Something Old, Something Blue: An Analysis of Identification Methods for Determining the Presence of Indigo Within Historic Textiles for The Charleston Museum

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SOMETHING OLD, SOMETHING BLUE: AN ANALYSIS OF IDENTIFICATION
METHODS FOR DETERMINING THE PRESENCE OF INDIGO WITHIN HISTORIC
TEXTILES FOR THE CHARLESTON MUSEUM

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Historic Preservation

by
Michael Christopher Cone
May 2024

Accepted by:
Dr. Stéphanie Cretté, Committee Chair
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ABSTRACT

Identification methods for the analysis of indigo dye within textiles have been compiled in multiple studies. Though few examples demonstrate chemical analysis and phase separation as a technique that can assist in the identity of the dyestuff. The inclusion of nontechnical methods, techniques that require little knowledge of chemistry and can be completed without the assistance of high-tech machinery, can contribute toward these efforts. The goal of this thesis was to provide a simple testing procedure where eight historic textile samples dating from the mid-eighteenth to the mid-nineteenth centuries were subjected to chemical analysis and phase separation to determine the presence of indigo for The Charleston Museum. A reducing solution of 1.25 g sodium hydroxide, 1.25 g sodium dithionite, and 25 mL deionized water was combined and introduced into Eppendorf tubes containing the historic samples to facilitate a chemical reaction. The tubes were added to a water bath at 40 C for 2-3 minutes to assist in the reaction, an observable color change that could be recorded as part of phase 1. Phase 2 consisted of adding ethyl acetate following chemical analysis to bring about phase separation, where the internal components would separate and result in two layers. Provided phase separation occurred, the top layer was extracted, and the color was noted. Findings suggest that these methods are relatively effective in determining the identity of indigo dyestuffs within historic textiles since six out of the eight samples tested were found to be positive for indigo. The data recorded in this thesis can be used to assist
future museum professionals, and conservators when examining historic textiles and determining best methods of indigoid dye analysis.
DEDICATION

To Mom, Dad, & Carol — I don’t ever expect you to want to read this, but I have appreciated the love and support throughout this experience.

To Susan, & Hannah — Thank you for being the best of friends and keeping me sane. Love you!
ACKNOWLEDGMENTS

I would like to extend my gratitude toward several individuals that assisted in the completion of this thesis. Thank you to my committee chair, Dr. Stéphanie Cretté, for taking time to meet with me, and offering advice and guidance throughout this process. Thank you to Lisa Arslaner and Kate Dieringer at the Warren Lasch Conservation Center. You both were there for me when this was first being formed and worked with me to determine if it was possible. Thank you for your patience, counseling, and being there to guide me. I learned to have fun and just enjoy the science and have gained two friends as a result.

Thank you to Virginia Theerman at The Charleston Museum for giving my initial thesis topic consideration and moving it forward through the proper channels. You invited me into the Museum and took the time to navigate this field with me. I appreciate your guidance during the research portion and your edits only strengthened the content, making me a better writer in the end. Thank you to The Charleston Museum for approving my request and allowing me to work with you. It has been a privilege.

Thank you to Dr. Jon Marcoux, Amalia Leifeste, and Carter Hudgins, who were all either part of the initial idea or supported this journey by asking questions, offering insight and just being available. I really appreciate it.

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CHAPTER ONE
INTRODUCTION

Within the world of collection management, museum workers often rely upon archived information to provide insight into an object’s historical background or provenance. These pieces, often bestowed to organizations, might lack fundamental knowledge that would otherwise indicate the object’s origins and overall makeup. In particular, these records can contain clues about original materials, patterns, colors and/or dye makeup. While these values can also be distinguished through simple observation, scientific analysis provides empirical evidence on which to classify and categorize items. Through the dye analysis of historic textile fibers, this research will examine the veracity of written records by determining if indigo is present.

The primary goal of this thesis is to ascertain whether each of The Charleston Museum’s eight historic textile samples, dating from the late 18th to mid 19th century, were dyed with indigo; and the subsequent significance of the dye within the local contemporary landscape. These pieces have been selected in consultation with Virginia Theerman, Curator of Historic Textiles for The Charleston Museum, due to the assumption that most, if not all, contain indigo. The range of textiles include women’s garments, block print fragments, and coverlets that originate from the Lowcountry, England, and France. The majority predominantly point to a higher-class status; however, it does not entirely exclude other socioeconomic factors. While most of these fabrics may have been intended for an elite class, some if not all would have been produced by enslaved hands.

By subjecting each sample to chemical analysis, a method that when exposed to an aqueous reducing solution of sodium dithionite and sodium hydroxide, results in an observable color change that will determine phase 1 of testing. In phase 2, samples are exposed to ethyl
acetate resulting in phase separation. The top separated layer is extracted, and the color noted. If blue, a positive identification for indigo will be recorded.

Before synthetic dyes were implemented in the mid-to-late 19th century, natural dyes were gathered from plant life, insects, and some bodily fluids. These consisted of common and wild varieties such as woad, indigo, saffron, alkanet, henna, and turmeric, to name a few. While methods differ on the procurement of the dye, typically the items were dried or fermented and ground up using a mortar or stone prior to boiling. Fermentation or the vatting process, which is done for the indigo plant, assists in the releasing of sugars and extraction of the physical dyes, turning the dye into a water-soluble compound.¹ Depending on the source and culture, dyes were used for multiple purposes including art, religious associations, and adornment on the body in the form of makeup and garments.²

Dyes are labeled according to the chemical composition or their properties. However, some dyes can be found within multiple chemical classes and thus makes the process more difficult. As for the actual name of each individual dye, it is given either from the method of application, the associated molecules that dictate the color, or the chemicals and/or matter involved within the process. There are multiple diverse types of dyes. These consist of direct, acid, basic, disperse, vat, sulfur, azo, mordant, and reactive. Each has different chemical structures, methods of application, and particular fibers that the dye is more suited to than others. For example, acid dyes are highly soluble while disperse dyes are less water soluble. Basic, sulfur, and vat dyes are all insoluble in water. Direct and reactive dyes are both water soluble. However, some dye classes share common characteristics and are suitable for many of the same

types of materials. The most common can be found within direct, vat, sulfur, azo, and reactive dyes, which all share an ability to adhere to cotton and rayon, another cellulosic fiber.³

The cultivation of indigo had been attempted in the North American colonies as early as the 17th century but did not become successful until the mid-18th century. In South Carolina, rice production was the main form of agriculture, though indigo thrived where rice could not. This meant that, unlike rice production, the land and labor needed for indigo did not have to be fashioned or changed and therefore could function easily in the natural environment.⁴

From 1740-1744, Eliza Lucas Pinckney (1722-1793) attempted to produce indigo at Wappoo Plantation, what is today known as West Ashley, Charleston, South Carolina. According to local lore, Pinckney and indigo are almost synonymous with one another, for she has been celebrated as the main contributor to the success of indigo within South Carolina. However, this view is shortsighted and slightly more complicated when assessing the realities of the historical narrative. Assuredly, her upbringing on a Caribbean plantation and knowledge of botany assisted in Pinckney’s cultivation of the crop, but she was not the only person or gentry farmer attempting to do so.⁵ It must be stated that the enslaved persons forcibly mandated to work in the fields had vital knowledge of rice and indigo production and were the majority parties responsible for its success.⁶ In response to Pinckney's attempts and eventual shipment of indigo, England devised a bounty for the Carolina farmers in an attempt to outsource the French and raise interest in it.

From 1739 to 1748, the War of Jenkins' Ear, an outright trade war between Great Britain and

³ Timár-Balázs Ágnes et al, pp. 70-71.
Spain, disrupted Britain’s indigo production that had long been supplied via St. Domingue, a French colony within the Caribbean. New supplies of indigo were needed to keep up with European demand and the North American colonies were now a viable and reliable option.\(^7\)

The three main variations of indigo consisted of *Indigofera tinctoria*, also known as “True Indigo or French Indigo,” which was derived from India, *Indigofera suffruticosa*, sourced from Guatemala and West Indies, and *Indigofera caroliniana*, the local native wild species of indigo in South Carolina. The first two were predominantly used and preferred over the native species due to the quality of the dye, as *Indigofera caroliniana* was not competitive on the same standards; it neither produced the lasting deep blue color nor was readily available.\(^8\)

To assist in the development of experimental protocols, research, and analysis, a literature review was compiled for Chapter Two. The gathered literature focuses on materials, methodologies, and case studies that cover the chemical composition and analysis of indigo. These studies allowed for the successful implementation of dye analysis and interpretative data. Further literature was sourced that provided an overview of the history of indigo, its roots both globally and locally in the Carolinas, and how it affected the late 18th and early 19th century economy. Moreover, more research was compiled examining the role that enslaved people played in indigo production along with the political and socioeconomic themes. In Chapter Three, the methodology is discussed in more detail as it relates to the sampling, techniques, and scientific data analysis of the museum textiles. In particular, this chapter elaborates on materials and usage, individual solvents, and chemical analysis. Chapter Four covers the results and discussion of each experiment. Finally, Chapter Five lists the conclusions, interpretations, and potential future

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research. Each chapter is intended to solidify and expand upon one another to better assist the Museum and subsequently future visitors, as they seek to understand the cultural and historical significance of indigo, the importance it served to multiple pockets of society, and its roots that extend far beyond South Carolina.
CHAPTER TWO
LITERATURE REVIEW

Introduction

While there are many case studies that examine different methods of dye analysis, very few focus on indigo within historic textiles. For those that do, the methods are often highly technical, have varied success rates, and show stark contrasts in both the types and quantities of solvents that should be used. With that in mind, chemical analysis is favored as an efficient model for the identification of indigo. In order to inform this study, the literature review was compiled thematically, first focusing on the history of the dyestuffs. This included its preparation, mechanization, and the European trade network from the 17th through the 19th century. Second, the major wars of the mid-eighteenth century that aided in the rise of indigo as a cash crop within the Carolinas was researched alongside established gentry within the South and the enslaved people who were crucial to indigo production. Lastly, the analysis of multiple case studies were reviewed that examine different identification methods. These methods are compared to the standards used that informed the methodological approach.
The Origins of Indigo

There are over 700 species of indigo-producing plants worldwide. The most common to Europe and parts of Asia during the eighteenth and nineteenth centuries include: *Indigofera tinctoria*, *Isatis tinctoria* (woad), *Indigofera suffruticosa*, and *Polygonum tinctoria*. Indigo found its way into European culture beginning in the sixteenth century through trade with the Portuguese and Dutch. Prior to this, woad had been the main source of blue in Europe for centuries. Woad was slowly losing popularity to the more prominent and reliable Indian indigo (*I. tinctoria*), which had higher levels of indican and were easier to transport. In response to this, woad producers and local dyers dubbed indigo the “devils' dye.” This campaign incited political upheaval and social commentary within Europe. The resulting legislation established the import of indigo as an illegal act and, depending on the country, a death sentence. In 1577, France and Germany banned the import of indigo in favor of local woad producers, while England banned imports only from 1581 to 1660. By 1609, France amended its ban to include execution. By the eighteenth century, indigo bans were lifted, and trade was once again established. This was due to the shifting of attitudes toward indigo and the trade industry for the dyestuff as European dyers began to comprehend the benefits of indigo versus woad. Indigo produced a more prominent blue color and could be sourced cheaper than the prized European woad. The woad industry had created a system of wealth since the medieval period. As indigo emerged, it disrupted this wealth, pulling money from woad merchants and tested loyalties. The nature of protectionist laws worked

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10 Amelia Peck et al, 128.
to preserve the woad industry, but the prioritization of one and the exclusion of another served to be its downfall.\textsuperscript{12}

\textit{Indigo Trade Routes:}

The original driver of the trade between the European and Indian markets was not the textile industry, but spices. In 1600, the English formed the Governor and Company of Merchants of London Trading in the East Indies and later shortened it to the East India Company (EIC). By 1708, it was changed to the United East India Company (UEIC). In 1858, it ceased operations in India and was decommissioned officially in 1873. Like the English, Dutch traders established their company in 1602 under the name Verenigde Oost-Indische Compagnie (the Dutch East India Company; VOC) and ended it in 1800. Denmark, France, Austria, and Sweden all followed suit and formed their own trade companies to compete. These trade routes combined with the added slave labor in India and across the Atlantic, drove the price of indigo down, and allowed for its use to be adopted in Western Europe and North America. Established trade pathways are depicted in Figure 1.\textsuperscript{13}

\begin{flushright}
\textsuperscript{12} Noor FK Iqbal, “Ambivalent Blues: Woad and Indigo in Tension in Early Modern Europe,” \textit{Constellations} 4, no. 1 (February 22, 2013), 287-288. \url{https://doi.org/10.29173/cons19050}
\end{flushright}
Vat Preparation of Indigo

Unlike most dyes that require a mordant, typically a metallic salt fixative, to secure the dye to the fabric, indigo does not. It relies on multiple chemical reactions that when introduced to oxygen, attach the color to the item. After the leaves have been removed from the plant, they must be crushed in water. This reveals a sometimes colorless or greenish-yellow color to the dye bath and releases the indican present within. The addition of soda ash raises the pH making the solution alkaline and transforms the indican into indoxyl. Through agitation and the introduction of oxygen, the indoxyl molecules join to form indigotin or indigo pigment, resulting in the blue color. As this happens, the previous solution morphs into a more solid or paste-like state that is

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then pressed into cakes or bricks and dried. During this process, the bricks of indigo were placed in a two-foot mold and allowed to cure. The bricks were then cut into two-inch squares and packed into barrels for transport. This was a systematic approach toward an efficient packing and transportation protocol.

The next stage in the dyeing process returns the insoluble indigotin back to its soluble form through vat dyeing. The pigment is submerged in an alkaline solution, using a reducing agent that removes the oxygen from the vat. The reducing agent strips the indigo molecule of its weakly attached oxygen. This turns indigo into leuco-indigotin, which is also referred to as indigo white, a yellowish soluble dye. Once it has fully reduced, wool, cotton, silk, hemp, or linen can be immersed into the vat solution so that the leuco-indigotin can penetrate the material. Provided the fabric consists of natural fibers and not synthetic, the color will adhere to the textile. Synthetic fibers will still produce the blue hue, but the color will not be as rich and result in a paler blue. As it is removed from the vat, leuco-indigotin reacts with oxygen to become indigo and attaches itself to the fibers. The color change from greenish yellow to blue is instantaneous.

It should be noted that distinct cultures utilized different methods, materials, ingredients, and tools that all culminated in the production of indigo dye. The dyeing process reflected the region in which it was conducted. Indigo was placed in clay pots, empty tree trunks, holes in the ground, or wooden vessels built for dyeing (Figure 1.1).

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17 Amelia Peck et al, p. 132.
19 Amelia Peck et al, p. 132.
dye-works, multiple spaces were designated to ensure there was enough room for the division of multi-color dyes, vat dyeing for indigo and woad, and a spacious drying room.\(^{21}\)

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Textile Printing & Manufacturing

Indigo printing on uncolored cotton cloth in many patterns and methods was called calico printing. Calico originated from Calicut, India, a notable example of globalization in language. Indigo could be fashioned into many varieties of textiles and was used more predominantly than any other. By 1750, it comprised more than half of England’s imports of the dyestuff. Prior to the nineteenth century, calico had been known as any cotton cloth that was derived from India. In the nineteenth century, Americans began to associate the name calico with printed textiles as opposed to its country of origin. The demand for these goods increased and printing shops appeared both in Britain and America. This was evident in the calico workshops that were seen from Boston to Charleston after the American Revolution.

The methods of calico printing varied. The most common was the resist or reserved style. Resist fabrics were made by coating areas of the fabric with wax to prevent or block the dye from settling into the fiber. Through this process, patterns could be formed and were referred to as paste prints. These patterns tended to be white while the remaining cloth was dyed blue. However, other designs implemented figures or flowers in multiple hues of blue amidst a white background. These variations depended upon personal as well as regional tastes.

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25 Susan W Greene, 70.
One of the earliest printing techniques used wooden printing blocks. This consisted of a printing table lined with a layer of protective felt used as an underlay for the intended printed cotton cloth. The cloth was secured to the table and an outline was etched with a pencil on the fabric. Oftentimes, it would be roughed up prior to printing, as it was believed that this would help the paste attach easier and prevent it from bleeding through the fabric. A “stamp pad,” or cushion made of linen held the paste and was stuffed with potato flour or feathers and had a protective oiled coating. A generous amount of paste was poured onto the pad and a wooden scraper was used to spread the paste until the desired thickness was achieved. The block was readied and pressed against the pad and was then quickly moved to the cloth. The craftsmen applied great force to the wooden block by hammering it multiple times. This added pressure ensured an even pattern and was repeated until the printing was completed. Afterwards, the textile was hung to dry for a few weeks before the paste print could be readied for transport.

