[redacted]

11 MAY 18

Michael Mansell, LTC, DC
DIRECTOR, FORT HOOD AEGD-2yr RESIDENCY
Committee Chairperson

[redacted]

11 MAY 18

Zachary Highberger, LTC, DC
DEPARTMENT OF ORAL MAXILLOFACIAL SURGERY
Committee Member

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Dr Michael Rice
Fort Hood AEGD-2yr Residency
Uniformed Services University
11 June 2018

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Differing Acidity Levels of Five Dipping Tobacco Products and the Possible Dental Implications

A Thesis
Presented to the Faculty of the Advanced Education in General Dentistry, Two-Year Program,
United States Army Dental Activity, Fort Hood, Texas
And the Uniformed Services University of the Health Sciences – Post Graduate Dental College
In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Oral Biology

By
Michael R. Rice, CPT, USA, DC
May 2018

Differing Acidity Levels of Five Dipping Tobacco Products and the Possible Dental Implications

A REPORT ON

Research project investigating differences in pH of aqueous mixtures of five different dipping tobacco products.

By
Michael R. Rice, CPT, DC, USA
D.D.S., University of Southern California - 2014

Staffed By
Zachary Highberger, LTC, DC, USA
D.M.D., University of Pittsburgh - 2008

Fort Hood, Texas
May 2018

ABSTRACT

Purpose: The purpose of this study is to compare pH differences of different dipping tobacco products and provide a literature based discussion of potential dental implications.

Methods: Fifty (50) total samples prepared from five dipping tobacco products. For each sample, 2.00 grams of dipping tobacco weighed using a scientific scale and weighing paper. The dipping tobacco was then transferred to a 25-mL graduated cylinder and 20 mL of reagent grade water was added. pH readings were then taken for each sample. For the first sample of each tobacco product, pH was measured at 5, 15, and 30 minutes. If there was no systematic variation in pH with time, the subsequent nine samples were measured at 5 minutes. Final pH determination was recorded to an accuracy of two decimal places for each sample.

Results: Of the five products tested, Grizzly Long Cut Wintergreen showed the highest mean pH of 8.19. Skoal Long Cut Classic Wintergreen had the lowest mean pH at 6.88. The thus null hypothesis is rejected. In other words, a significant difference exists in the measured pH between the different aqueous mixtures of dipping tobacco products. No significant difference between the mean pH for the Skoal Long Cut Berry Tobacco Blend dipping tobacco and the Skoal Long Cut Classic Wintergreen tobacco. However, all other means are statistically different.

Conclusion: Statistically significant differences exist between the pH levels of different dipping tobacco products when prepared in aqueous mixture.

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DISCLAIMER

The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the view of the Department of Defense or the Uniformed Services University of the Health Sciences.

TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION

1.1 Background

This study examines the acidity, or basicity, of smokeless tobacco in aqueous solution, measured using the pH scale.

This study will focus on the variety of smokeless tobacco known as dipping tobacco (also known as moist snuff, or dip) as this is the most common form, traditionally accounting for over 90% of the smokeless tobacco market share[1]. Furthermore, sales of dipping tobacco in the US increased by 65.6% between 2005 and 2011. (3) Finally, this study focuses specifically on flavored dipping tobacco products. Sales of flavored forms of dipping tobacco increased by 72.1%.9 between 2005 and 2011[2].

Comprehensive reports are available regarding the physical and chemical properties of tobacco products, including pH[1,2.] However, due to the continued introduction of new flavors of dipping tobacco to the market, it is incumbent on the scientific community to continue the study of contemporary products.

1.2 Military Relevance

Significant differences in rates of smokeless tobacco use exist between military and civilian populations. In 2011, the overall prevalence of smokeless tobacco use among military personnel was 12.8%., compared to 2.7% amongst all US adults[3].

A recent survey regarding smokeless tobacco use was completed by dental patients at Army installation Fort Bragg, North Carolina. Just over half of smokeless tobacco users reported initiation of use during or after basic training. More than thirty percent reported never having been talked to about quitting, and Army reports suggest zero percent utilization of smokeless tobacco cessation programs. Finally, respondents cited stress as the primary barrier to quitting[4].

In order to maximize military relevance of this study, all dipping tobacco products tested were purchased at a Post Exchange store within Fort Hood, Texas.

1.3 Purpose and Hypothesis

The purpose of this study is to compare pH differences of different dipping tobacco products and provide a literature based discussion of potential dental implications.

Aqueous mixtures of five dipping tobacco products will be prepared, and mean pH values will be analyzed.

The hypothesis is that there will be a difference in pH between the different varieties of dipping tobacco.

The null hypothesis is that there will be no difference in pH between the different varieties of dipping tobacco.

1.4 Study Limitations

There are three major limitations to this study.

The first major limitation is that sample mixtures for pH measurement are created by mixing dipping tobacco and water, thus poorly simulating conditions *in vivo*. Human saliva importantly possesses pH buffers, primarily in the form of phosphate buffers. Previous in vitro studies have used over-the-counter (OTC) sialagogues for sample fabrication. Unfortunately, these products serve to replace certain functions of salivary flow, but don't actually resemble the physical characteristics of human saliva. For example, OTC sialagogues may have lubricating proteins that render them acidic. Thus the experimenter has chosen to instead, per federal guidelines, to use reagent grade water in mixture preparation[5,6].

The next major limitation is that the study focuses on pH as opposed to dental caries. Thus any results of this study cannot be used to suggest clinical implications. However, this study may serve as a pilot study for future research into the product-specific cariogenicity of dipping tobacco.

The last major limitation is the limited number of study samples. This study focuses on only five different dipping tobacco products.

CHAPTER 2 LITERATURE REVIEW

2.1 Foodstuffs and Dental Caries

The importance of alkalinity and acidity in the oral environment has long been studied in the field of dentistry[7]. Past literature shows a relationship between dental caries and an acidic oral environment[8,9]. Additionally, literature shows that the pH of the oral cavity is affected by things introduced to the oral cavity[10], including foodstuffs, beverages, sweets, and possibly dipping tobacco.