Another dye process was referred to as copper plating. During the seventeenth century, this technique had been commonplace for printing with ink on silk and linen. It was designed to replicate an entire image with accuracy and often depicted intricate portrayals of country scenes, fable imagery, and battles. The design was carved out in the copper plate and was positioned within the rolling press via a metal carrier. The plate moved up and down just beneath a cylinder that was covered in a woolen material. The nature of the cylinder was to impart the mordant necessary to fuse the design to the material. As the plate moved back and forth, it was primed with mordant and passed under the cylinder to stamp the fabric. Mordant acted as a binder and

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32 Ibid, 50-52.
was necessary to secure the dye. The process of copper plate printing during the 1700s can be seen in Figure 1.2. The design Les Travaux de la Manufacture (The Activities of the Factory) was created by Christophe-Philippe Oberkampf, owner of the Royal factory specializing in the technique from the 1700s to the 1950s. This technique was difficult and time-consuming, for the plate and fabric both needed to be repositioned to ensure accuracy.

![Figure 1.2: 1700s copper plate printing design, known as “Les Travaux de la Manufacture”, Courtesy of The Cleveland Museum of Art](image)

By the eighteenth century, many developments were implemented into rolling presses. The implementation of engraved copper cylinders along with the invention of the “doctor” blade by a Scotsman named Thomas Bell, enabled the entire process to transition easily. The engraved cylinder and blade increased production and had the capability of producing upwards of

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35 Linda Eaton et al, 140.
36 Linda Eaton et al, 140.
37 Linda Eaton et al, 142.
200 to 500 calico prints per day.\textsuperscript{38} As previously stated, the cloth was coated with a mordant so that the paste could penetrate the fiber. As the fabric sat upon the roller, the excess mordant was scraped off by the blade. The cloth moved down the line through the rolling press due to the pressure of the cylinders and the pattern was applied. There was no need to reposition the cloth or machine. Despite these technological advances, copper plating and wooden block printing were still used widely and in combination with other methods of printing within Europe and North America. While most patterns were monochrome, polychrome patterns existed and were facilitated through multiple printing techniques throughout Europe and North America during the eighteenth and nineteenth centuries. This required a phase of block printing followed by cylinders that were coated with multiple colors. Though vibrant patterns could be achieved by a series of blocks, each coated in a separate color. As time progressed, the ability to print polychrome colors increased.\textsuperscript{39}

\textsuperscript{38} Giorgio Riello, 44.
\textsuperscript{39} Linda Eaton et al, 142-143.
Emergence of Indigo within the Carolinas

During the eighteenth century, warfare was rampant among the English, French, Spanish, and other European monarchs in an attempt to seize and control land as well as merchant trading. The wars that ensued affected the colonists of South Carolina but also presented opportunities for England to capitalize on trade with the colonies. These included the War of Jenkin’s Ear (1739-1748), the War of Austrian Succession or King George’s War (1740-1748), and the Seven Years War (1756-1763).

The War of Jenkin’s Ear was aptly named after the laceration of British captain Robert Jenkin’s ear by Spanish forces. The British used it as a mean to justify war against the Spanish to lay claim to Caribbean trade routes. King George’s War defended against the invasion of the Austrian Netherlands (now Belgium) by the French and its allies to protect British territory within Hanover. The Seven Years War primarily focused on the territory rights between Great Britain and France within the North American colonies. However, it was fought on the global stage, employing armies from across the North American and European landscape.

In 1739, South Carolina’s rice industry began to suffer and by the mid-1740s, it brought very little income. This was due to an overproduction of the crop, but more so rising freight costs due to war that crippled the markets of Europe and the West Indies. For plantation owners to move rice, it required lower shipping rates. Though in times of war, maritime insurance rates accelerated due to the dangers of the open seas and caused the price of rice to fall. This prompted the need for other staples that could secure income for the colony. In a spirit of

entrepreneurship, planters looked to the cultivation of indigo in hopes of revitalizing the local economy.\footnote{R. C. NASH, 363.} Indigo seeds had been introduced to South Carolina beginning in 1670 when the original English settlers had arrived. Different crops such as tobacco, rice, cotton and indigo were attempted in an effort to understand the effects that the South Carolina climate and soil had on the crop(s).\footnote{Nic Butler, “Indigo in the Fabric of Early South Carolina,” Charleston County Public Library, February 16, 2023, https://www.ccpl.org/charleston-time-machine/indigo-fabric-early-south-carolina#_edn6.} Between 1690 and 1700, experimentation with rice production had become more profitable and replaced indigo in the market. French Huguenots had also experimented with indigo upon arrival to the Lowcountry in parts of the Santee.\footnote{Nic Butler, “Indigo in the Fabric of Early South Carolina,” Charleston County Public Library, February 16, 2023, https://www.ccpl.org/charleston-time-machine/indigo-fabric-early-south-carolina#_edn6.}

The production of indigo took time to successfully implement for Lowcountry planters. While indigo could be grown on the higher lands above the rice fields and the soil was amicable to its growth, it involved a great deal of trial and error.\footnote{R. C. NASH, 386.} France had been the primary importer of indigo beginning in 1720. Spain was second, importing its Guatemalan indigo from Central America. In 1740, English import and re-export records show that France and Spain continued to supply England’s textile industry with indigo.\footnote{R. C. NASH, 363.} It was needed to dye the wool used for the Royal Army uniforms.\footnote{Margaret F. Pickett, “The Young Adult Years (1739–1744),” essay, in Eliza Lucas Pinckney: Colonial Plantation Manager and Mother of American Patriots, 1722–1793 (Jefferson, NC: McFarland & Company, Inc., Publishers, 2016), 41.} When England could not or did not seek to deal directly with the French, it procured small quantities from the American colonies. This product was of both the French and Spanish variety but was shipped through Jamaica before being received in North America.\footnote{R. C. NASH, 366.}

As Lowcountry planters found success with scaling up their indigo production in the succeeding decade, England ramped up its trade network with the Carolinas. In 1747, 135,000
pounds of indigo was exported to England. By 1748, a bounty was instituted by Parliament for the exportation of colonial indigo to England with a reward of six pence per pound. It was reported that planters doubled and tripled profits during this time. In 1750, England took notice of the colony’s native indigo plant (*Indigofera caroliniana*) that was widely available and could be used as a cheaper alternative over French indigo.

The native indigo plant was not universally used amongst planters, as other species of indigo were later preferred due to the quality of the dyestuff. There are varying and often contradictory accounts on this matter, and it is more likely that the native variety was used briefly. Otherwise, it may have been an option for the lower social classes including planters of limited means, indigenous tribes, and enslaved persons before being eclipsed by the imported species. However, some prominent members of society did attempt to cultivate it despite the poor quality yields and associations it earned abroad. Between 1766 and 1769, Henry Laurens of South Carolina, boasted that his crops matched that of the best French product. During this time, the future American Revolutionary leader, slave trader, and merchant sold over 4,000 lbs of indigo in Charleston. Copper indigo, as it was sometimes referred to, fetched lower prices in London than the French indigo. While it was true the evaluation of the dyestuff was often considered lower quality than other species, this was likely a tactic used by England to drive its cost down. South Carolina had an abundance of land, but lacked the professional knowledge or

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50 Margaret F. Pickett, 61.
51 R. C. NASH, 386-387.
53 R. C. NASH, 384.
54 R. C. NASH, 384
methods needed to ensure a good crop. Despite further advances in the quality, planters decidedly focused on quantity. For profits to be realized, production had to increase, and England was willing to purchase in bulk.55

By 1770, South Carolina was responsible for more than half of the total indigo exports from all the British colonies combined.56 The War of Jenkin’s Ear (1739-1748), King George’s War (1740-1748) and The Seven Years War (1754-1763) proved beneficial for indigo agriculturalists within Colonial America, assisting in the demand for the dyestuff. As the American Revolution (1775-1783) occurred, it dramatically altered planters' ability to properly grow indigo. With British troops at war with American Patriots, plantations and fields were burned or left to neglect. Enslaved workers succumbed to disease or were seized by the British Army, halting all production. Continental Congress established that after the 10th day of September 1775, that no exports of any kind would be sent to Great Britain, Ireland, or the West Indies. In an attempt to compromise with the pleas of Carolinians who had urged the council to approve the export of indigo and rice, the Continental Congress agreed to allow the export of rice to Europe. For a majority of planters, the capital and protection that had once been received through the connections with Great Britain and the British Army were no more. All ties for a prosperous harvest had been severed.57

Following the American Revolution, indigo saw a brief resurgence with more than one million pounds of indigo being exported to England.58 However, the Carolina indigo market

55 R. C. NASH, 385-386.
56 R. C. NASH, 386-387.
declined by the mid-1790s and the rise of India’s indigo resurgence within Bengal and Bihar took shape.\textsuperscript{59} At the same time, the cotton gin was invented by Eli Whitney which had the ability to triple the amount of cotton that could be harvested, assisting in the move toward supplanting cotton as a staple for the American South.\textsuperscript{60}

Historians claim that the War of Jenkin’s Ear cut off the supply of French and Spanish indigo for England thus creating a monopoly for South Carolina. According to these accounts, this is not entirely accurate. It is evident that South Carolina indigo prospered, not because of trade route blockades, but choices made by the British government. British forces chose South Carolina indigo over the French and Spanish imports due to its quantity and price and continued to do so despite the supposedly poor reputation it gained in the quality of its crop. Therefore, it highlighted the probability of an open and competitive market despite the limitations that war might have brought.\textsuperscript{61}

\textsuperscript{59} R. C. NASH, 387. \\
\textsuperscript{61} R. C. NASH, 384.
Eliza Lucas Pinckney

Eliza Lucas Pinckney is a prominent figure in the history of South Carolina’s indigo cultivation. While French Huguenots had a hand in its early experimental development in the Lowcountry beginning in the late-seventeenth century, Pinckney fostered its full potential. With assistance from her plantation upbringing and love of botany, she aided in its progression as a cash crop for the many planters seeking alternatives to the low-income producing rice plant. In publications and exhibits, her contributions are remembered—though the enslaved labor force that made her success and fortune possible are often only a footnote.

Eliza Lucas was born on the Caribbean Island of Antigua as the eldest daughter of George Lucas and Anne Mildrum. Her father owned a sugar plantation on the island and served as lieutenant governor for the British Army. After receiving an education in London, in March 1739 Eliza Lucas sailed with her family to Charleston, South Carolina. The Lucas family had acquired a great deal of land including the 600-acre plantation on Wappoo Creek, a 1500-acre plantation named Garden Hill near the Combahee River and 3000 acres of land along the Waccamaw for the purpose of growing rice. By November 1739, Major George Lucas was required to return to Antigua to assist in the war efforts. The War of Jenkins Ear, a trade war between England and Spain was in full force. Eliza Lucas was entrusted to care for her family and manage their lands at the young age of sixteen.

63 Margaret F. Pickett, 23.
Each plantation grew rice, but was also in the business of exporting pitch, tar, lime, lumber, beef, and pork. Their yield of rice was low and the price at market was even lower. By spring of 1740, Eliza began experimenting with different seeds sent by her father in Antigua in hopes of diversifying production. This included indigo, ginger, cotton, a type of alfalfa called lucerne, and cassava, a popular starchy root vegetable from the West Indies.

In 1741, George Lucas hired Nicholas Cromwell to supervise the production of indigo for the Wappoo plantation. Cromwell immediately began building the necessary vats to facilitate the dyeworks. Only a small batch of the dye was made that year. The indigo bricks, which are made after the vatting and drying process, were considerably different from the standard bricks. When added to fabric, it left a reddish overtone. This was likely due to the construction of the vats using brick rather than the traditional wood. It was also proposed that the addition of lime might have interacted with the structure, causing the mortar to leach into the solution. Cromwell attempted to blame the Lowcountry climate, but it has been theorized that he wished not to compete with his home country of Montserrat. By 1744, he was replaced by his brother Patrick, who helped to improve conditions and was successful in producing an ample amount of dye.

Eliza Lucas married nearby neighbor Charles Pinckney in 1744, just four months after the passing of Charles’ first wife, Elizabeth Lamb. This arrangement came at the request of Elizabeth, hoping for Charles to find happiness after her passing. Charles was a well-known lawyer in South Carolina and owned a vast amount of land. He was also a staunch advocate for

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65 Margaret F. Pickett, 29-30.
66 Margaret F. Pickett, 40.
67 Margaret F. Pickett, 40-41.
68 Margaret F. Pickett, 42.
69 Margaret F. Pickett, 44-45.
Eliza’s agricultural pursuits. In 1744, he shipped six pounds of indigo produced at Wappoo Plantation to James Crokatt, the South Carolina agent in London, to have its quality authenticated.

I have shown your INDIGO to one of our most noted Brokers in that Way, who tried it against some of the Best FRENCH, and in his opinion it is as GOOD….When you can in some measure supply the British Demand, we are persuaded, that on proper Application to Parliament, a Duty will be laid on Foreign Growth, for I am informed that we pay for INDIGO to the French £200,000 per annum. (Pickett 59-60)\textsuperscript{71}

Following this report, Charles and Eliza worked to ensure that planters had access to the seeds from the 1744 batch. Charles kept a small amount to grow at Auckland, his plantation on the Ashepoo River. His intent was to prepare more seeds that could be sold the following year. Seeds from the West Indies were harder to obtain in wartime and its exportation was eventually criminalized by the French once indigo was established within the colonies. The local availability of seeds proved a resourceful tactic and a necessary one.\textsuperscript{72}

To produce the indigo crops and seeds, an enslaved labor force was required. At least twenty enslaved people were documented living and working at Wappoo plantation and each property belonging to the Pinckney's held a multitude of enslaved peoples. Quash (Christian name, John Williams) was an enslaved laborer and carpenter responsible for the construction of the wooden vats that assisted in the production of the quality dye.\textsuperscript{73} It is believed that Quash was

\textsuperscript{71} Margaret F. Pickett, 59-60.
\textsuperscript{72} Margaret F. Pickett, 60.
a trusted servant to Eliza, managing the plantation and working with her to cultivate the indigo crops. Eliza took great interest in Quash and must have developed a good deal of trust in their relationship, for his part furthered Eliza’s endeavor to produce indigo on South Carolina soil. Eliza granted Quash his freedom many years later following her marriage to Charles Pinckney. He would later become part of Charleston’s free Black society working as a master carpenter.

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75 Margaret F. Pickett, 30.
Enslaved Cultural Knowledge

Indigo has been firmly planted within African culture for centuries. In Mali, archaeological evidence of indigo-dyed textile fragments dating between the eleventh and twelfth centuries showed that textiles were used for everyday clothing, but also spiritual and ceremonial purposes.\(^{76}\) Primarily women undertook the art of dyeing, but depending on the area of West Africa, these gender roles varied.\(^{77}\) The most prominent species and commonly used were *Indigofera tinctoria*, *Indigofera guatemalensis* and *Lonchocarpus cyanescens*, a native indigo-bearing plant in parts of Nigeria, Sierra Leone, and the Côte D'Ivoire.\(^{78}\)

The methods involved in producing the dye differed depending on the region and what was available. In eighteenth century Senegambia, what today would be considered the coastal areas between Senegal and Sierra Leone, indigo leaves were first cut then pounded with a wooden mortar (Figure 1.3).\(^{79}\) It would leave behind a paste that would be formed into balls and left to dry. After drying, it would be vatted, or fermented through an alkaline solution that consisted of water and a lye made of burned wood and ash. These items were placed inside clay pots and filtered into a dyeing vat. As the solution was readied for the indigo balls, each would be dissolved, and the dyeing could proceed.\(^{80}\)

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The dyestuff was not only fashioned into West African fabrics, but also beads, amulets, and homes. Beads were used as currency and were made from shells, bones, and seeds. The adornment of plant and animal fibers along with beads in hues of blue, furthered opportunities for trade and represented an individual’s status within the community. It also represented physical

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and spiritual protection and was therefore used in both life and death. Indigo dye was also found along the windows and door trims to ward against evil spirits. This tradition was carried across the Atlantic and survived into modern day Gullah cultures.82

While blue was significant, it was neither the only color of importance nor the only one worn. In South Carolina, enslaved people were limited to certain fabrics, colors, and patterns. The 1740 Slave Code dictated what could be worn. These included checked cottons, scotch plaids, coarse garlix, blue linen, checked linen, calicoes, coarse kersies, duffelds, and lowell cloth. Many were solid blue, checked, or striped.83 Despite these strict outlines, the enslaved continued to embrace their heritage through textiles and the use of indigo. Access to the dyestuff enabled slaves to accentuate their own clothing and often incorporate elements of their culture. The plangi, or tie-dyeing, that is a familiar technique to modern society, was used throughout parts of West Africa. Nigeria is often credited as being the first to partake in this artform, though plangi can be traced to parts of South Asia, in particular Indonesia where it is referred to as shibori. Numerous variations of patterns and designs could be achieved and was a technique used to bring bits of the enslaved homeland to the Lowcountry.84

The production of indigo within the British colonies of North America and the West Indies required a labor force that could not only perform the work but pull from an existing knowledge base that the planters lacked. Early plantation owners recognized the familiarity that

enslaved Africans had with staple crops such as rice, cotton, millet, tobacco, and indigo. Native American groups like that of the Edisto, Kussah, St. Helena, Wimbee, Ashepoo, and Combahee, to name a few, had no evident ties to the plant, but occupied lands that planters quickly encroached upon. Whether through trade or violent force, British colonists established contact that resulted in enslavement of many peoples. The contributions of both Africans and indigenous enslaved people are seldom discussed yet are important in understanding the world in which indigo was produced within the Lowcountry.

Early imagery of enslaved people, both indigenous and African, were depicted in the form of cartouches on colonial maps of the territory. These images highlighted the roles of early settlers and enslaved people in the seventeenth and eighteenth centuries. A 1773 map of South Carolina conveyed the importance of indigo and the system of slavery needed for its production (Figure 1.4). The drawing showed an enslaved African carrying a cargo of indigo aboard the docks of Charles Town and an Indian alongside two white colonists supervising him. The enslaved were barely clothed while the other two were dressed in the finest clothing, showing the implied superiority of the white men and the wealth that oppression created. As the image suggested, Native Americans were used to enforce these conditions upon enslaved Africans though they were considered on the same level and were often victims of the same system. They were enslaved alongside their African counterparts, on both local plantations within South

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85 “In an Ocean of Blue,” Working the Diaspora, 2020, 91, https://doi.org/10.18574/nyu/9780814748183.003.0005
Carolina and those in the West Indies. Although the enslaved native trade declined by the mid-eighteenth century, they were not exempt from the harsh realities of plantation life. Interestingly, women and children were absent from these maps. The art of producing indigo was a combined effort. It included women, men, and children but not all were represented in the subsequent propaganda.\(^89\)

![Map of South Carolina](https://www.loc.gov/item/74692124/)

**Figure 1.4:** James Cook, *A Map of the Province of South Carolina* (1773). Courtesy of South Caroliniana Library, University of South Carolina\(^90\)

In all stages of production, the enslaved were forcibly controlled and subjected to cruelty at the hands of a white overseer who had full authority over every aspect of plantation life. The

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overseer was at the top of the hierarchy, directly under the plantation owner. Drivers, enslaved black laborers who were tasked with managing the enslaved workers, reported directly to the overseer. Enslaved people had no autonomy and no choice in what their role within the plantation would be. Representations in images from the Colonial era were nothing more than falsehoods, portraying enslaved African and natives as savage, ignorant, and overly satisfied with their circumstances. Despite the appearance of submission, the pursuit of self-emancipation was ever-present.91

Chemical Composition of Indigo

Indigo is a dark blue crystal-like structure that melts between 390°C to 392°C. In its natural state, it is a pigment and is insoluble in water, alcohol, and ether. As a dye, it is soluble in water. The main differences between dyes and pigments lies in its solubility (the ability to be dissolved), and the method of application. For indigo, the transition from insoluble (pigment) to soluble (dye), a reducing agent is used that removes the oxygen. This process results in the transformation of indigotin into leuco-indigo, otherwise known as indigo white. The chemical structure of indigo and its leuco form, or indigo white can be seen in Figure 2. Indigo white refers to “the compounds relative lack of color” and the solution appears in various degrees of yellow or yellow-green. This compound is achieved through the reduction of indigo blue and through oxidation, can be reversed. Dyes chemically bind with the textile, combining and settling into the fibers whereas pigments are applied on the surface of the material. Most dyes require a mordant to secure to the fibers; however, indigo does not.

The chemical formula for indigo is C\textsubscript{16}H\textsubscript{10}N\textsubscript{2}O\textsubscript{2}. Indican is a “colorless crystallized formation” found in indigoid plant species and is soluble in water. Indican can be broken down in water to β-D-glucose and indoxyl through chemical reactions. Exposure to oxygen transforms the

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indoxyl to indigo. Moreover, indican is the compound that produces the blue colorant and is considered a glycoside. Glycoside is a sugar which for indigo is in the form of glucose and is connected to another molecule, indoxyl. When the glycosidic connection breaks, the indoxyl is released. When that release occurs and the indoxyl is exposed to oxidation, it turns blue.99

Figure 2: Chemical structure of indigo and its leuco form, or indigo white, Courtesy of Ali Tehrani Bagha & Krister Holmberg via Research Gate100

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Methods of Identification

Chemical Analysis

To extract indigo dye, Abrahams prescribed a steam bath after removing the indigo from the textile with boiling glacial acetic acid. Following the steam bath, the indigo was to be washed with water and alcohol and then dried.101 A more simplified method was conducted by Judith H. Hofenk-De Graaf in A Simple Method for the Identification of Indigo (1974) involving the use of thin-layer chromatography in the identification of indigo vat dyes within textiles.102 Before chromatographic analysis, indigo dye was identified through its coloration from wet chemical analysis. A small sample (0-3 mg) was combined with a few drops of reducing solution that consisted of 50 grams sodium hydroxide, 50 grams sodium hydrosulphite or sodium dithionite, and one liter of water. The solution and sample were shaken, and color noted. When indigo was present, the color turned yellow, revealing its leuco-form or indigo white. If not immediate, the solution was heated to accelerate the color change. Upon adding a few drops of ethyl acetate, the color turned blue. The color change with ethyl acetate was caused by an exposure to oxygen when indigo was present. This color change is specific to indigo vat dyes when exposed to ethyl acetate, but similar blue colors can appear in modern synthetic dyes.

In Material Characterization Tests for Objects of Art and Archaeology by Odegaard et al, chemical analysis procedures for the testing of indigo using sodium hydrogen sulfite were further broken down. Odegaard cited De Graaf’s original paper and provided more context on the procedure. For the reducing solution, 1.25g of sodium hydroxide and 1.25g of sodium hydrogen sulfite or sodium dithionite were added to 25mL of distilled water and dissolved within an

appropriate container. A small amount of the sample was placed inside of a test tube and three to five drops of reducing solution were added. If the sample was a textile, it should be inserted in a warm water bath to help extract the dye and color change. For phase separation, three to five drops of ethyl acetate were added to the test tube. The tube was stoppered and shaken. Phase separation occurred when the solution split into two visibly distinct layers. If indigo was present, the top layer of phase separation will turn blue. Odegaard further claimed that the experiment was sensitive to 5 mg of indigo and that for much smaller samples, the observable changes in color may be harder to discern. Odegaard set up testing protocols to instruct others on the proper processes of chemical analysis and phase separation though no actual tests were performed.\textsuperscript{103}

**Thin-Layer Chromatography**

Following extraction methods in phase separation, another useful method of authentication of dyestuff is through thin-layer chromatography (TLC). It is a chromatographic technique that separates the components of a mixture (Figure 2.1).\textsuperscript{104} It uses a TLC plate made of glass, plastic, or aluminum that is non-reactive and contains a thin layer of adsorbent material which makes up the stationary phase. The plate is marked with a pencil, drawing a line close to the bottom. A pen should never be used, as the ink may travel across the plate when exposed to the solvent.\textsuperscript{105} The appropriate measurement between the line and the bottom of the plate depends on the amount of solvent that will interact with it once it has been placed inside a container. The plate should be labeled at the bottom directly underneath the line so that it is understood what is being tested. The plate is spotted with the solvent or solution using a capillary tube and then


moves up the plate, referred to as the mobile phase. The movement is possible through capillary action. The plate is placed inside and leaned against the side of a container with a small amount of solvent at the bottom. A glass beaker is sufficient. Using a glass watch plate, place it over the top of the beaker to prevent evaporation of solvent. The solvent in the container should not touch the spotted solution and should rest below the penciled line. As the solvent meets the plate, it will interact with the solution and travel across the plate. Different compounds will move along the plate at different speeds. As the solvent approaches the top of the plate, ensuring it never reaches the edge, remove the plate and immediately draw a line with a pencil across the solvent line at the top. Never allow the solvent to travel past the edge of the plate. The results will be compromised, and the procedure will need to be redone.\textsuperscript{106}

If the spots are colored, outline the spots in pencil so that it can be marked, should the spots fade or disappear. The nature of the compounds (organic or inorganic) will determine how effective visualization methods will be. Using ultraviolet light, inorganic compounds shine bright whereas organic compounds appear as a dark spot on the TLC plate. Another method to visualize the spots on the TLC plate is to place it inside a container with some crystals of iodine. Organic compounds typically reveal as yellow-brown to purple when exposed to iodine vapor. If a color observation is not seen, the container can be warmed by placing it in your hand or through a steam bath.\textsuperscript{107} Other methods utilize reagents that react chemically. Potassium permanganate can be effective when sprayed on the dried TLC plate within a fume hood. The plate should turn dark purple with yellow spots.\textsuperscript{108}

\textsuperscript{106} Ralph J. Fessenden et al, 186-188.
\textsuperscript{107} Ralph J. Fessenden et al, 188.
\textsuperscript{108} Thin layer chromatography, accessed February 19, 2024, \url{https://scs.illinois.edu/system/files/inline-files/ThinLayerChromatography.pdf}. 

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Once visualization of spotting is complete, the distance that the compound has traveled across the plate must be recorded. This measurement is from the original spot at the base of the plate to the final spot before the solvent front line. The solvent distance must also be recorded. This measurement begins with the original solvent line and ends with the solvent front line at the top of the plate. The distance traveled by the compound will be divided by the distance traveled by the solvent and will provide the retention factor, or Rf value (Figure 2.2). This provides real quantitative data that can be used and compared to known compounds. TLC is a relatively cost effective and fairly simple method requiring little knowledge of chemistry to conduct.

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111 Ralph J. Fessenden et al, 184-185.
Thin-layer chromatography has been used as an identification method to determine the types of dyes present within textiles. For chromatographic analysis, DeGraaf proposed an eluent of ethyl acetate, ethyl alcohol, and water in a 2:1:1 ratio in combination with acetylated cellulose plates. This system was used after chemical analysis and phase separation, wherein the top blue layer would be extracted to spot on the cellulose plate. DeGraaf failed to mention the extraction or any protocols on how to successfully perform thin-layer chromatography. However, the results determined that indigo gave a blue spot with an Rf value of c. 0.5. Two spots in blue and violet were also seen with an Rf value of c. 0.55, presenting the possibility for another type of indigo.\textsuperscript{113}

In 1992, MJ Bide and H. Choi looked closer at thin-layer chromatography, particularly TLC of vat dyes using reducing and non-reducing systems, since these had not been fully


\textsuperscript{113} Judith H. Graaff, 54.
investigated. There was a need to explore further chromatographic methods, as vat dyes were often relegated to paper chromatography or ignored entirely. The methods involved the use of (1) high boiling point solvents at different higher temperatures to separate unreduced dyes and (2) the reduction of vat dyes with multiple reducing agents with the combined chromatography of the alkali leuco compounds.

TLC plates consisted of standard glass silica gel plates, 50 x 200 mm in size and over 30 eluents were tested. The reducing agents were sodium dithionite, sodium borohydride, hydrazine hydrate, tetramethylammonium borohydride, and tetraethylammonium borohydride (TEAB). To transition the acid leuco vat dyes into the water-soluble alkali leuco compound, sodium hydroxide and tetraethylenepentamine (TEP) were used as bases. Samples (0.5µl) consisting of a 1 percent dispersion in dimethylformamide were applied to the TLC plate. Each plate primed with the sample and upon being fully dry, were entered into the TLC chamber with appropriate eluent. A flow of nitrogen was added to the chamber to eliminate all oxygen. Increased temperatures were achieved by placing the chamber on top of a hot plate, but more controlled parameters were achieved through the use of a heat mantle combined with a voltage regulator for the reducing system and adding the chamber into the laboratory oven for the non-reducing system. Following the plate's development, TLC plates were removed from the chambers and allowed to dry completely. Improper handling or inconsistencies with the TLC plate removal found that migration continued.114

Sodium dithionite and TEAB, in combination with either sodium hydroxide or TEP, reduced each dye despite the temperature, provided it did not exceed 50°C. As temperatures climbed past 50°C, testing was unstable, and the results were inconclusive. Reducing agents

proved inadequate in the separation layers of vat dyes. Despite initial positive migration along the plate for all dyes using a mixture of N-isopropyl-2-pyrrolidone, n-butylbenzoate, TEP and TEAB (5:6:2:0.4 g), it could not be reproduced. Variations in temperature, especially during the introduction of nitrogen, the complex structure of the eluent, and the mixture of solvent and dye, were all mentioned as potential factors.¹¹⁵

Non-reducing systems incorporated multiple mixtures of two or more solvents at high boiling points, temperatures that did not exceed 150°C. Chromatograms were tested between 100 and 120°C. Over 100 solvents were tested as well as different TLC plates and temperatures. Nine eluents proved fruitful in the separation of vat dyes, but one proved successful in the separation of indigoid vat dyes. The eluent was nitrobenzene: diphenyl ether: benzophenone in a 3:7:1 ratio. Silica gel plates were shown as favorable with all eluents tested. Mixtures that consisted of polar and non-polar solvents produced better separation and migration on the plate. Only 10 to 20 percent of 2-hydroxyethyl ether or 2-methoxyethyl ether were added into the mixtures and proved successful for vat dye separation, for the greater percentage of these chemicals resulted in the increased demixing of the solvents. With elevated temperatures, these procedures were reproduced multiple times with satisfactory results, and the development of the plates for vat dyes took less time than the reducing agents. This also allowed for chromatography to take place without the need for the nitrogen stream, provided an appropriate oven and ventilation system was available.¹¹⁶

¹¹⁵ M J Bide and H Choi, 133-138.
¹¹⁶ M J Bide and H Choi, 134-137.
Paper Chromatography

Techniques like paper chromatography are related or similar to thin-layer chromatography. Rather than a glass plate with an adsorbent front, paper with water acting as the adsorbent is used. This constitutes a liquid-liquid partition technique over the previous liquid-solid example seen in TLC, though much of the same concepts apply. Paper chromatography is considered a simple and efficient model for identifying organic and inorganic compounds. The cellulose layer within the filter paper acts as the stationary phase while the solvent is considered the mobile phase. The sample is carried along the filter paper and separates into multiple components (Figure 2.3). The separation depends on how well the sample adsorbs or dissolves.

Figure 2.3: Paper-chromatography process, Courtesy of Sagar Aryal et al via Microbenotes

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117 Ralph J. Fessenden et al, 189.
119 Ibid.
Previous work has been fairly successful in the identification of indigosol dyes which were soluble derivatives of vat dyes by using paper chromatography. This method by Sramek (1958) tested numerous indigosol dyes with Whatman no. 1 paper and an aqueous pyridine solution as the solvent. These original results were not discussed; however, were reworked to include two additional solvent systems. This was referred to as the descending technique which utilized Whatman no. 1 paper and three solvents since some dyes were deemed to react better with one over another.

The solvents were categorized as S1, S2, & S3. The S1 system consisted of ammonia (25%), methanol, and water in a 1:2:3 ratio while the S2 system utilized pyridine, isoamyl alcohol, and ammonia (25%) in a 1:3:1:1 ratio. The S3 system used methanol, acetic acid, and water in a 4:1:1 ratio. The first system (S1) greatly depended on the chemical structure of the dye as to whether it would work, and therefore, Rf values varied. The second (S2) proved adequate for separations, in understanding the identity of the dyes, especially dyes where the other systems failed. However, S2 was not as precise in determining the identity as was S1. The third system (S3) was found to be successful for only a small amount of indigosol dyes. Each of these were tested on 160 commercial brands of indigosol dyes. For comparison, centrifugal chromatography was used for each of the indigosol dyes and Rf values were listed.