2.2 Federal Law

Under the Comprehensive Smokeless Tobacco Health Education Act of 1986, tobacco manufacturers report annually to the Centers for Disease Control and Prevention (CDC) on the pH of their smokeless tobacco products. This information however is considered confidential and in accordance with federal law cannot be released to the public[11,12,13]. Therefore, it is incumbent upon the scientific community to ascertain this information.

2.3 Nicotine Chemistry

Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a pKa of 8.0. In its ionized state, such as in acidic environments, nicotine does not rapidly cross biologic membranes. However, at a higher pH a greater proportion of nicotine exists in the un-ionized state (“free nicotine”), which more readily crosses biologic membranes[14].

Smokeless tobacco is known to contain a number of additives, including bicarbonate salts[2]. It has been suggested that during smokeless tobacco production, various alkaline agents are added that boost pH and increase the amount of free nicotine that can be delivered to the user[15]. Prior studies examining pH levels and levels of free nicotine in different smokeless tobacco products show a large variance from product to product[1].

2.4 Dental Effects of Dipping Tobacco

2.4.1 Carcinogenicity

Extensive literature exists about the possible carcinogenicity of dipping tobacco products. This topic is outside of the scope of the present study.

2.4.2 The 1988 Spring Training Study

The most robust clinical study to-date regarding the oral effects of smokeless tobacco use comes from the study of professional baseball players participating in Spring Training in 1988[16]. Data collected from this study lead directly to the 1990 publication of separate Journal of Periodontology (JOP) and Journal of the American Medical Association (JAMA) articles about the oral effects of smokeless tobacco use[17,18].

This study involved 1109 subjects, from seven major league organizations, playing in the Phoenix and Tucson areas. Participants received extensive medical and dental evaluations.

Analysis adjusted for age, race, cigarette smoking, alcohol consumption, and dental hygiene practices. The overall prevalence of dental caries, gingivitis, and plaque did not differ between ST users and nonusers. However, in analysis confined to the facial surfaces of mandibular incisor teeth, where ST is most commonly used, there were significant increases among users in both gingival recession and attachment loss[16,17].

2.4.3 Effects on the Periodontium

Various studies have contradicted the findings of the spring training studies, instead failing to show any significant differences between users and non-users of smokeless tobacco[19,20].

Systematic reviews conducted in 1986, 2003, and 2007 all note the inconclusively of past studies. These reviews do however note that limited evidence does suggest an association between smokeless tobacco use and gingival recession[21,22,23].

2.4.4 Effects on the Dentition

Similarly to the periodontal effects of smokeless tobacco, studies reveal differing results as to the possible effects on the dentition of smokeless tobacco use[24]. Again, multiple published reviews reflect these incongruities[21,22,23].

Perhaps the strongest evidence of the possible risk of dipping tobacco use to caries development comes from 1999 JADA article titled "Chewing Tobacco Use and Dental Caries Among U.S. Men." This study took data from the Third National Health and Nutrition Examination Survey. Studying 14807 dentate adults, researchers found a dose-dependent relationship between weekly tobacco use and the likelihood of having a decayed or filled root surface[25].

2.4.5 The 1980 JADA Articles

It has been speculated that smokeless tobacco may have both cariogenic and cariostatic properties[26,27].

In 1980, JADA published two articles assessing and discussing the dental implication of two particular components of smokeless tobacco: sugar (sucrose) and fluoride. Researchers used gas liquid chromatography to measure the sugar content of various smokeless tobacco products, and a pH meter with a fluoride ion activity electrode to measure fluoride content.

The researchers found large variations in both sugar and fluoride content from product to product. Researchers concluded that the ideal smokeless tobacco should contain a relatively low-sugar/high-fluoride content to minimize promotion of dental caries[26,27].

2.4.6 Present Study

The present study seeks to build upon the 1980 studies by assessing and discussing a third property of dipping tobacco which may affect dental caries susceptibility: acidity.

CHAPTER 3 MATERIALS AND METHODS

3.1 Product Selection

In order to maximize military relevance of this study, all dipping tobacco products tested were purchased at a Post Exchange store within Fort Hood, Texas.

The varieties of dipping tobacco to be tested were carefully selected based on popularity, as judged by market share.

The three largest selling dipping tobacco brands are: Grizzly, Copenhagen, and Skoal[28]. The most popular flavor of dipping tobacco is Wintergreen. Three of the five products tested are the wintergreen varietals from each of the top-selling brands[29]. Two additional flavors have been included, both of which are manufactured by the Skoal label.

3.2 Experimental Design

Experimental materials and methods will adhere to guidelines set forth in the Federal Register regarding measurement of the pH of smokeless tobacco products[5,6].

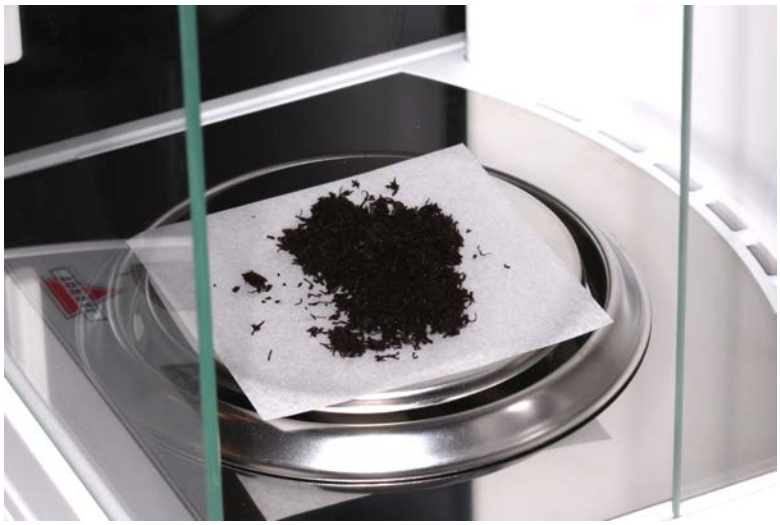
3.3 Sample Preparation

Fifty (50) total samples prepared. Ten (10) samples using each of the following five dipping tobacco products were prepared:

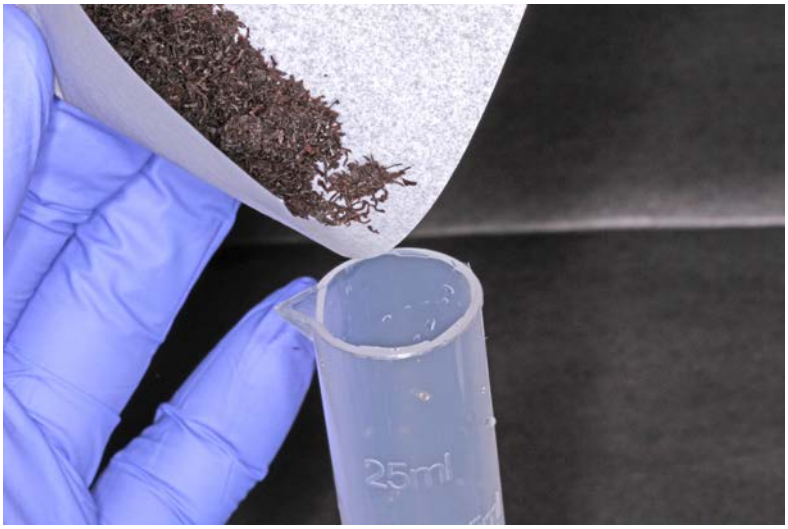
1) Copenhagen Long Cut Wintergreen
2) Grizzly Long Cut Wintergreen
3) Skoal Long Cut Classic Wintergreen
4) Skoal Long Cut Berry Tobacco Blend
5) Skoal Long Cut Citrus Tobacco Blend

A single canister of each product was used for sample collection.

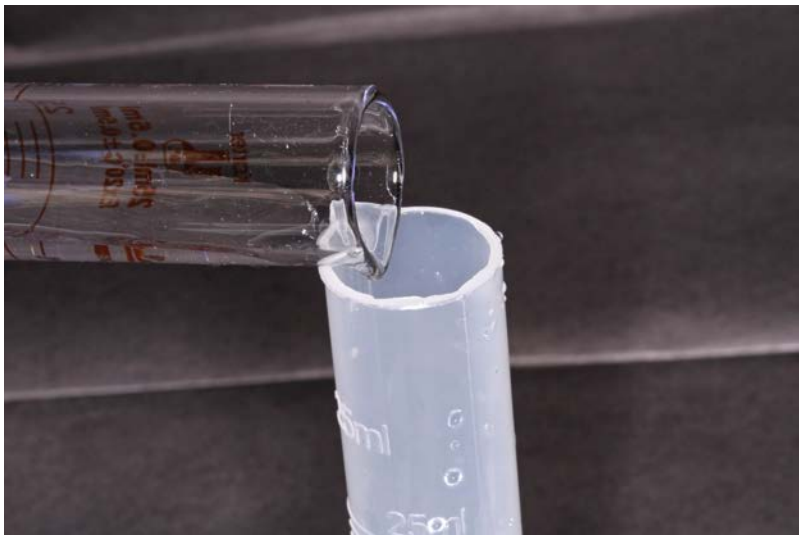
For each sample, 2.00 grams of dipping tobacco weighed using a scientific scale and weighing paper.



The dipping tobacco was then transferred to a 25-mL polypropylene graduated cylinder.



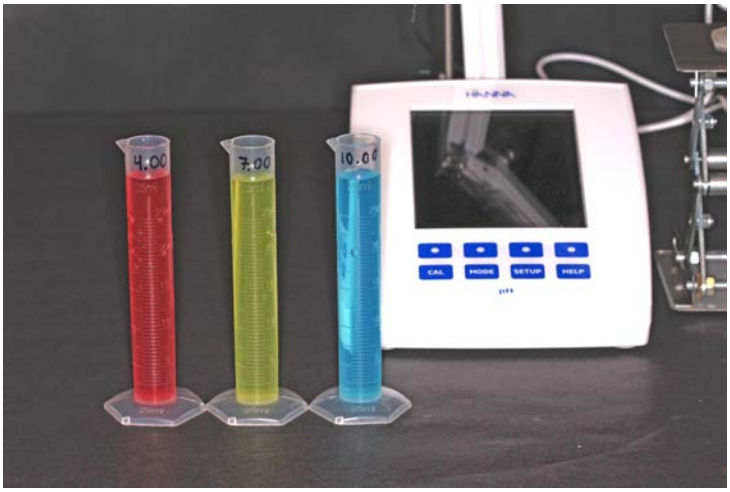
For each sample, 20 mL of reagent grade water was measured using a 25-mL borosilicate glass graduated cylinder and poured into the graduated cylinder containing the measured dipping tobacco.



Each mixture was created immediately before data collection.

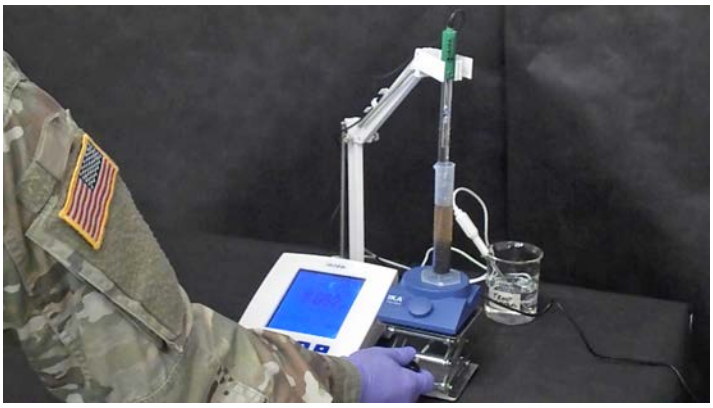
3.4 pH Meter Calibration

A three-point calibration of the pH meter will be performed to an accuracy of two decimal places using standard pH buffers (4.00 and 7.00 or 7.00 and 10.00).