Centrifugal chromatography, like paper chromatography, is a liquid-liquid partition technique that acts as both the mobile and stationary phase. For this to work, columns or CPC cells are combined in stages through ducts attached to a large rotor. The liquid stationary system

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is then applied, which is halted by a centrifugal force within the rotor-cell system. The mobile phase that holds the sample is made pure and is delivered via pressure through the rotor and the stationary phase as droplets. This study was performed using a pressureless apparatus with central spot development. This technique had been associated with Pavlicek, Rosmus, and Dyel. It is unknown if the protocols match the systems of the previous centrifugal chromatography setup. Whatman no. 3 papers were used, and the separation was conducted at 600 r.p.m. for 45 minutes. The chromatograms were sprayed with a warm (40°C) solution of NaNO₂ (2%) in 2% hydrochloric acid to assist in the visual confirmation of the indigosol dye. Once dried, the chromatograms were placed under ultraviolet radiation to reveal the dye. Results showed that both paper chromatography and centrifugal chromatography were successful and identical in the separation of indigosol dyes when using the S1 system. This technique was not applied to anthraquinoid or indigoid vat dyes, but suggested that in principle, the technique could be applied.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is similar in many ways to the previous chromatographic methods. However, the stationary phase is placed into a column rather than on a plate. The solvent is also introduced into the column at much higher pressure. The dissolved sample (dye) is inserted into a valve via a syringe prior to reaching the column (Figure 2.4). Depending on the sample, each component passes through the column at its own length of time. The detection of the sample is typically made possible through the absorption of ultraviolet

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light as it passes through the detector. Data is processed as visible peaks at a specific retention time.\textsuperscript{127}

HPLC has been used to determine the presence of indican and indigo precursors in shoot extracts of European (\textit{I. tinctoria}) indigo and Chinese (\textit{I. indigotica}) woad. This method was in combination with an evaporative light scattering detector. For confirmation, a particle beam mass spectrometer was connected. Leaves of indigo were extracted via boiling for five minutes in 5mL of water. The extract was centrifuged for 5 min (10000g) or filtered through a 0.22 mm Millipore membrane filter. Then, the filtrate was diluted and processed by HPLC and the evaporative light scattering detector (ESLD) or a mass spectrometer. Each analysis had its own set of criteria. ESLD used a Ramona 5 data logger. 20 unit liters of the methanol extract were inserted into a


Rheodyne valve onto a Econosphere C18 (EC18) column which was connected to a second EC18 column and shielded by a 5 unit guard column. The nitrogen flow assisted in the detection of the compounds, flowing at 2.00 standard liters per minute with the drift tube controlled at 92°C. The solvent system consisted of methanol, water, and acetonitrile in a 15:60:25 ratio with 0.2% formic acid at a flow rate of 0.4mL per minute.\textsuperscript{129}

As an additional layer of confirmation, HPLC was connected to a Thermoquest LCQ mass spectrometer with a source voltage of 2kV where the atmospheric pressure chemical ionization source was tested. The vaporizer temperature was maintained at 450°C and capillary temperature at 150°C. Methanol extracts and injection sequences used the same protocols for solvents and construction of columns previously reported. This acquired a spectra over the range of 50-800 amu in the negative ion mode. These experiments proved that indigo, \textit{isatan B}, and indican were found in plant extracts and were identified through comparisons of retention times. HPLC-ELSD concluded that both the European and Chinese sourced indigo plants contained indican and \textit{isatan B} though the data was not shown. This method proved to be a successful analytical tool for identifying indican and \textit{isatin B} from woad leaf extracts.\textsuperscript{130} While HPLC is an efficient model for the identification of dyes, it is much more intricate and highly technical than the previous methods.

\textsuperscript{129} KG Gilbert (nee Stoker) et al., “Qualitative Analysis of Indigo Precursors from Woad by HPLC and HPLC-MS,” PEARL Home, January 1, 2000, https://pearl.plymouth.ac.uk/handle/10026.1/9314.
\textsuperscript{130} KG Gilbert, 19.
Gas Chromatography

Gas chromatography (GC) has many other names including gas-liquid chromatography (GLC), gas-liquid phase chromatography, gas-liquid partition chromatography (GPLC), and vapor phase chromatography.\textsuperscript{131} The liquid sample is inserted inside the gas chromatograph via a syringe (Figure 2.5).\textsuperscript{132} As it enters through the rubber septum, the sample travels through the heated column where it is vaporized by an inert gas. This typically consists of helium or nitrogen. Located at the end of the column is the detector where all components within a given sample must pass. The detector is attached to a recorder that tracks the amount of each component and when it passes through it. This technique allows for the detection of specific compounds within a mixture. Gas chromatography can be coupled with mass spectrometry (GC-MS), where the compounds can be further examined.\textsuperscript{133}

\textbf{Figure 2.5: Gas chromatography process, Courtesy of Sagar Aryal et al via Microbenotes}\textsuperscript{134}

\textsuperscript{131} Ralph J. Fessenden et al, 152-153.
\textsuperscript{133} Ralph J. Fessenden et al, 152-153.
GC-MS has been used to examine an eighteenth-century blue silk bodice from Genoa, Italy and a sixteenth century Brussels wool tapestry believed to have been dyed with indigo. Through the analysis of chromatograms, the identification of indigotin and indirubin were catalogued. To do this, a 6890 Network GC System was used in conjunction with a methyl-phenyl-polysiloxane cross-linked 5% phenyl-methyl silicone capillary column. The mass spectrometer that was added to the GC system was a 5973 Network Mass Selective Detector. Mass spectra were measured according to the electron impact that remained a constant 70 eV with a scan range of 40-700 m/z. The interface was maintained at 280°C, 230°C for the ion source and 150°C for the quadrupole mass analyzer. An example of similar applications can be seen in Figure 2.6. The starting temperature for the gas chromatogram was 57°C and was constant for two minutes. The temperature increased by 10°C every minute until 200°C was reached. This was repeated at a three-minute constant with a 20°C increase every minute until 300°C was met for a period of twenty minutes. The gas that facilitated this process was helium and kept a steady flow rate of 1.2 mL per minute.

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Before examining historic textiles with GC-MS, wool samples were dyed with both indigoid extracts to match the amount of dye that historic counterparts might typically contain. The dyebaths consisted of *Indigofera tinctoria* L. and *Isatis tinctoria* L., where 40 mL of demineralized water, 13 g of sodium hydrosulphite, 2 g of sodium hydroxide and 1.5 g of indigoid extract were added. Prior to adding the extract, 1.5 mL of ethanol was used to dissolve it. The dye bath was maintained at 50°C for approximately 15 minutes and was constantly stirred. The wool was first cleaned with water and soap and was washed. To prepare the wool, water and soap were used to clean it. It was washed and allowed to dry. Before the wool samples could be added to the dye bath, it was immersed in a solution at 40 mg/L of sodium hydrosulphite and

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deminerlized water for 15 minutes. The wool was removed from the solution and added to the dye bath for 10 minutes at 50°C.  

In preparation of the samples, indigotin and indirubin were examined separately. The standard molecules were administered in a 30 ppm solution of pyridine. For 100 μL of solution, 200 μL N, O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% of trimethylchlorosilane (TMCS) were added. BSTFA was used as a derivatizing agent. It assisted in transitioning compounds into the gaseous phase at lower temperatures, otherwise this would not be visible. This was heated for 75 minutes at 70°C. The plant extracts were received as powder and did not require an extraction method. The sample size of the extracts consisted of 0.01 mg and were administered through the same protocols described earlier. The injection volume for each was 2 μL.  

In the analysis of the plant extracts, the chromatograms showed di-trimethylsilyl indigotin, trimethylsilyl and tri-trimethylsilyl. Both Isatis and Indigofera peaked on the chromatogram, showing indigotin present. Indirubin was found to be present in Isatis only when examining the total ion current (TIC). However, both plant extracts were found to contain small amounts of indirubin when analyzed through selected ion monitoring (SIM). This led to the conclusion that indirubin was indeed present within Indigofera, but only in traces. The wool samples were cataloged into different weights consisting of 4, 3, 2 and 1 mg. Indigotin was only detected in the 4 mg samples when examined in TIC mode, revealing the limited capabilities of TIC applications when examining smaller sample sizes. SIM proved more efficient for detecting


indigotin in smaller samples since this technique was more sensitive in the determination of ions.\textsuperscript{141}

Visual peaks were observed within the chromatogram that indicated the presence of indigotin within the silk sample in the forms of trimethylsilyl derivatives. This conveyed that the blue colorant was due to indigoid dye. However, indirubin was not found to be present in the silk sample, likely due to the species of indigo since some contain much less of the compound than others. For the Brussels tapestry, indirubin and indigotin were not detected with GC analysis in the TIC mode because of the sample amount. With SIM acquisition, trimethylsilyl indigotin derivatives were also found to be present. This proved that the wool fibers obtained from the tapestry were indeed dyed with a source of indigo.\textsuperscript{142}


Conclusion

Indigo has been used as a dye and fashioned into numerous media throughout multiple parts of the world for centuries and the practice of identifying the different species of indigo has been an elaborate and ever-changing field. As evidenced, chemical analysis and phase separation have proved useful in that aspect. Further chromatographic techniques such as thin-layer chromatography and paper chromatography can be incorporated after extraction methods when combined with appropriate solvents. Further testing can be initiated through centrifugal chromatography, high performance liquid chromatography, gas chromatography, mass spectrometry and ultraviolet spectra yet requiring much more dedication in the operation of multiple processes, equipment, and chemicals. As this body of work has tried to contribute toward best methods for the identification of indigo within textiles for a museum professional that may not be knowledgeable in chemistry, these analytical techniques are not recommended despite the additional data that can be achieved. If dedicated technicians have access to the technology, further research can be pursued. The next chapter will cover the Methodology section where the preferred methods for identification of indigo within historic textiles are discussed. This will provide an overview and step-by-step guidelines on the processes that were followed.
CHAPTER THREE
METHODOLOGY

Introduction

Through the study of dye from historic textiles for The Charleston Museum (TCM) using chemical analysis, the goal of this thesis has been to identify the presence of indigo, as well as its significance to the South Carolina Lowcountry, and to provide an appropriate identification technique for museum professionals without a background in chemistry. While there were various methods for identifying indigo, the majority were highly technical and did not align with the premise of this work. The test method chosen for this work was published in *Material Characterization Tests for Objects of Art & Archaeology* by Odegaard, Carroll, & Zimm.\(^\text{143}\) This method identified indigo within textiles using chemical analysis and phase separation.

The methodology primarily consisted of data collection and its subsequent sections within. It began with the sampling material history section where each textile sample was closely examined to provide necessary context. The material selection process portion discussed the reasoning behind each sample chosen for this experiment, including the controls. The chemical schedule table listed all chemicals, along with their usage, quantities, and providers. Following this, individual sections on preparing the reducing solution and testing protocols for chemical analysis and phase separation have been documented. This was a determination of color change followed by solvent layer separation that signified the presence or absence of indigo.

Sampling Material History

Object Number: HT 634

Object number HT 634 was a piece of cream-colored linen that has been dyed with dark and light blue indigo designs in a resist dye floral motif (Figure 3.1). Resist fabrics were made by coating areas of the fabric with wax to prevent or block the dye from settling into the fiber. Through this process, patterns could be formed and were called paste prints. These patterns tended to be in white while the remaining cloth was dyed blue. Other designs implemented figures or flowers in multiple hues of blue amidst a white background. This fragment was donated to The Charleston Museum by Mrs. George Aldret of Florida. Based on its description, it was “flax grown, carded, spun and woven by Sally Sitter in France.” The exact timeline was believed to be mid-to-late 18th century. In consultation with Virginia Theerman, Historic Textiles Curator at The Charleston Museum, it was chosen due to the type of print and the high probability of indigo that was traditionally used in such prints.

Figure 3.1: HT 634 18th century resist dye floral print, provided by The Charleston Museum

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144 From the Accession Records of The Charleston Museum
146 From the Accession Records of The Charleston Museum
Object Number: HT 573b

The sample taken from object number HT 573b was likely produced in the 1820s (Figure 3.2). The block print consisting of two large pieces of chintz fabric depicted a floral pattern of pinks, blues, and reds atop a beige background. Chintz referred to a printed multicolored cotton fabric that on most occasions was glazed. This would have been done by stamping the assorted colors in segments utilizing blocks that were carved out with patterns. By the eighteenth century, methods improved within Europe and North America and employed cylinder printing that assisted in printing patterns quicker and with more vibrancy. These pieces were donated by Simons Vanderhorst Waring in 1926.

Figure 3.2: HT 573b, 1820s block print pattern, provided by The Charleston Museum

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147 From the Accession Records of The Charleston Museum
148 From the Accession Records of The Charleston Museum
150 From the Accession Records of The Charleston Museum
Object Number: HT 3226

Object number HT 3226 was a toile fabric of cream linen and blue design believed to be a 1780s copperplate print (Figure 3.3).\textsuperscript{151} It depicted a rural scene of a man fishing with a maiden in the foreground amidst a riverbed or lake. In the background, it featured a barn and other buildings among the trees and rolling hills. Near the barn, two people relaxed under the canopy of the trees. The imagery was highly detailed and repeated throughout. The copperplate print was donated to the Museum by Mrs. Jason R. Westerfield in 1949.\textsuperscript{152} A variation of this print was contained within The New England Historic Archives and in a red colorway instead of blue.\textsuperscript{153}

![Figure 3.3: HT 3226, 1780s copperplate pattern, provided by The Charleston Museum](image-url)

\textsuperscript{151} From the Accession Records of The Charleston Museum
\textsuperscript{152} From the Accession Records of The Charleston Museum
The sample from the archaeology department, provided by Martha Zierden, was an eighteenth-century cotton fragment and was recovered from the southwest part of the Exchange Building in Charleston, South Carolina, at the central entrance to the cellar (Figure 3.4). The overall measurements were eleven by six centimeters. Its primary color was white and/or tan with spots of green, and black. Though to the naked eye, it appeared a darker blue than green. The black was attributed to pine pitch, a mixture of turpentine and tar that was boiled and mixed to form a hard substance. This was traditionally used on the hulls of ships as a weathering agent and securement. The item was determined to be made of homespun, or locally produced fiber, with 2-twist yarn in an over-one-under-one weave. It was previously identified as indigo by Natalie Rothstein of the Victoria & Albert Museum in London, England in 1980. However, this was not scientifically tested but rather identified by sight. Natalie Rothstein believed that due to the rust marks and holes present on the fragment, this might suggest it was a lining that was hammered into place within a chest or similar furniture.
Object Number: 1987.10a-b

This sample was taken from a two-piece dress (bodice and skirt) of flowered brocade in gold, cream, peach, purple, and two shades of blue, with black shading (Figure 3.5).\textsuperscript{156} The materials of the dress consisted of cotton, silk, and linen. The bodice had short sleeves and a scoop neck. It contained three darts fashioned at the center of the basque bodice with wooden stays. Along the armscye, lower bodice, and neckline, bright blue silk cording lined the trim work. The underlining consisted of brown cotton and the shoulders and upper bust were softly padded with cotton batting. At the back of the dress, twelve metal hooks span the close with smaller ones at the edge of the opening. Jessie Edmondston, for her marriage to Dr. Amory Coffin on December 25, 1836, commissioned this “second day dress” to be made in Paris, France. Second day dresses were a common addition to the wedding dress during the 19th century. It was appropriate for travel, greeting guests and as the name implied, to be worn the day after the ceremony. In 1852, Mary Coffin, the daughter of Dr. & Mrs. Coffin brought it to London, England upon her marriage to her cousin, Thomas Edmondston. It was handed down to their daughter, Mary Charlotte Edmonston, then willed to Alida Harper Fowlkes. During the 1970s, the dress was used by the Historic Charleston Foundation during a photoshoot. It was later gifted to The Charleston Museum by William Harper in 1987.\textsuperscript{157}

\textsuperscript{156} From the Accession Records of The Charleston Museum
\textsuperscript{157} From the Accession Records of The Charleston Museum
Figure 3.4: ARL-11894, 18th century cotton fragment, provided by The Charleston Museum

Figure 3.5: 1987.10a-b, 1836 Parisian second day dress, provided by The Charleston Museum
Object Number: HT 6652

Another sample was taken from a c. 1857 woman’s slate blue silk jacket (Figure 3.6). While the jacket’s exterior was described as slate blue, the interior lining was shown as an off-white silk satin. The back (not shown) had a peplum with four weights at the interior corners. A peplum is a “short flared, gathered, or pleated strip of fabric attached at the waist of a woman’s garments” designed to move outwards from the body. It also had two rosettes attached at the back with tassels. In the trim work, small bias ruffles and tucks of blue fabric were present. Material was taken from the top right nearest the sleeve and collar where the blue fabric had worn away. This item would have been worn by a woman of higher standing within society, perhaps middle or upper class. This item was donated by Susan Sanders in 1980. It was originally worn by Merry Mitchell Moorhouse (1874-1955).

Figure 3.6: HT 6652, 1857 woman’s slate blue silk jacket, provided by The Charleston Museum

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158 From the Accession Records of The Charleston Museum
159 From the Accession Records of The Charleston Museum
160 From the Accession Records of The Charleston Museum
Object Number: 1984.67.1

This c. 1854 sample came from a coverlet composed of blue indigo wool and natural cotton (Figure 3.7). The overall geometric pattern was called Cat Track & Snails Trail. While the exact origins of the pattern were unknown, it could be traced to the American South and had many variations on its name including Winding Vine, Trailing Vine, Twining Vine, and Dogwood Blossom. It had three 27” widths that were sewn together. At the ends, narrow hems had been sewn by hand and were believed to have been woven by enslaved hands on a plantation within Greenville or Newberry County, SC. The coverlet was received by The Charleston Museum in 1984, having been gifted to The Museum by Miriam Ashley Anderson. The original ownership was ascribed to Nancy Clary Hair (1835-1918) and her husband Wiliam Goldsmith (1819-1911). William Goldsmith had owned two large plantations. One near Newberry and the other near Greenville. Both contained numerous enslaved people and were worth thousands of dollars.

161 From the Accession Records of The Charleston Museum  
162 Eliza Calvert Hall, A book of hand-woven coverlets (Classic reprint); ..., accessed February 18, 2024, 46.  
163 From the Accession Records of The Charleston Museum
Object Number: 2009.20.1

The final sample consisted of a nineteenth century blue wool and natural cotton woven coverlet that was in a “Lee’s Surrender” pattern (Figure 3.8).\(^{164}\) The pattern had multiple stars that were repeated and separated by small two-step crosses. An elaborate border with a sunrise and four tables surrounded it. The two panels had been stitched together and at the edges of the sides, had been salvaged and hemmed. The coverlet was gifted and transferred to the museum in 2009 by the Witte Museum in San Antonio, Texas. It was originally owned by Marjorie Guillory of Texas.\(^{165}\) There was not a lot of information regarding the pattern and sources appeared to differ. In some instances, Lee’s Surrender referred to the time the coverlet was made rather than the design itself.\(^{166}\) Whereas Lee’s Surrender had also been noted as a variation on Braddock’s Defeat, a popular weave pattern that could be dated to nineteenth century New York and New Jersey. From observing other patterns, Lee’s Surrender appeared to incorporate patterns such as Pine Bloom, Whig Rose, and Sunrise.\(^{167}\) It did not appear to be an exact replica of each pattern, but perhaps a modification of each.

\(^{164}\) From the Accession Records of The Charleston Museum

\(^{165}\) From the Accession Records of The Charleston Museum


Figure 3.7: 1984.67.1, 1854 blue indigo wool coverlet, provided by The Charleston Museum

Figure 3.8: 2009.20.1, 19th c. blue wool & cotton coverlet, provided by The Charleston Museum
Material Selection Process

A majority of the samples from The Charleston Museum were chosen due to the probability of indigo dye being present within the fibers. Since the Museum had not previously conducted scientific testing for the identity of the dye within these objects, archive records that stated indigo as the dyestuff relied on assumption rather than scientific evidence. Other samples were selected based on similar blue coloration, though the records made no mention of indigo and lacked any consensus on the type of dye used. Additional determining factors included the provenance of the items, the time period of creation, their origin location, and the methods of production. Each sample ranges from the mid-eighteenth century to the mid-nineteenth century. Each item required a sampling of two to four pieces each. These were taken from the frayed edges or loose threading in the individual piece to allow for sizable sampling without destroying the visual appeal or structural condition of the object. Each sample was then bagged and photographed. The polyethylene, chemically inert bag was marked with a set of characters that indicated the sample’s object identification number, correlating to the records of The Charleston Museum. These samples were housed and analyzed at the Warren Lasch Conservation Center (WLCC), where methods and protocols were established in consultation with conservation scientist Lisa Arslaner.

The selection process for the control fiber samples, both indigo and non-indigo, were chosen with assistance from WLCC conservation scientist Lisa Arslaner. For the indigo control, a tea towel was purchased from Caroline Harper, who is a local dyeworks artisan and owner of CHI Design Indigo. It had been dyed with *Indigofera suffruticosa*, also known as Guatemalan indigo. As for the non-indigo variety, a commercially available blue towel, likely dyed with synthetic blue dye, was selected.
The necessary tools and equipment were chosen based on consultation with the scientists at the WLCC and those that were prescribed in procedural documentation. These consisted of glass beakers, glass watch plates, scoopulas, tweezers, glass pipettes, stir plate, hot plate and scale. These items were provided by the Warren Lasch Conservation Center and did not require purchase.

**Chemical Schedule**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Quantity</th>
<th>Provider</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>50 mg</td>
<td>Warren Lasch Conservation Center</td>
<td>Wet chemistry analysis</td>
</tr>
<tr>
<td>Sodium dithionite</td>
<td>50 mg</td>
<td>Fisher Scientific</td>
<td>Wet chemistry analysis</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4 L</td>
<td>VWR Chemicals</td>
<td>Chemistry analysis</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td></td>
<td>Warren Lasch Conservation Center</td>
<td>Sterilization of Tools</td>
</tr>
</tbody>
</table>

Table 3.1 Chemical Schedule

**Preparation of Reducing Solution**

As seen in *Material Characterization Tests For Objects of Art & Archaeology* by Odegaard, Carroll, & Zimmt, the following methods were adhered to for the preparation of a reducing solution in the identification of indigo within textiles. The first step in producing the reducing solution was to gather all necessary items and begin the setup within the fume hood. The Fisher Scientific IsoTemp stir plate was plugged in and turned to 100 rpm speed. A 25 mL

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graduated cylinder was filled with deionized water and poured into a plastic bottle labeled with the date and marked as a reducing solution. A magnetic stirrer was dropped into the bottle and sealed. A 20 mL beaker was placed inside the scale and the door was closed. The scale was tared and the overhead fume hood was turned on. Using a scoopula, 1.25 g of sodium dithionite was measured then added to the reducing solution bottle. A clean 20 mL beaker was placed on the scale and tared. Next, 1.25 g of sodium hydroxide pellets were weighed and added to the reducing solution. This solution dissolved for a few minutes on the stir plate.

![Reducing solution setup on stir plate (left) & water bath on hot plate (right)](image)

Figure 3.9: Reducing solution setup on stir plate (left) & water bath on hot plate (right)

Directly next to the stir plate, a hot plate was plugged in and set to 40°C. A 40 mL glass beaker was filled with water and placed onto the hot plate (Figure 3.9).\(^{169}\) This was used as a water bath for chemical analysis of the samples. Another 40 mL beaker was placed nearby in the

\(^{169}\) Chris Cone, Reduction solution setup image, 2023.
fume hood and filled with a small amount of ethyl acetate. Glass pipettes and pipette balloons were assembled, along with a larger beaker for the used pipettes.

**Testing Protocol: Chemical Analysis**

A 20 mL Pyrex glass beaker with an empty Eppendorf tube inside the beaker was placed inside a scale. The door was closed and the scale was tared. The Eppendorf tube was removed from the beaker, labeled with a corresponding sample number and placed on the workstation. Using curved tweezers, fiber samples were extracted and laid onto Kimtech Kimwipes. The sample was cut into smaller pieces and inserted into the Eppendorf tube. The tube was placed back into the beaker on the scale and the door was closed to ensure accurate weight (Figure 3.10). Depending on the sample quantity and size, the weight varied between 0.000 g and 0.004 g.

![Figure 3.10: OHAUS scale & portable fume hood with Eppendorf tube & beaker](image)

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170 Chris Cone, OHAUS scale & portable fume hood image, 2023.
Within the fume hood, three to six drops of the reducing solution were added to the Eppendorf tube which was placed inside the water bath that rested on the hot plate at 40°C for 2-3 minutes until an observable color change was visible. The intended color change was yellow or yellow-green, signifying the indigo’s transition to its leuco-form, or indigo white. Once this had been observed, or if no color was observed after five minutes, the Eppendorf tube was removed from the water bath. Three to six drops of ethyl acetate were added using a glass pipette and the tube was agitated and shaken to assist any possible indigo to be extracted through phase separation.

Following this step, the very top layer was extracted with a glass pipette and inserted into an additional Eppendorf tube labeled with the corresponding sample number + extract. If the color was blue or light blue, it signaled the presence of indigo. Upon exposure to oxygen, ethyl acetate adopted a blue color, provided the dye contained within was indigo. If it was clear, then indigo was not present. It should be noted that some synthetic blue dyes can result in a false positive; however, none of the historic samples contained any synthetic dyes since all but one sample predated the emergence of synthetic dyes beginning in 1856. In fact, synthetic dyes were not widely used or available until the close of the nineteenth century.
CHAPTER FOUR

FINDINGS

Introduction

The ten samples were analyzed both visually and analytically using multiple methods. This consisted of eight historic textile samples and two control samples. The information for this portion has been gathered into tables that classified the visual evidence of color change through chemical analysis and phase separation extraction for all samples (Table 4.1). The purpose of this chapter was to convey the data accordingly and to provide an analysis of the results. Observations were recorded for the two control samples, following drops of reducing solution that enabled a chemical reaction and ethyl acetate that facilitated extraction of indigo through phase separation (Table 4.2). These controls acted as a reference point on which to judge all other samples. The same protocols were observed for the historic textiles and all observations were recorded in this chapter. The weight of the sample determined the amount of reducing solution and ethyl acetate that would be added. Transitions from one color to another during the chemical analysis portion have been documented and the potential reasoning behind these unexpected observations. When positive identification for indigo was not successful, an inconclusive result was warranted due to differing factors that might have affected the outcome of the experiments. For each inconclusive result, an analysis of the fiber and dye in the chemical analysis and phase separation has been conducted to provide a broader understanding for possible contributory factors that might have hindered these processes. For each sample, material test identification forms were filled out and can be found in Appendix A. Photomicrographs of each historic textile sample have been added to aid in this matter.
### Analysis of Historic Textile Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight in grams</th>
<th>Drops of Reducing Solution</th>
<th>Chemical Reaction Color Observed</th>
<th>Drops of Ethyl Acetate</th>
<th>Phase Separation Extraction Color</th>
<th>Positive for Indigo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT 634</td>
<td>&lt;0.001</td>
<td>3</td>
<td>Faint Yellow</td>
<td>3</td>
<td>Faint Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>1987.10a-b</td>
<td>&lt;0.001</td>
<td>4</td>
<td>Yellow-Green followed by Pinkish Purple</td>
<td>4</td>
<td>Faint Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>HT 6652</td>
<td>0.001</td>
<td>3</td>
<td>Yellow</td>
<td>3</td>
<td>Clear</td>
<td>No</td>
</tr>
<tr>
<td>HT 573b</td>
<td>0.002</td>
<td>3</td>
<td>Dark Yellow</td>
<td>3</td>
<td>Light Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>ARL 11894</td>
<td>&lt;0.001</td>
<td>3</td>
<td>Yellow</td>
<td>3</td>
<td>Light Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>HT 3226</td>
<td>0.004</td>
<td>6</td>
<td>Yellow</td>
<td>3</td>
<td>Clear</td>
<td>No</td>
</tr>
<tr>
<td>1984.67.01</td>
<td>&lt;0.001</td>
<td>3</td>
<td>Yellow-Green to Blue</td>
<td>3</td>
<td>Light Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>2009.20.1</td>
<td>0.002</td>
<td>3</td>
<td>Yellow</td>
<td>3</td>
<td>Light Blue</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 4.1: Analysis of Historic Textile Samples
### Analysis of Control Textile Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight in grams</th>
<th>Drops of Reducing Solution</th>
<th>Chemical Reaction Color Observed</th>
<th>Drops of Ethyl Acetate</th>
<th>Phase Separation Extraction Color</th>
<th>Positive for Indigo</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI Design Indigo Tea Towel</td>
<td>0.004</td>
<td>6</td>
<td>Yellow</td>
<td>6</td>
<td>Blue</td>
<td>Yes</td>
</tr>
<tr>
<td>BIG ONES Blue Bath Towel</td>
<td>0.004</td>
<td>6</td>
<td>Clear</td>
<td>6</td>
<td>Clear</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 4.2: Analysis of Control Textile Samples

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**Chemical Analysis Color Observation of Historic Textile Samples**

- Yellow-Green to Pink-Purple: 12.5%
- Yellow-Green to Blue: 12.5%
- Faint Yellow: 12.5%
- Dark Yellow: 12.5%
- Yellow: 50.0%

Figure 4.1: Chart showing the observable color change for the historic textile samples when subjected to chemical analysis
Control Systems

Threads from the indigo tea towel were cut into tiny pieces and inserted into the Eppendorf tube which was placed inside a beaker and weighed on a scale. The weight was 0.004 g. Six drops of the reducing solution were added. Upon contact with the reducing solution, the indigo tea towel fibers immediately began to change color (Figure 4.3) The Eppendorf tube was placed inside the water bath at 40℃ for two-three minutes on the hot plate. A vibrant yellow coloration was observed. The tube was removed from the warm water bath and six drops of ethyl acetate were added. The tube was shaken vigorously by hand for a few seconds and positioned within a tray until layer separation developed. Phase separation took less than a minute to occur, revealing a blue layer at the top of the tube. This was extracted using a glass pipette and inserted into another Eppendorf tube labeled Tea Towel Extract. The blue color signified that it was indigo. Since this was one of two control systems, the type of dye was known, and a positive
result was expected. The tea towel had been dyed with *Indigofera suffruticosa* by Chi Design Indigo in Edisto, South Carolina.

For the second control, the same protocol was followed as described in the first control. Threads were extracted from the commercially available blue bath towel, purchased from Kohls, and cut into smaller pieces placed inside an Eppendorf tube. Commercial towels use chemical dyes and not organic indigo dyes. The exact dye was unknown since the manufacturer did not list it. This towel was chosen to test against the known indigo dyed tea towel. The fibers weighed 0.004 g on the scale and six drops of the reducing solution were added. The tube was placed into the warm water bath at 40°C though no color was observed. Instead, the fibers remained unchanged, and the liquid showed clear. A period of five minutes passed until the tube was taken out of the water bath. Six drops of ethyl acetate were added to the tube and were shaken by hand to facilitate phase separation. The liquid remained clear despite all attempts to separate the layers. Since there had been no chemical reaction to the reducing solution, there was no reason to believe there would be any reaction or separation of layers when exposed to ethyl acetate. The chemical reaction of the indigo dyed tea towel versus the non-reactive blue bath towel can be seen in a side-by-side comparison in Figure 4.4.
Historic Textile Samples

HT 634

Fibers from HT 634 were carefully laid out and cut into smaller pieces. The fibers were placed inside the Eppendorf tube and onto the scale; they were too light to be weighed and were recorded as <0.001 g. Three drops of the reducing solution were added to the tube and placed inside the water bath at 40°C for two-three minutes. The fibers remained a dark blue while the liquid surrounding it turned a faint yellow. The tube was removed from the water bath and three drops of ethyl acetate were added. The tube was agitated (shaken by hand) and phase separation occurred within one minute. The very top layer of the tube appeared to be a light blue. Using a glass pipette, the top layer was extracted and placed inside an empty Eppendorf tube labeled HT 634 Extract. All attempts to capture the image were unsuccessful as the blue coloration did not translate well on the camera. This was very subtle and hard to discern from the image. The linen
fibers displayed multiple colorways, revealing a darker blue and lighter blue atop a cream-colored background when viewed under the microscope. HT 634 tested positive in both the chemical analysis and phase separation, revealing that it was conclusively indigo. The chemical reaction, photomicrograph, and positive identification of HT 634 can be seen in Figure 4.5.