3.5 Procedure

A teflon-coated magnetic stirring bar was placed in each sample. The mixture was stirred using a magnetic stirrer placed atop a laboratory jack. The laboratory jack was elevated to allow immersion of the pH probe into the mixture.



For the first sample of each tobacco product, pH measured at 5, 15, and 30 minutes. If there was no systematic variation in pH with time, the subsequent nine samples were measured at 5 minutes. All five dipping tobacco products showed no systematic variation with time.

Between each sample measurement the pH electrode was rinsed thoroughly with reagent grade water.

Final pH determination recorded to an accuracy of two decimal places for each sample.

CHAPTER 4 RESULTS

4.1 Mean pH Values

IBM SPSS software used for statistical analysis. Results for the mean pH for each tobacco brand and flavor are shown below:

Final pH

Brand and Flavor	Mean	N	Std. Deviation
Grizzly LC Wintergreen	8.19340	10	.029345
Copenhagen LC Wintergreen	7.56310	10	.023974
Skoal LC Classic Wintergreen	6.87760	10	.034225
Skoal LC Berry Blend	6.90590	10	.021362
Skoal LC Citrus Blend	7.29590	10	.039884
Total	7.36718	50	.491304

4.2 One-way ANOVA

One-way ANOVA was performed to analyze the differences in the mean pH values for each tobacco product:

ANOVA

Final pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.786	4	2.946	3165.219	.000
Within Groups	.042	45	.001		
Total	11.828	49			

The significance value obtained from one-way ANOVA allows for the rejection of the null hypothesis ($p<0.001$).

4.3 Post-hoc Testing

Post-hoc testing was performed using Tukey's Range Test:

Dependent Variable: Final pH

Tukey HSD

(I) Brand and Flavor	(J) Brand and Flavor	Mean Difference (I–J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Grizzly LC Wintergreen	Copenhagen LC Wintergreen	.630300*	.013645	.000	.59153	.66907
	Skoal LC Classic Wintergreen	1.315800*	.013645	.000	1.27703	1.35457
	Skoal LC Berry Blend	1.287500*	.013645	.000	1.24873	1.32627
	Skoal LC Citrus Blend	.897500*	.013645	.000	.85873	.93627
Copenhagen LC Wintergreen	Grizzly LC Wintergreen	-.630300*	.013645	.000	-.66907	-.59153
	Skoal LC Classic Wintergreen	.685500*	.013645	.000	.64673	.72427
	Skoal LC Berry Blend	.657200*	.013645	.000	.61843	.69597
	Skoal LC Citrus Blend	.267200*	.013645	.000	.22843	.30597
Skoal LC Classic Wintergreen	Grizzly LC Wintergreen	-1.315800*	.013645	.000	-1.35457	-1.27703
	Copenhagen LC Wintergreen	-.685500*	.013645	.000	-.72427	-.64673
	Skoal LC Berry Blend	-.028300	.013645	.249	-.06707	.01047
	Skoal LC Citrus Blend	-.418300*	.013645	.000	-.45707	-.37953
Skoal LC Berry Blend	Grizzly LC Wintergreen	-1.287500*	.013645	.000	-1.32627	-1.24873
	Copenhagen LC Wintergreen	-.657200*	.013645	.000	-.69597	-.61843
	Skoal LC Classic Wintergreen	.028300	.013645	.249	-.01047	.06707
	Skoal LC Citrus Blend	-.390000*	.013645	.000	-.42877	-.35123
Skoal LC Citrus Blend	Grizzly LC Wintergreen	-.897500*	.013645	.000	-.93627	-.85873
	Copenhagen LC Wintergreen	-.267200*	.013645	.000	-.30597	-.22843
	Skoal LC Classic Wintergreen	.418300*	.013645	.000	.37953	.45707
	Skoal LC Berry Blend	.390000*	.013645	.000	.35123	.42877

*. The mean difference is significant at the 0.05 level.

The results of the Tukey's range show no significant difference between the mean pH for the Skoal Long Cut Berry Tobacco Blend dipping tobacco and the Skoal Long Cut Classic Wintergreen tobacco. All other means are statistically different. These same relationships can also be seen below in a homogenous groups analysis of the above post-hoc testing:

Final pH

Tukey HSD[a]

Brand and Flavor	N	Subset for alpha = 0.05			
		1	2	3	4
Skoal LC Classic Wintergreen	10	6.87760			
Skoal LC Berry Blend	10	6.90590			
Skoal LC Citrus Blend	10		7.29590		
Copenhagen LC Wintergreen	10			7.56310	
Grizzly LC Wintergreen	10				8.19340
Sig.		.249	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

CHAPTER 5 DISCUSSION

5.1 Interpretation of Results

Of the five products tested, Grizzly Long Cut Wintergreen showed the highest mean pH of 8.19. Skoal Long Cut Classic Wintergreen had the lowest mean pH at 6.88. This equates to a pH range of 1.31. The mean pH of all 50 samples was 7.37 with a standard deviation of 0.49.