**HT 573b**

Fibers from HT 573b were placed inside the Eppendorf tube and onto the scale. They weighed 0.002 g. The weight was achieved because there was more of the sample available compared to others and not all of the original sample was used. Three drops of the reducing solution were added to the tube and placed inside the warm water bath at 40°C for two to three minutes. The fibers turned a muddy brown color after a minute. The chemical analysis revealed a dark yellow liquid surrounding the fibers when exposed to the reducing solution. The tube was removed from the water bath and three drops of ethyl acetate were added. The tube was agitated (shaken by hand) and phase separation occurred within a minute. The very top layer of the tube was blue. Using a glass pipette, the top layer was extracted and placed inside an empty Eppendorf tube labeled HT 573b Extract. When viewed under the microscope, the cotton chintz over-under weave features a soft blue colorant interwoven with a darker beige or brown. HT 573b gave the corresponding yellow colorant during the chemical analysis and blue colorant during the phase separation, providing a positive identification for indigo. The chemical reaction, extraction following phase separation, photomicrograph, and positive identification of HT 573b can be seen in Figure 4.6.
Figure 4.5: HT 634 Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 18th century resist dye print, positive for indigo (right).

Figure 4.6: HT 573b Chemical analysis (left), extraction following phase separation (center left), 5x magnification, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 1820s block print pattern, positive for indigo (right).
HT 3226

Fibers from HT 3226 were carefully trimmed to limit the amount of non-blue thread, as most of the sample was cream-colored with small amounts of blue dotted amongst the material. The fibers were placed inside the Eppendorf tube and weighed 0.004 g on the scale. Six drops of the reducing solution were added, and the tube was placed inside the warm water bath at 40°C for two to three minutes. The fibers weight generally dictated the amount of reducing solution added. If the weight was less than 0.004 g then only three drops were added. The blue colorant within the fibers faded and left only a darker tan. The chemical reaction resulted in a yellow liquid surrounding the fibers. The tube was removed from the warm water bath and three drops of ethyl acetate were added. Typically, the amount of ethyl acetate matched the amount of reducing solution used. However, the amount was limited because the more diluted the ethyl acetate layer was, the harder it was to see the resulting color. The tube was agitated (shaken by hand) but no phase separation occurred. After five minutes, the layer remained clear. This experiment could not definitively identify indigo within the sample and was therefore inconclusive. The fibers were taken from the edge of the textile to limit any damage. Due to the location of extraction and amount of blue colorant on the fibers, there might not have been enough to accurately test. It was unknown if the sample contained indigo or another blue dye. When examined under the microscope, the linen fibers appeared to be an over-under weave with a predominant cream and darker beige colorant behind or entangled within the fibers. Two shades of blue, one dark and one light, were shown. The darker beige might indicate the original color and over time, the fibers might have lightened likely due to light exposure. The chemical reaction, photomicrograph, and inconclusive result for HT 3226 can be seen in Figure 4.7.
ARL-11894

The fibers from sample ARL-11894 were cut into smaller pieces to increase the amount of thread containing a blue colorant since most of the material was cream and/or tan. The fibers were placed in an Eppendorf tube. Like HT 634, they were too light to be weighed and were recorded as <0.001 g. Three drops of the reducing solution were added to the tube and were placed inside a warm water bath at 40°C for two to three minutes. The chemical analysis occurred after a minute, revealing a yellow liquid. The tube was removed from the water bath and three drops of ethyl acetate were added. The sample was agitated (shaken by hand) and phase separation occurred within a minute. The top layer was a light blue and was extracted and placed inside another tube labeled ARL-11894 Extract. Both the chemical analysis and phase separation proved successful, resulting in a positive identification for indigo. When examined under a microscope, the cotton fibers in an over-under weave pattern could be seen in a light blue and cream colorant. The pattern was not as apparent since the fibers had been cut. The chemical reaction, photomicrograph, and positive identification for ARL-11894 can be seen in Figure 4.8.
Figure 4.7: HT 3226 Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), 1780s copper plate pattern, inconclusive result (right)

Figure 4.8: ARL-11894 Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 18th c. cotton fragment, positive for indigo (right).
The fibers from sample 1987.10a-b were cut into small pieces and placed inside the Eppendorf tube. Like HT 634 and ARL-11894, they were too light to be weighed and were recorded as $\leq 0.001$ g. Three drops of the reducing solution were initially added to the tube, but the fibers were not fully immersed in the solution and another drop was added. The tube was placed into the warm water bath at 40°C for two to three minutes. An immediate color change was observed. The fibers remained blue while the liquid surrounding it turned a yellow-green color. Photographs were not taken due to the rate of color change. The tube transitioned from yellow-green to a pinkish-purple color. Once removed from the water bath, the color was photographed. The purple colorant was hard to discern in the photograph, but a thin layer of purple could be seen at the very top just above the blue fibers. Four drops of ethyl acetate were added to the tube and agitated (shaken by hand) to assist in phase separation. Phase separation occurred within a minute and the top layer was extracted. The faint blue layer was added to an additional Eppendorf tube and was labeled 1987.10a-b Extract. Both the chemical analysis and phase separation proved successful, resulting in a positive identification for indigo. However, the change in color from yellow-green to pinkish-purple could only be theorized. Possibilities included the potential that other dyes may have reacted with the indigo dye since the sample came from a multicolored dress. There was also the idea that it might be a certain species of indigo that reacted differently when exposed to the reducing solution. Both proposals were unknown and required further investigation. When examined under a microscope, linen, cotton, and silk fibers were shown in a multidirectional pattern. There were three main colorants, a predominant dark blue, a cream or off-white, and black. The chemical reaction, photomicrograph, and positive identification for 1987.10a-b can be seen in Figure 4.9.
The fibers from HT 6652 were cut into small pieces, placed inside the Eppendorf tube and weighed 0.001 g on the scale. Three drops of reducing solution were added to the tube and were placed in the warm water bath at 40°C for two to three minutes. Within a minute the color began to change. The fibers became a darker tan color while the liquid surrounding it turned yellow. Three drops of ethyl acetate were added to the tube and were agitated (shaken by hand) to assist in phase separation. Phase separation did not occur after five minutes. The ethyl acetate layer remained clear. The experiment proved to be inconclusive for indigo. The sample was rather degraded. The brittle nature of the fibers as well as the muted color of the dye compared to other portions of the jacket, might be cause for the inconclusive result. Silk may react differently than other fibers when exposed to such tests. In addition to this, the color may indeed be a slate blue as described in the accession records or some other non-indigo dye. This would need to be investigated further to determine if any of the proposed theories were accurate. It was also recommended that a sample be obtained from a different area for any future studies. When examined under a microscope, silk satin fibers in an over-under patterned weave in a dark blue colorant were shown. It was hard to determine if the areas where the blue dye had been lifted revealing a cream colorant indicates degradation of the dye or fibers. The edges appeared to be broken, either due to degradation or when cutting the sample from the textile. However, the severity cannot be determined at this magnification. The chemical reaction, photomicrograph, and inconclusive result for HT 6652 can be seen in Figure 4.10.
Figure 4.9: 1987.10a-b Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 1836 gold brocade two-piece dress (bodice and skirt), positive for indigo (right).

Figure 4.10: HT 6652 Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 1857 blue silk jacket, inconclusive result (right).
The fibers from 1984.67.01 were cut into small pieces and placed inside an Eppendorf tube to be weighed on a scale. Due to the small amount, the fibers weight could not be determined and was recorded as <0.001 g. Three drops of reducing solution were added to the tube and were placed inside a warm water bath at 40℃ for two to three minutes. Within a minute, an immediate color change was observed. The liquid surrounding the fibers began to turn yellow-green and transitioned to dark blue. The tube was removed from the water bath and photographed. Three drops of ethyl acetate were added to the tube and were agitated (shaken by hand) until phase separation occurred. After a minute, phase separation of the top layer occurred, revealing a light blue layer. The top layer was extracted and added to another Eppendorf tube that was labeled 1984.67.01 Extract. Both the chemical analysis and phase separation proved successful, resulting in a positive identification for indigo. However, the change in color from yellow-green to blue could only be theorized. Perhaps the sample that was obtained from a coverlet might have had other dyes intermingled and upon chemical analysis, reacted. There was also the possibility that it might have been another species of indigo and reacted differently when exposed to the reducing solution. Both theories were unknown and required further study. When examined further under a microscope, wool fibers in a dark blue colorant were shown. It was woven within natural cotton fibers that were not depicted. The fibers were extracted from small piling at the edge of the material and were reflected in the circular shape of interconnected thread. The chemical reaction, extraction process, photomicrograph, and positive identification for 1984.67.01 can be seen in Figure 4.11.
The fibers from 2009.20.1 were cut into small pieces and placed inside an Eppendorf tube to be weighed on a scale. The fibers and tube weighed 0.002 g. Three drops of reducing solution were added to the tube and were placed inside a warm water bath at 40°C for two to three minutes. Within a minute, a visual color change was observed. The liquid surrounding the fibers began to turn yellow. The fibers became a much darker green, almost black. Three drops of ethyl acetate were added to the tube and were agitated (shaken by hand) until phase separation occurred. After a minute, the top layer of ethyl acetate appeared light blue and was extracted. The extraction layer was added to another Eppendorf tube that was labeled 2009.20.1 Extract. Both the chemical analysis and phase separation proved successful, resulting in a positive identification for indigo. When examined under a microscope, the wool fibers showed a dark and light blue colorant with a single black thread. The wool fibers were interwoven with natural cotton that are not shown. At 5x magnification, the ridges or scales that are present on wool fibers are slightly noticeable. The chemical reaction, photomicrograph, and positive identification for 2009.20.1 can be seen in Figure 4.12.
Figure 4.11: 1984.67.01 Chemical analysis (left), extraction following phase separation (center left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (center right), and 1854 woven, blue indigo wool and natural cotton overshot coverlet, positive for indigo (right).

Figure 4.12: 2009.20.1 Chemical analysis (left), 5x magnification 2D, photomicrograph, Courtesy of the Warren Lasch Conservation Center (middle), and 19th century blue wool and natural cotton woven coverlet, “Lee’s Surrender” pattern, positive for indigo (right).
The efficiency of chemical analysis and phase separation as a trusted method for the identification of indigo within historic textiles relies upon multiple factors. These characteristics include the quantity and condition of the materials being tested, the amount and type of dye present in the fibers and the degradation levels of both the fibers and dye. Environmental conditions are an important consideration. Light absorption in textiles results in pigment loss when overexposed to ultraviolet rays. Fading of the dye or decomposition of the material will further decrease the likelihood of an observable reaction. Indigo, regardless of an organic or synthetic variety, yields identical positive results. Therefore, the provenance of the material as well as any alterations over time must be considered to distinguish between the two. Provided these criteria have been accounted for, identification results should prove favorable. This study was intended to contribute to the existing available knowledge and be accessible to museum professionals and conservators that require alternative nontechnical methods of testing for indigoid dyes. The inclusion of more technical methods has been provided to supply additional research pathways. Further determinations can be made into the species of indigo through chromatographic techniques.

Most of the historic textile samples tested positive for indigo, indicating chemical analysis and phase separation as a relatively efficient model in the identity of indigo dyestuffs. This experiment did record inconclusive results for two of the historic samples despite each having a reaction upon introduction to the reducing solution. There were no observable changes as ethyl acetate was administered for phase separation. Contributing factors are unknown but have been
proposed. These include the amount of dye available, the sample's quantity and the possible degradation levels of the fibers and dye. Non-indigo blue dyes are another consideration as it would not react in the same manner given the current testing protocols.

Modifications were made toward the method of chemical analysis and phase separation. With the chosen methodology, acid digestion was not needed. Known fiber samples containing indigo were inserted into Eppendorf tubes and subjected to chemical analysis. This procedure was repeated with samples known to be a non-indigo blue dye source. There was uncertainty on if the dye would react while being contained within the fiber but was necessary to test before moving to the historic samples. Both controls reacted accordingly. The indigo dyed tea towel gave a positive indicator of yellow while the blue bath towel had no reaction and remained clear. When ethyl acetate was added to facilitate phase separation, the tea towel had a blue separation layer indicating a positive result while the bath towel had none.

The suggested minimum weight for each sample was at least 5 mg to achieve an observable color change when conducting chemical analysis and phase separation. Smaller quantities only lessened the reality of this occurrence. The historic samples varied in size from <0.001 g to 0.004 g. To increase these odds, fibers were cut into smaller pieces and positioned inside the tube. The entirety of each sample was not tested, should the initial attempt result in failure. Each sample reacted to chemical analysis. In some instances, the dye reacted to the reducing solution by being pulled outward from the fibers. In at least two samples, one cotton and the other linen, the fibers resulted in a much darker muddy brown appearance. This initially inhibited the view of the reaction but could be seen once the tubes were removed from the water bath. During phase separation, ethyl acetate layers gave a majority light blue and faint blue

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colorant while others were non-reactive and remained clear. As evidenced, greater quantities garnered better results in both chemical analysis and phase separation. However, smaller samples were still noticeable. While it is recommended that tests be conducted on larger samples, it has been proven that much smaller amounts can also provide visible data.

The extraction process proved to be more difficult following phase separation given the faint colorant of the ethyl acetate layer and the amount of fibers present within the tube. This required a concerted effort to extract only the topmost layer. The extractions were stored in individual Eppendorf tubes with parafilm securing the lid to decrease evaporation. Despite all attempts, evaporation did occur over time. Ethyl acetate was reintroduced into the extraction tubes but was unsuccessful in producing the intended results. However, phase separation was successful when adding a drop of ethyl acetate into the original tube containing the fibers. The extraction's purpose was to test the type of indigo through thin-layer chromatography (TLC). Chemical analysis and phase separation allowed for the successful identification of indigo, whereas TLC could provide much more specificity.

Based on the evidence compiled throughout this study, chemical analysis and phase separation proved to be a successful identification method for most of the historic textile samples. Previously listed factors likely contributed to indeterminate results. While each sample differed slightly, the majority performed well under the testing conditions and the information assembled can be used toward future applications. Through these simplified testing protocols, and the modifications enacted, this study served to aid the conservation field and museum professionals with limited knowledge of chemistry for future indigo dye analysis. Current techniques and products have been documented to assist future users since these systems can change over time.
For reference, safety data sheets were compiled in Appendix B to better equip professionals later, should materials differ.

**Limitations**

The limitations that have been identified during this process can likely be traced to the degradation levels of some of the samples. The fibers in combination with the dyestuff for at least two samples were either in a brittle state or provided very little amount of dye in contrast to the amount of surrounding fibers or contained a much weaker dye within. While each fiber reacted to chemical analysis, the observable reaction was more prominent in other samples. The size of the sample was another factor. As the first round of testing was underway, it was quickly realized that the sample size needed to be a certain weight (0.002 g) rather than a certain length (3 mm). When the samples were first collected from The Charleston Museum, at least four lacked necessary weight. This prompted a return visit to the Museum to collect more. Another factor is the timeline of this thesis, for additional methods could not be pursued to further the study of the type of indigo that might be present within the fibers. As certain methods were shelved, further research went into understanding the process of chemical analysis and phase separation. These techniques were not fully discussed in previous case studies and needed additional resources to fill in the gaps where the data was lacking. This required additional research time and trial and error within the laboratory setting. Thin-layer chromatography was the original course for this work following the phase separation and extraction; however, it ended up not being feasible. The sourcing of the silica or cellulose plates that were compatible with indigo proved difficult. Previous studies suggested items that were no longer available. As the deadline neared, the approach had to pivot. This method was still plausible but was suggested for future studies.
Recommendations for Future Research

Early testing with indigoid pigments comprising different species of indigo were completed using silica gel plates and an eluent of ethyl acetate, ethyl alcohol and water in a 2:1:1 ratio as suggested in De Graaf’s work where she cited Kolsik’s methods. The pigments were to serve as a control on which to judge the outcomes of the historic counterparts. The selection process for the control pigments looked at the popular species of indigo within Europe, Asia, Africa, the West Indies and North America. Decidedly, seven species of indigo were chosen based on publications, their popularity and availability throughout the eighteenth and nineteenth centuries. Rebekah Compton, an Art History professor at the College of Charleston provided one ounce of *Indigofera tinctoria*. Small amounts of green indigo pigment, *Indigofera longeracemosa* and *Isatis tinctoria* were purchased from Suzanne Dekel, a dyeworks artisan within Israel. *Polygonum tinctoria* was purchased from Kremer pigments, an online pigment supplier and *Indigofera suffruticosa* was received from Yarn Tree, a dyeworks company within Central America. *Indigofera caroliniana*, a local indigoid shrub found in parts of the Lowcountry during the eighteenth century could not be procured. In consultation with the International Center for Indigo Culture, a nonprofit organization working to generate interest and awareness to the importance of indigo culture within the Lowcountry, have found no physical remnants of this particular species. From all available information, it would appear that *I. caroliniana* is no longer produced. However, the exact history of this species of indigo would require further study.