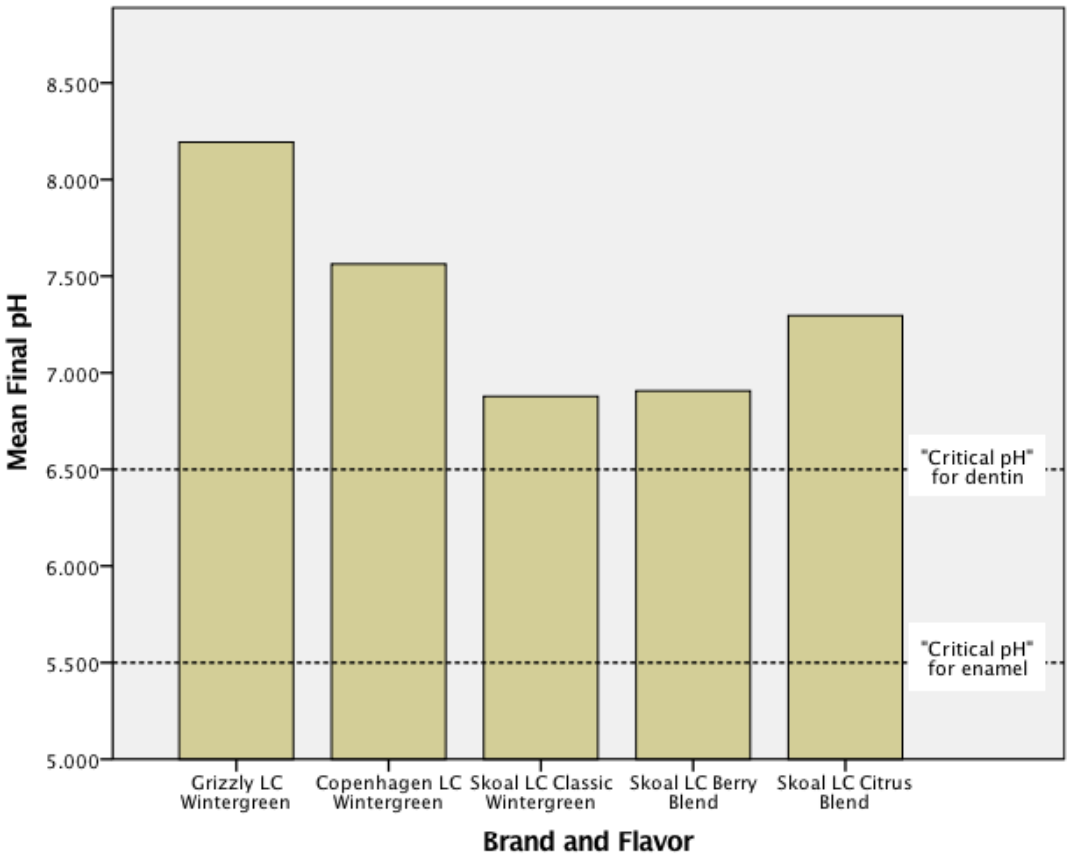
The standard deviations within each group (product) were relatively low, the highest being 0.4 in the Skoal Long Cut Citrus Tobacco Blend group. These small standard deviations can be attributed to the precision of sample preparation. Additionally, these small standard deviation values suggest large homogeneity within a particular canister of dipping tobacco.

One-way ANOVA yielded a p-value of 0.00 when measured to two decimal places. This is less than the significance value of 0.05. The thus null hypothesis is rejected. In other words, a significant difference exists in the measured pH between the different aqueous mixtures of dipping tobacco products.

Post-hoc testing was performed using Tukey's range test to analyze to the specific relationships between groups. The results show no significant difference between the mean pH for the Skoal Long Cut Berry Tobacco Blend dipping tobacco and the Skoal Long Cut Classic Wintergreen tobacco. However, all other means are statistically different.

5.2 Relationship of Mean pH Calculations to "critical pH" Levels

The graph below shows how the mean pH values of each product relate to literature reported values for approximate "critical pH" of both enamel and dentin[30,31].



None of the five products tested had a measured pH below the critical pH levels for enamel and dentin. This suggests that these dipping tobacco products are unlikely to cause tooth structure dissolution by means of chemical erosion alone.

5.3 Conflicting Prior Studies

The 1980 JADA studies, which discussed the opposing effects of sugar and fluoride content in smokeless tobacco, attempted to quantify the cariogenicity of different dipping tobacco products relative to each other. This study did conclude that wide differences may exist in cariogenicity from one product to the next[26,27].

Considering the results of the 1980 JADA studies, it is possible that the differences amongst the different dipping tobacco products is contributory towards the varying results of clinical studies. According to the results of a CDC study, pH is a primary factor in the amount of nicotine that is

in the most readily absorbable, unprotonated form, and that the brands of dipping tobacco with the largest amount of unprotonated nicotine also are the most frequently sold brands[15]. Thus it may be that clinical studies are predisposed to fail to show a link between dipping tobacco use and dental caries if the study subjects are in fact using the most frequently sold brands.

5.4 Ethical Considerations

There are five fundamental principles that form the foundation of the American Dental Association Code of Professional Conduct: patient autonomy, nonmaleficence, beneficence, justice and veracity[32].

Practitioners may face ethical dilemmas when discussing the effects of dipping tobacco with patients. A practitioner may fail to discuss the inconclusiveness of the literature regarding the adverse oral effects of smokeless tobacco. In this situation, a practitioner may experience a conflict between the principle of veracity and the principles of beneficence and nonmaleficence.

Practitioners may also face ethical dilemmas when comparing different tobacco products with a patient who is unwilling to quit, and who inquires as to which products are less detrimental. Based on the results of this study, a dentist may be apt to advise a higher pH product over a lower one in hopes of minimizing caries susceptibility. Many higher pH products however are likely to allow more free nicotine absorption, placing the patient at an increased risk of harm. Thus it is prudent for the dental practitioner to be well informed and advise only within his or her scope of practice.

CHAPTER 6 CONCLUSION

Statistically significant differences exist between the pH levels of different dipping tobacco products when prepared in aqueous mixture.