As the silica gel plates were spotted with the pigments, the solvent moved them across but did not spot along the plate. Instead, it carried each pigment the full length of the plate. Potassium

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permanganate was used as a reagent and sprayed onto the plates to reveal potential spotting. A heat gun on the lowest setting was carefully moved back and forth to assist in the acceleration, though both were unsuccessful. Without spotting, there were no markers to measure and compare for later analysis. Likely a different type of plate could produce more favorable results. The intention was to use acetylated cellulose plates, in particular Polygram Cel AC-30 by Macherey-Nagel; however, these are no longer produced. Polyester cellulose plates were available and appeared to be comparable, but no official testing was completed to confirm this. As previously mentioned, TLC was shelved due to the timeline of this thesis and the initial unsuccessful outcome with the silica gel plates. Chromatographic techniques are recommended to better understand the individual compounds and can provide more information. While this study identified indigo within textiles, others suggested the potential to investigate wallpaper and paint samples since these should give similar reactions under chemical analysis. A diverse set of substrates can be explored to see how it would compare to current systems.
### Spot Test Identification Forms

**Spot Test for Material Identification Form**

**Trial # 1**

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed less than 0.001 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 5 minutes on hot plate.</td>
</tr>
<tr>
<td>Observations:</td>
<td>No visible reaction or color change.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Minimal amount of sample and degradation of fibers are likely factors for inconclusive result.</td>
</tr>
</tbody>
</table>
## Spot Test for Material Identification Form

**Trial #** 2  
**Test title:** Chemical Analysis & Phase Separation  
**Date:** 1.17.2024  
**Sample:** HT-6652

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.001 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test data:</strong></td>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 1-2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td><strong>Observations:</strong></td>
<td>Prior to the water bath, the solution gave a reaction of yellow colorant and fibers turned dark tan. Phase separation occurred after introduction of ethyl acetate &amp; agitation. The top layer was clear.</td>
</tr>
<tr>
<td><strong>Remarks:</strong></td>
<td>Test inconclusive. Potential factors: degradation of sample, amount of dye present within the fibers, and the possibility of non-indigoid sample.</td>
</tr>
</tbody>
</table>
Spot Test for Material Identification Form

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Test title: Chemical Analysis &amp; Phase Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Sample: HT-634</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed less than 0.001 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>3 drops of reducing solution added to tube.</td>
</tr>
<tr>
<td></td>
<td>Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of faint yellow colorant Phase separation did not occur initially. Additional agitation prompted separation layers. The top layer upon extraction gave a faint blue colorant.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Positive for indigo.</td>
</tr>
</tbody>
</table>
## Spot Test for Material Identification Form

**Trial # 1**

**Test title:** Chemical Analysis & Phase Separation

**Date:** 1.17.2024

**Sample:** 1987.10a-b

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed less than 0.001 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>4 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 4 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of yellow-green colorant followed by pinkish-purple colorant. Phase separation did not occur initially. Additional agitation prompted separation layers. The top layer upon extraction gave a faint blue colorant.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Positive for indigo. As the yellow-green colorant transitioned to a pinkish-purple colorant, it likely reacted with another dye present in the sample since the dress had multiple different colors/dyes, or is a certain species of indigo that reacts different under certain conditions.</td>
</tr>
</tbody>
</table>
### Spot Test for Material Identification Form

**Trial # 1**  
Test title: Chemical Analysis & Phase Separation

**Date:**  1.18.2024  
**Sample:** HT-573b

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.002 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of dark yellow colorant. Phase separation occurred. The top layer upon extraction gave a light blue colorant.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Positive for indigo.</td>
</tr>
</tbody>
</table>
### Spot Test for Material Identification Form

#### Trial # 1

<table>
<thead>
<tr>
<th>Test title: Chemical Analysis &amp; Phase Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date: 1.31.2024</td>
</tr>
<tr>
<td>Sample: ARL-11894</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample preparation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed less than 0.001 grams.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test data:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Observations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The solution gave a reaction of yellow colorant. Phase separation occurred. The top layer upon extraction gave a light blue colorant.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Remarks:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for indigo.</td>
</tr>
</tbody>
</table>
**Spot Test for Material Identification Form**

**Trial # 1**  
**Test title: Chemical Analysis & Phase Separation**

**Date:** 1.31.2024  
**Sample:** HT-3226

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.004 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>6 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of yellow colorant. Phase separation occurred. The top layer upon extraction was clear.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>The test was inconclusive. Potential factors: Smaller amount of dye present in the fibers needed to test, or non-indigo sample.</td>
</tr>
</tbody>
</table>
## Spot Test for Material Identification Form

**Trial # 1**  
**Test title:** Chemical Analysis & Phase Separation  
**Date:** 1.31.2024  
**Sample:** 1984.67.01  

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed less than 0.001 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of yellow-green colorant followed by blue colorant. Phase separation occurred. The top layer upon extraction gave a light blue colorant.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Positive for indigo. As the yellow-green colorant transitioned to a blue colorant, it likely reacted with another dye present in the sample or is a certain species of indigo that reacts different under certain conditions.</td>
</tr>
</tbody>
</table>
### Spot Test for Material Identification Form

**Trial # 1**  
**Test title:** Chemical Analysis & Phase Separation  
**Date:** 1.31.2024  
**Sample:** 2009.20.1  

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.002 grams.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test data:</td>
<td>3 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 3 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
<tr>
<td>Observations:</td>
<td>The solution gave a reaction of dark yellow colorant. Phase separation occurred. The top layer upon extraction gave a light blue colorant.</td>
</tr>
<tr>
<td>Remarks:</td>
<td>Positive for indigo.</td>
</tr>
</tbody>
</table>
**Spot Test for Material Identification Form**

**Trial #1**

**Test title:** Chemical Analysis & Phase Separation

**Date:** 1.17.2024

**Sample:** Indigo-dyed tea towel

<table>
<thead>
<tr>
<th>Sample preparation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.004 grams.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test data:</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2 minutes on hot plate. Removed from water bath after observable color change. Added 6 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Observations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The solution gave a reaction of yellow colorant. Phase separation occurred. The top layer upon extraction gave a blue colorant.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Remarks:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for indigo.</td>
</tr>
</tbody>
</table>
**Spot Test for Material Identification Form**

**Trial # 1**  
**Test title: Chemical Analysis & Phase Separation**

**Date:** 1.17.2024  
**Sample:** Blue bath towel

<table>
<thead>
<tr>
<th>Sample preparation:</th>
<th>Beaker and empty Eppendorf tube placed on scale and tared. Sample cut into small pieces and placed inside Eppendorf tube. Eppendorf tube placed inside beaker on scale. Measurement weighed 0.004 grams.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Test data:</th>
<th>6 drops of reducing solution added to tube. Eppendorf tube placed inside water bath at 40 C for 2-5 minutes on hot plate. Removed from water bath after. Added 6 drops of ethyl acetate. Agitated tube by hand to initiate phase separation.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Observations:</th>
<th>The solution gave no reaction, remained clear. Phase separation did not occur. The top layer was clear, no extraction done.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Remarks:</th>
<th>Not positive for indigo.</th>
</tr>
</thead>
</table>
1. Identification

Product Name: Sodium hydrosulfite
Cat No.: S310-100; S310-500; S80-182
CAS No: 7775-14-6
Synonyms: Sodium dithionite

Recommended Use: Laboratory chemicals.
Uses advised against: Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company: Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

<table>
<thead>
<tr>
<th>Hazard Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-heating substances and mixtures</td>
</tr>
<tr>
<td>Acute oral toxicity</td>
</tr>
<tr>
<td>Serious Eye Damage/Eye Irritation</td>
</tr>
</tbody>
</table>

Label Elements

Signal Word: Danger

Hazard Statements
Self-heating, may catch fire
Harmful if swallowed
Causes serious eye irritation
Sodium hydrosulfite

Precautionary Statements
Prevention
Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Keep cool. Protect from sunlight
Wear protective gloves/protective clothing/eye protection/face protection
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Ingestion
IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
Rinse mouth
Storage
Maintain air gap between stacks/pallets
Store away from other materials
Do not expose to temperatures exceeding 50 °C/122 °F
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
Contact with acids liberates toxic gas

3. Composition/Information on Ingredients

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>&gt;85</td>
</tr>
</tbody>
</table>

4. First-aid measures

General Advice
If symptoms persist, call a physician.

Eye Contact
Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact
Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.

Inhalation
Remove to fresh air. If not breathing, give artificial respiration. Get medical attention if symptoms occur.

Ingestion
Clean mouth with water and drink afterwards plenty of water. Get medical attention if symptoms occur.

Most important symptoms and effects
None reasonably foreseeable.

Notes to Physician
Treat symptomatically

5. Fire-fighting measures
Sodium hydrosulfite

Unsuitable Extinguishing Media
No information available

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flash Point Method</td>
<td>No information available</td>
</tr>
<tr>
<td>Autoignition Temperature</td>
<td>&gt;80 °C / &gt;176 °F</td>
</tr>
<tr>
<td>Explosion Limits</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>No data available</td>
</tr>
<tr>
<td>Lower</td>
<td>No data available</td>
</tr>
<tr>
<td>Sensitivity to Mechanical Impact</td>
<td>No information available</td>
</tr>
<tr>
<td>Sensitivity to Static Discharge</td>
<td>No information available</td>
</tr>
</tbody>
</table>

Specific Hazards Arising from the Chemical
Self-heating; exposure to air may cause substance to self-heat without an energy supply.

Hazardous Combustion Products
Sulfur oxides.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

<table>
<thead>
<tr>
<th>NFPA</th>
<th>Health</th>
<th>Flammability</th>
<th>Instability</th>
<th>Physical hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

6. Accidental release measures

Personal Precautions
Use personal protective equipment as required. Ensure adequate ventilation. Avoid dust formation.

Environmental Precautions
Should not be released into the environment. See Section 12 for additional Ecological Information. Do not flush into surface water or sanitary sewer system.

Methods for Containment and Clean Up
Sweep up and shovel into suitable containers for disposal. Keep in suitable, closed containers for disposal.

7. Handling and storage

Handling
Wear personal protective equipment/face protection. Ensure adequate ventilation. Do not get in eyes, on skin, or on clothing. Avoid ingestion and inhalation. Avoid dust formation.

Storage.

8. Exposure controls / personal protection

Exposure Guidelines
This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Engineering Measures
Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal Protective Equipment

Eye/face Protection
Tight sealing safety goggles.

Skin and body protection
Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection
Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard
EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

**Recommended Filter type:** Particulates filter conforming to EN 143.

**Hygiene Measures** Handle in accordance with good industrial hygiene and safety practice.

## 9. Physical and chemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical State</td>
<td>Powder Solid</td>
</tr>
<tr>
<td>Appearance</td>
<td>White</td>
</tr>
<tr>
<td>Odor</td>
<td>Rotten-egg like</td>
</tr>
<tr>
<td>Odor Threshold</td>
<td>No information available</td>
</tr>
<tr>
<td>pH</td>
<td>8-9.5, 50 g/l aq.soi</td>
</tr>
<tr>
<td>Melting Point/Range</td>
<td>300 °C / 572 °F</td>
</tr>
<tr>
<td>Boiling Point/Range</td>
<td>No information available</td>
</tr>
<tr>
<td>Flash Point</td>
<td>No information available</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Flammability (solid, gas)</td>
<td>No information available</td>
</tr>
<tr>
<td>Flammability or explosive limits</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>No data available</td>
</tr>
<tr>
<td>Lower</td>
<td>No data available</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>No information available</td>
</tr>
<tr>
<td>Vapor Density</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.4</td>
</tr>
<tr>
<td>Solubility</td>
<td>No information available</td>
</tr>
<tr>
<td>Partition coefficient; n-octanol/water</td>
<td>No data available</td>
</tr>
<tr>
<td>Autoignition Temperature</td>
<td>&gt;80 °C / &gt;176 °F</td>
</tr>
<tr>
<td>Decomposition Temperature</td>
<td>No information available</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>Na2 O4 S2</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>174.1</td>
</tr>
</tbody>
</table>

## 10. Stability and reactivity

**Reactive Hazard** Yes

**Stability** Stable under normal conditions. Moisture sensitive. Strong reducing agent. Fire and explosion risk in contact with oxidizing agents.

**Conditions to Avoid** Incompatible products. Excess heat. Avoid dust formation. Exposure to moist air or water.

**Incompatible Materials** Acids, Oxidizing agent

**Hazardous Decomposition Products** Sulfur oxides

**Hazardous Polymerization** Hazardous polymerization does not occur.

**Hazardous Reactions** None under normal processing.

## 11. Toxicological information

### Acute Toxicity

<table>
<thead>
<tr>
<th>Component</th>
<th>LD50 Oral (mg/kg (Rat))</th>
<th>LD50 Dermal (g/kg (Rat))</th>
<th>LC50 Inhalation (mg/L/4h (Rat))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionate</td>
<td>LD50 = 2500 mg/kg (Rat)</td>
<td>&gt;2 g/kg (Rat)</td>
<td>&gt;5.5 mg/L/4h (Rat)</td>
</tr>
</tbody>
</table>

**Toxicologically Synergistic Products** No information available
Sodium hydrosulfite

**Delayed and immediate effects as well as chronic effects from short and long-term exposure**

**Irritation**
Irritating to eyes

**Sensitization**
No information available

**Carcinogenicity**
The table below indicates whether each agency has listed any ingredient as a carcinogen.

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>IARC</th>
<th>NTP</th>
<th>ACGIH</th>
<th>OSHA</th>
<th>Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
</tr>
</tbody>
</table>

**Mutagenic Effects**
No information available

**Reproductive Effects**
No information available.

**Developmental Effects**
No information available.

**Teratogenicity**
No information available.

**STOT - single exposure**
None known

**STOT - repeated exposure**
None known

**Aspiration hazard**
No information available

**Symptoms / effects, both acute and delayed**
No information available

**Endocrine Disruptor Information**
No information available

**Other Adverse Effects**
The toxicological properties have not been fully investigated.

---

### 12. Ecological information

**Ecotoxicity**
Do not empty into drains. The product contains following substances which are hazardous for the environment. Harmful to aquatic organisms. Contains a substance which is:

<table>
<thead>
<tr>
<th>Component</th>
<th>Freshwater Algae</th>
<th>Freshwater Fish</th>
<th>Microtox</th>
<th>Water Flea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>EC50: = 87 mg/L, 96h (Desmodesmus subspicatus) EC50: = 120 mg/L, 72h (Desmodesmus subspicatus)</td>
<td>Not listed</td>
<td>EC50 = 107 mg/L, 17h</td>
<td>EC50: = 96 mg/L, 48h (Daphnia magna Straus)</td>
</tr>
</tbody>
</table>

**Persistence and Degradability**
Persistence is unlikely

**Bioaccumulation / Accumulation**
No information available.

**Mobility**
Will likely be mobile in the environment due to its water solubility.

<table>
<thead>
<tr>
<th>Component</th>
<th>log Pow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>4.7</td>
</tr>
</tbody>
</table>

---

### 13. Disposal considerations

**Waste Disposal Methods**
Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

---

### 14. Transport information

**DOT**

UN-No UN1384
Sodium hydrosulphite

Proper Shipping Name: SODIUM DITHIONITE
Hazard Class: 4.2
Packing Group: II

IATA
UN-No: UN1384
Proper Shipping Name: Sodium dithionite
Hazard Class: 4.2
Packing Group: II

IMDG/IMO
UN-No: UN1384
Proper Shipping Name: Sodium dithionite (Sodium hydrosulphite)
Hazard Class: 4.2
Packing Group: II

15. Regulatory Information

United States of America Inventory

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>TSCA</th>
<th>TSCA Inventory notification - Active-Inactive</th>
<th>TSCA - EPA Regulatory Flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>X</td>
<td>ACTIVE</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend:
TSCA US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)
X - Listed
* - Not Listed

TSCA - Per 40 CFR 751, Regulation of Certain Chemical Substances & Mixtures, Under TSCA Section 6(h) (PBT) Not applicable
TSCA 12(b) - Notices of Export Not applicable

International Inventories
X = listed.