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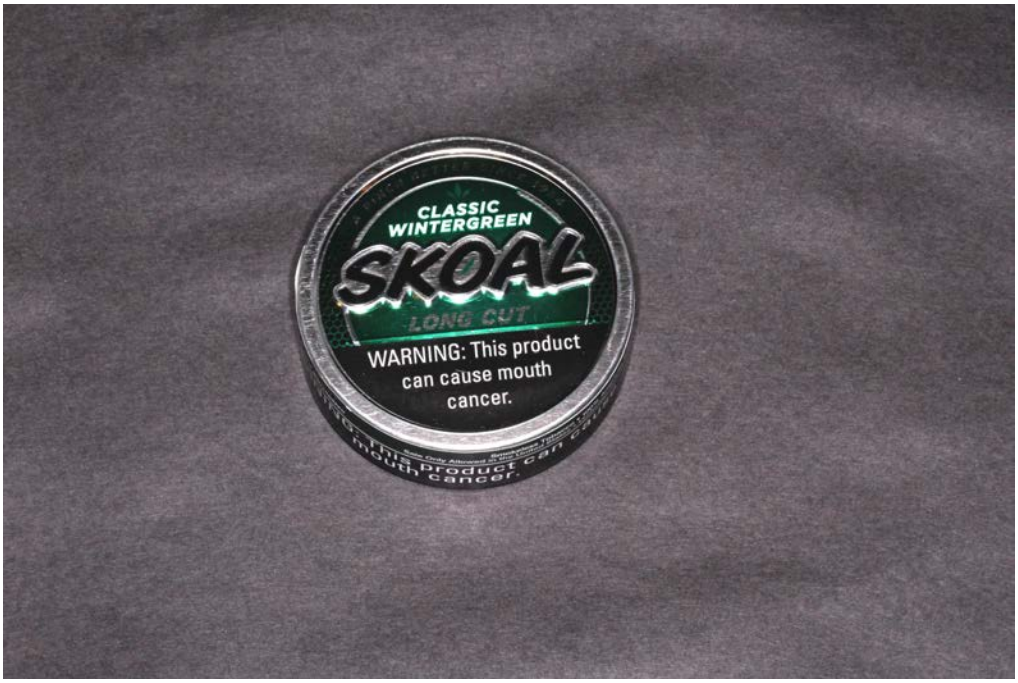
APPENDIX A MATERIALS

1) Grizzly Long Cut Wintergreen

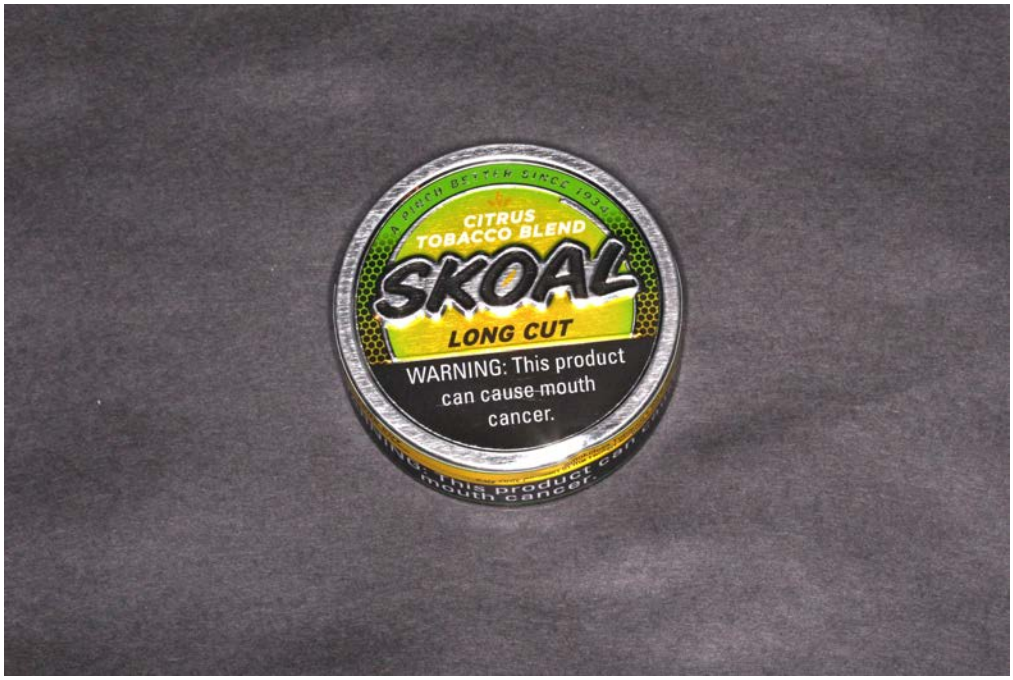
2) Copenhagen Long Cut Wintergreen

3) Skoal Long Cut Classic Wintergreen



4) Skoal Long Cut Berry Tobacco Blend

5) Skoal Long Cut Citrus Tobacco Blend

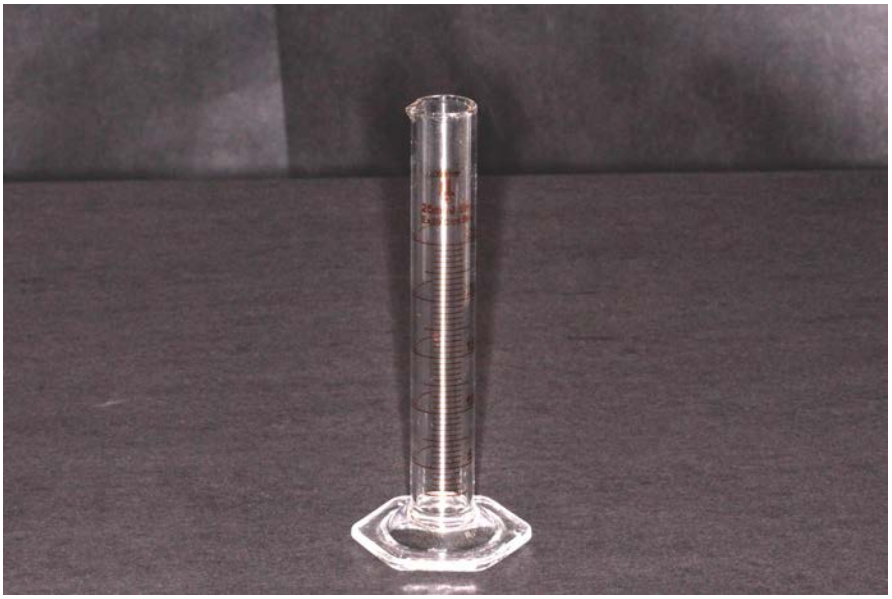


6) 100-gram calibration weight

7) Torbal Model AGZN100 precision scientific scale

8) 3”x3” glassine weighing paper

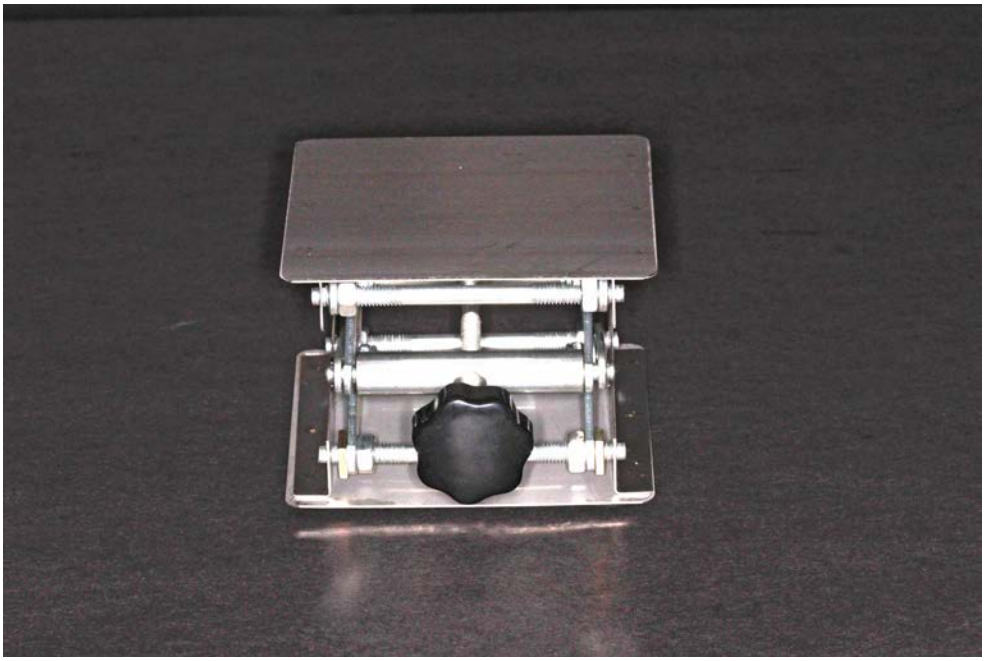
9) Karter Scientific 25-mL borosilicate glass graduated cylinder



10)) 25-mL polypropylene graduated cylinders

11) Laboratory wash bottle

12) 4”x4” Aluminum Scientific Lab Jack



13) IKA Topolino magnetic stirrer

14) 6x15mm Teflon (PTFE) coated octagonal stirring rods

15) RICCA Chemical Company ACS Reagent Grade Water (ASTM Type I, ASTM Type II) with MSDS

RICCA CHEMICAL COMPANY®

Safety Data Sheet

SECTION 1: Identification

1.1. Product Identifier

Trade Name or Designation: Water, ACS Reagent Grade, ASTM Type I, ASTM Type II
Packaged in plastic containers

Product Number: 9150

Other Identifying Product Numbers: 9150-1, 9150-1CS, 9150-1CT, 9150-2.5, 9150-32, 9150-5, 9150-500, 9150-55, 9150-5HP

1.2. Recommended Use and Restrictions on Use

General Laboratory Reagent

1.3. Details of the Supplier of the Safety Data Sheet

Company: Ricca Chemical Company

Address: 448 West Fork Drive
Arlington, TX 76012 USA

Telephone: 888-467-4222

1.4. Emergency Telephone Number (24 hr)

CHEMTREC (USA) 800-424-9300
CHEMTREC (International) 1+ 703-527-3887

SECTION 2: Hazard(s) Identification

2.1. Classification of the Substance or Mixture (in accordance with OSHA HCS 29 CFR 1910.1200)

For the full text of the Hazard and Precautionary Statements listed below, see Section 16.

This product is not categorized as hazardous in any GHS hazard class.

2.2. GHS Label Elements

Pictograms: None required.

Signal Word: None required.

Hazard Statements: None required.

Precautionary Statements: None required.

16) Innovating Science pH Buffer Calibration Kit with MSDS

SDS No.: BBR404 **SAFETY DATA SHEET** **GENERAL STORAGE CODE GREEN**

Section 1 **Chemical Product and Company Identification** Page E1 of E2

Innovating Science® by Aldon Corporation
"cutting edge science for the classroom"

221 Rochester Street
Avon, NY 14414-9409
(585) 226-6177

CHEMTREC 24 Hour Emergency
Phone Number (800) 424-9300
For laboratory use only.
Not for drug, food or household use.

Product	**BUFFER SOLUTION, PH4 (RED COLOR CODED)**
Synonyms	Standard Buffer Solution, pH 4.00

Section 2 **Hazards Identification**

Signal word: WARNING
Pictograms: None required
Target organs: None known

GHS Classification:
Skin irritation (Category 3)
Eye irritation (Category 2B)

GHS Label information: **Hazard statement(s):**
H316: Causes mild skin irritation.
H320: Causes eye irritation.

Precautionary statement(s):
P264: Wash hands thoroughly after handling.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313: If skin irritation occurs: Get medical attention.
P337+P313: If eye irritation persists: Get medical attention.

Ca Prop 65: This product does not contain any chemicals known to the State of California to cause cancer or reproductive toxicity.

Section 3 **Composition / Information on Ingredients**

Chemical Name	CAS #	%	EINECS
Water	7732-18-5	98.52%	231-791-2
Acetic acid, glacial	64-19-7	0.99%	231-913-4
Sodium acetate	127-09-3	0.49%	215-185-5
FD&C Red #40 (C.I.No. 16035)	25956-17-6	Trace	247-368-0

Section 4 **First Aid Measures**

INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

EYE CONTACT: Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention.

SKIN ABSORPTION: Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention.

Section 5 **Fire Fighting Measures**

Suitable Extinguishing Media: Use any media suitable for extinguishing supporting fire

Protective Actions for Fire-fighters: In fire conditions, wear a NIOSH/MSHA-approved self-contained breathing apparatus and full protective gear. Use water spray to keep fire-exposed containers cool.

Specific Hazards: During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.

Section 6 **Accidental Release Measures**

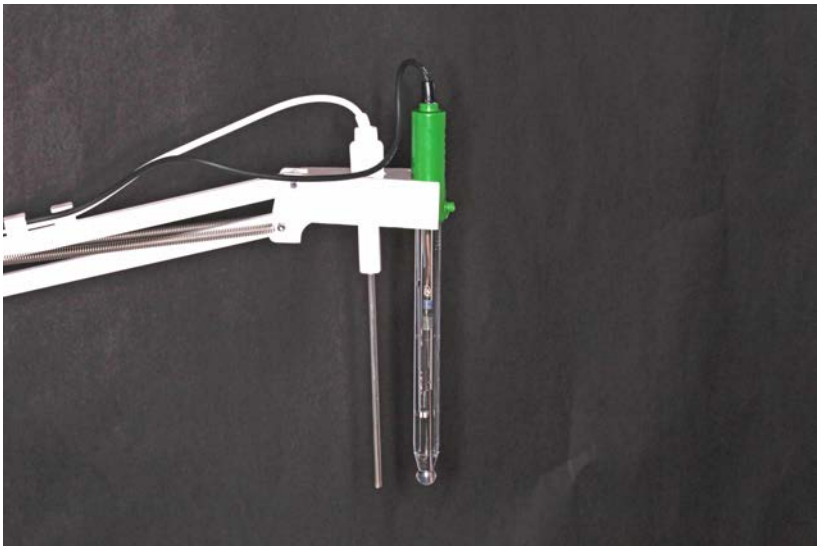
Personal Precautions: Evacuate personnel to safe area. Use proper personal protective equipment as indicated in Section 8. Provide adequate ventilation.

Environmental Precautions: Avoid runoff into storm sewers and ditches which lead to waterways.

Containment and Cleanup: Absorb with inert dry material, sweep or vacuum up and place in a suitable container for proper disposal. Wash spill area with soap and water.

17) Hanna instruments HI5221-01 Laboratory Research Grade Benchtop pH/mV Meter with 0.001 pH Resolution

18) HI1131B-Refillable Combination pH Electrode with BNC Connector



APPENDIX B CALIBRATION PHOTOS

1) Scale Calibration:

2) pH Meter Calibration:

APPENDIX C STATISTICAL ANALYSIS

1) Levene's test was performed to verify homogeneity of variance:

Test of Homogeneity of Variances

Final pH

Levene Statistic	df1	df2	Sig.
.806	4	45	.528

A measured significance value of 0.528 does not allow for the rejection of the null hypothesis that population variances are equal. In other words, homogeneity of variance is confirmed.

2) Below is a detailed reporting of the results of ANOVA:

Dependent Variable: Final pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	11.786[a]	4	2.946	3165.219	.000	.996
Intercept	2713.767	1	2713.767	2915277.52	.000	1.000
Brand	11.786	4	2.946	3165.219	.000	.996
Error	.042	45	.001			
Total	2725.595	50				
Corrected Total	11.828	49				

a. R Squared = .996 (Adjusted R Squared = .996)

The strength of association between the independent and dependent variable is measured as an effect size, partial eta squared. Partial eta squared equals 0.996. In other words, 99.6% of the variability in pH is explained by the type of tobacco used in the sample mixture.

3) The results of one-way ANOVA testing are reported as:

$$F(4,5)=3,165.219, p<.001, n^2_p=0.996$$

APPENDIX D DATA TABLES

1) Grizzly Long Cut Wintergreen

	pH		
Sample	Five minutes	Fifteen minutes	Thirty minutes
1	8.175	8.196	8.231
2	8.152		
3	8.243		
4	8.207		
5	8.169		
6	8.177		
7	8.192		
8	8.210		
9	8.165		
10	8.188		

2) Copenhagen Long Cut Wintergreen

	pH		
Sample	Five minutes	Fifteen minutes	Thirty minutes
1	7.565	7.572	7.588
2	7.550		
3	7.549		
4	7.57		
5	7.551		
6	7.543		
7	7.560		
8	7.601		
9	7.592		
10	7.527		

3) Skoal Long Cut Classic Wintergreen

	pH		
Sample	Five minutes	Fifteen minutes	Thirty minutes
1	6.884	6.886	6.893
2	6.900		
3	6.885		
4	6.922		
5	6.86		
6	6.817		
7	6.844		
8	6.915		
9	6.846		
10	6.894		

4) Skoal Long Cut Berry Tobacco Blend

	pH		
Sample	Five minutes	Fifteen minutes	Thirty minutes
1	6.858	6.865	6.878
2	6.928		
3	6.924		
4	6.918		
5	6.935		
6	6.906		
7	6.879		
8	6.915		
9	6.882		
10	6.894		

5) Skoal Long Cut Citrus Tobacco Blend

	pH		
Sample	Five minutes	Fifteen minutes	Thirty minutes
1	7.295	7.305	7.385
2	7.255		
3	7.308		
4	7.244		
5	7.297		
6	7.320		
7	7.280		
8	7.268		
9	7.291		
10	7.311		