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>DSL</th>
<th>NDSL</th>
<th>EINECS</th>
<th>PICCS</th>
<th>ENCS</th>
<th>ISHL</th>
<th>AICS</th>
<th>IECSC</th>
<th>KECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>X</td>
<td>-</td>
<td>231-890-0</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>KE-31508</td>
</tr>
</tbody>
</table>

KECL - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

U.S. Federal Regulations

SARA 313 Not applicable
SARA 311/312 Hazard Categories See section 2 for more information
CWA (Clean Water Act) Not applicable
Clean Air Act Not applicable
OSHA - Occupational Safety and Health Administration Not applicable
CERCLA Not applicable
Sodium hydrosulfite

California Proposition 65
This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

<table>
<thead>
<tr>
<th>Component</th>
<th>Massachusetts</th>
<th>New Jersey</th>
<th>Pennsylvania</th>
<th>Illinois</th>
<th>Rhode Island</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>X</td>
</tr>
</tbody>
</table>

U.S. Department of Transportation
Reportable Quantity (RQ): N
DOT Marine Pollutant: N
DOT Severe Marine Pollutant: N

U.S. Department of Homeland Security
This product contains the following DHS chemicals:
Legend - STQs = Screening Threshold Quantities, APA = A placarded amount

<table>
<thead>
<tr>
<th>Component</th>
<th>DHS Chemical Facility Anti-Terrorism Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>APA</td>
</tr>
</tbody>
</table>

Other International Regulations

Mexico - Grade
No information available

Authorisation/Restrictions according to EU REACH
Not applicable

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Safety, health and environmental regulations/legislation specific for the substance or mixture

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>OECD HPV</th>
<th>Persistent Organic Pollutant</th>
<th>Ozone Depletion Potential</th>
<th>Restriction of Hazardous Substances (RoHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>Listed</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Contains component(s) that meet a 'definition' of per & poly fluoroalkyl substance (PFAS)?
Not applicable

Other International Regulations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dithionite</td>
<td>7775-14-6</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com
Safety Data Sheet

according to the (US) Hazard Communication Standard (29 CFR 1910.1200)

Revision date: 23.04.2018 Version: 6.2 Print date: 23.04.2018

SECTION 1: Identification

Product identifier

Trade name/designation: Ethyl acetate ACS
Product No.: BDH1123
Synonyms: no data available
CAS No.: 141-78-6
Other means of identification:

Relevant identified uses of the substance or mixture and uses advised against

Recommended Use: For Further Manufacturing Use Only
Uses advised against: Not for Human or Animal Drug Use

Details of the supplier of the safety data sheet

Supplier

VWR International LLC
Street 100 Matsonford Road Radnor Corporate Center,
Postal code/city Building One, Suite 200 P. O. Box 6660
Telephone +1-800-932-5000 toll-free within US/Canada
Telefax: +1-610-728-2103
Emergency telephone
Telephone: +1-800-424-9300 (Chemtrec, 24 hrs/day, 7 days/week, USA)

Preparation Information
VWR International - Product Information Compliance
E-mail: sds@vwr.com

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture
   GHS Classification in accordance with 29 CFR 1910.1200 (OSHA HCS)

<table>
<thead>
<tr>
<th>Hazard classes and hazard categories</th>
<th>Hazard statements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flammable liquid, category 2</td>
<td>H225</td>
</tr>
<tr>
<td>Eye irritation, category 2</td>
<td>H319</td>
</tr>
<tr>
<td>Specific target organ toxicity (single exposure), category 3, narcotic effect</td>
<td>H336</td>
</tr>
</tbody>
</table>

2.2 Label elements
   Labelling in accordance with 29 CFR 1910.1200 (OSHA HCS)

Signal word: Danger

Hazard pictograms

Signal word: Danger

Hazard statements

<table>
<thead>
<tr>
<th>Hazard statements</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>H225</td>
<td>Highly flammable liquid and vapor.</td>
</tr>
<tr>
<td>H319</td>
<td>Causes serious eye irritation.</td>
</tr>
<tr>
<td>H336</td>
<td>May cause drowsiness or dizziness.</td>
</tr>
</tbody>
</table>

Precautionary statements

<table>
<thead>
<tr>
<th>Precautionary statements</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>P210</td>
<td>Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.</td>
</tr>
<tr>
<td>P280</td>
<td>Wear protective gloves/protective clothing/eye protection/face protection.</td>
</tr>
<tr>
<td>P305+P351+P338</td>
<td>IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.</td>
</tr>
</tbody>
</table>

Hazard not otherwise classified (HNOC)
none/none
SECTION 3: Composition / information on ingredients

3.1 Substances

<table>
<thead>
<tr>
<th>Substance name</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>CH3COOC2HS</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>88.11 g/mol</td>
</tr>
<tr>
<td>CAS No.</td>
<td>141-78-6</td>
</tr>
</tbody>
</table>

SECTION 4: First aid measures

4.1 General information

If exposed or if you feel unwell: Call a POISON CENTER or doctor/physician. If unconscious place in recovery position and seek medical advice. Never give anything by mouth to an unconscious person or a person with cramps. Change contaminated, saturated clothing. Do not leave affected person unattended.

After inhalation

Call a POISON CENTER/doctor. Remove casualty to fresh air and keep warm and at rest. If breathing is irregular or stopped, administer artificial respiration.

In case of skin contact

After contact with skin, wash immediately with plenty of water and soap. Remove contaminated, saturated clothing immediately. In case of skin reactions, consult a physician.

After eye contact

In case of contact with eyes flush immediately with plenty of flowing water for 10 to 15 minutes holding eyelids apart and consult an ophthalmologist. Protect uninjured eye. Remove contact lenses, if present and easy to do. Continue rinsing.

In case of ingestion

If accidentally swallowed rinse the mouth with plenty of water (only if the person is conscious) and obtain immediate medical attention. Do NOT induce vomiting. Give nothing to eat or drink.

4.2 Most important symptoms/effects, acute and delayed

no data available

4.3 Indication of any immediate medical attention and special treatment needed

no data available

4.4 Self-protection of the first aider

First aider: Pay attention to self-protection!

4.5 Information to physician

no data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Water spray

ABC-powder

Carbon dioxide (CO2)

Nitrogen
Extinguishing media which must not be used for safety reasons
no restriction

5.2 Specific hazards arising from the chemical
In case of fire may be liberated:
Carbon monoxide
Carbon dioxide (CO2)

5.3 Advice for firefighters
DO NOT fight fire when fire reaches explosives.
Protective equipment and precautions for firefighters
Wear a self-contained breathing apparatus and chemical protective clothing.

Additional information
Do not allow run-off from fire-fighting to enter drains or water courses.
Do not inhale explosion and combustion gases.
Use caution when applying carbon dioxide in confined spaces. Carbon dioxide can displace oxygen.
Use water spray/stream to protect personnel and to cool endangered containers.
In case of fire: Evacuate area.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
In case of major fire and large quantities: Remove persons to safety.

6.2 Environmental precautions
Discharge into the environment must be avoided.

6.3 Methods and material for containment and cleaning up
Spilled product must never be returned to the original container for recycling. Collect in closed and suitable containers for disposal.

6.4 Additional information
Clear spills immediately.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid: Inhalation Avoid contact with eyes and skin. Use extractor hood (laboratory). If handled uncovered, arrangements with local exhaust ventilation have to be used. If local exhaust ventilation is not possible or not sufficient, the entire working area must be ventilated by technical means. Keep away from sources of ignition - No smoking. Usual measures for fire prevention. Take precautionary measures against static discharges.

7.2 Conditions for safe storage, including any incompatibilities
Recommended storage temperature: Ambient temperature
Keep container tightly closed and in a well-ventilated place. Keep/Store away from combustible materials.

7.3 Specific end use(s)
no data available
SECTION 8: Exposure controls/personal protection

8.1 Control parameters

<table>
<thead>
<tr>
<th>Ingredient (Designation)</th>
<th>Regulatory information</th>
<th>Country</th>
<th>Limit value type (country of origin)</th>
<th>Limit value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>NIOSH</td>
<td>US</td>
<td>LTV</td>
<td>1400 mg/m³ - 400 ppm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>OSHA</td>
<td>US</td>
<td>LTV</td>
<td>1400 mg/m³ - 400 ppm</td>
</tr>
</tbody>
</table>

8.2 Engineering controls

**Appropriate engineering controls**
Technical measures and the application of suitable work processes have priority over personal protection equipment. If handled uncovered, arrangements with local exhaust ventilation have to be used.

**Personal protection equipment (PPE)**
Wear suitable protective clothing. When handling with chemical substances, protective clothing must be worn.

**Eye/face protection**
Eye glasses with side protection

**Skin protection**
Wear suitable gloves. When handling with chemical substances, protective gloves must be worn. In the case of wanting to use the gloves again, clean them before taking off and air them well. Check leak tightness/impermeability prior to use.

**By short-term hand contact**
Suitable material: NBR (Nitrile rubber)
Thickness of the glove material: 0,38 mm
Breakthrough time (maximum wearing time): -

**By long-term hand contact**
Suitable material: PE (polyethylene)
Thickness of the glove material: -
Breakthrough time (maximum wearing time): > 480 min

**Respiratory protection**
Respiratory protection necessary at: aerosol or mist formation if exposure limits are exceeded or irritation is experienced, NIOSH approved respiratory protection should be worn.

**Additional information**
Wash hands before breaks and after work. Avoid contact with skin and eyes. When using do not eat, drink or smoke. Provide eye shower and label its location conspicuously.

**Environmental exposure controls**
no data available
SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

(a) Appearance
   Physical state: liquid
   Color: colorless

(b) Odour: no data available
(c) Odour threshold: no data available

Safety relevant basic data

(d) pH: no data available
(e) Melting point/freezing point: -83 °C
(f) Initial boiling point and boiling range: 77.1 °C (1013 hPa)
(g) Flash point: -4 °C
(h) Evaporation rate: no data available
(i) Flammability (solid, gas): Highly flammable liquid and vapor.
(j) Flammability or explosive limits
   Lower explosion limit: 2.1 % (v/v)
   Upper explosion limit: 11.5 % (v/v)
(k) Vapour pressure: 93 hPa (20 °C)
(l) Vapour density: 3.04 (20 °C)
(m) Relative density: 0.902 g/cm³ (20 °C)
(n) Solubility(ies)
   Water solubility (g/L): 85.3 g/L (20 °C)
   Soluble (g/L) in Ethanol: no data available
(o) Partition coefficient: n-octanol/water: 0.73 (20 °C)
(p) Auto-ignition temperature: 460 °C (DIN 51794)
(q) Decomposition temperature: no data available
(r) Viscosity
   Kinematic viscosity: no data available
   Dynamic viscosity: 0.44 mPa*s (20 °C)
(s) Explosive properties: not applicable
(t) Oxidising properties: not applicable

9.2 Other information

Bulk density: not applicable
Refraction index: 1.3719 (589 nm; 20 °C)
Dissociation constant: no data available
Surface tension: no data available
Henry constant: no data available

SECTION 10: Stability and reactivity

10.1 Reactivity
   no data available
10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature).

10.3 Possibility of hazardous reactions
no data available

10.4 Conditions to avoid
no data available

10.5 Incompatible materials
no data available

10.6 Hazardous decomposition products
no data available

10.7 Additional information
no data available

SECTION 11: Toxicological information

11.1 Information on toxicological effects

**Acute effects**
*Acute oral toxicity:*
LD50: > 5620 mg/kg - Rat - (RTECS)

*Acute dermal toxicity:*
LD50: < 18000 mg/kg - Rabbit - (Merck KGaA)

*Acute inhalation toxicity:*
LC50: 1500 ppm - Mouse - (New Zealand Chemical Classification and Information Database)

**Irritant and corrosive effects**
*Primary irritation to the skin:*
not applicable

*Irritation to eyes:*
Causes serious eye irritation.

*Irritation to respiratory tract:*
not applicable

**Respiratory or skin sensitization**
In case of skin contact: not sensitising
After inhalation: not sensitising

**STOT-single exposure**
May cause drowsiness or dizziness.

**STOT-repeated exposure**
not applicable
CMR effects (carcinogenicity, mutagenicity and toxicity for reproduction)
Carcinogenicity
The table below indicates whether each agency has listed any ingredient as a carcinogen.

<table>
<thead>
<tr>
<th>no data available</th>
<th>ACGIH</th>
<th>IARC</th>
<th>NTP</th>
<th>OSHA</th>
</tr>
</thead>
</table>

Germ cell mutagenicity
No indications of human germ cell mutagenicity exist.

Reproductive toxicity
No indications of human reproductive toxicity exist.

Aspiration hazard
not applicable

Other adverse effects
no data available

Additional information
no data available

SECTION 12: Ecological information

12.1 Ecotoxicity

Fish toxicity:

Daphnia toxicity:

Algae toxicity:

Bacteria toxicity:
no data available

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
Partition coefficient: n-octanol/water: 0.73 (20 °C)
12.4 Mobility in soil:
no data available

12.5 Results of PBT/vPvB assessment
no data available

12.6 Other adverse effects
no data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Appropriate disposal / Product
Dispose according to legislation. Consult the appropriate local waste disposal expert about waste disposal.

Waste code product: 070104

Appropriate disposal / Package
Dispose according to legislation. Handle contaminated packages in the same way as the substance itself.

Additional information
no data available

SECTION 14: Transport information

Land transport (DOT)

UN-No.: 1173
Proper Shipping Name: ETHYL ACETATE
Class(es): 3
Classification code: F1
Hazard label(s): 3
Packing group: II
Environmental hazards: No
Marine pollutant: no data available
Special precautions for user:

Sea transport (IMDG)

UN-No.: 1173
Proper Shipping Name: ETHYL ACETATE
Class(es): 3
Classification code: 
Hazard label(s): 3
Packing group: II
Environmental hazards: No
MARINE POLLUTANT: No
Special precautions for user:
Segregation group: -
EmS-No. F-E S-D
Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code not relevant

Air transport (ICAO-TI / IATA-DGR)

UN-No.: 1173
Proper Shipping Name: ETHYL ACETATE
Class(es): 3
Classification code:
Hazard label(s): 3
Packing group: II
Special precautions for user:

SECTION 15: Regulatory information

Safety, health and environmental regulations/legislation specific for the substance or mixture

SARA 313 Components
Not listed.

Massachusetts Right To Know Components
Listed

Pennsylvania Right To Know Components
Listed

New Jersey Right To Know Components
Listed

California Prop. 65 Components
Not listed.
SECTION 16: Other information

Abbreviations and acronyms

ACGIH - American Conference of Governmental Industrial Hygienists
DOT - Department of Transportation
IARC - International Agency for Research on Cancer
IATA-DGR - International Air Transport Association-Dangerous Goods Regulations
ICAO-TI - International Civil Aviation Organization-Technical Instructions
IMDG - International Maritime Code for Dangerous Goods
LTV - Long Term Value
NIOSH - National Institute for Occupational Safety and Health
NTP - National Toxicology Program
OSHA - Occupational Safety & Health Administration
PBT - Persistent, Bioaccumulative and Toxic
PEL - Permissible Exposure Limit
STV - Short Term Value
SVHC - Substances of Very High Concern
TDG - Transport of Dangerous Goods
TLV - Threshold Limit Value
vPvB - very Persistent, very Bioaccumulative

Additional information

Indication of changes: general update

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guidance. The information in this document is based on the present state knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. VWR International and his Affiliates shall not be held liable for any damage resulting from handling.
1. Identification

Product Name: Ethanol Solution 96%
Cat No.: BP8202-1; BP8202-4; BP8202-500
CAS No: 64-17-5
Synonyms: Ethyl alcohol
Recommended Use: Laboratory chemicals.
Uses advised against: Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company:
Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number:
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) Identification

Classification:
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

<table>
<thead>
<tr>
<th>Flammable liquids</th>
<th>Category 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious Eye Damage/Eye Irritation</td>
<td>Category 2</td>
</tr>
</tbody>
</table>

Label Elements

Signal Word
Danger

Hazard Statements
Highly flammable liquid and vapor
Causes serious eye irritation
Precautionary Statements

Prevention
Use personal protective equipment as required
Wash face, hands and any exposed skin thoroughly after handling
Wear eye/face protection
Do not breathe dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area
Keep away from heat/sparks/open flames/hot surfaces. - No smoking
Keep container tightly closed
Ground/bond container and receiving equipment
Use explosion-proof electrical/ventilating/lighting equipment
Use only non-sparking tools
Take precautionary measures against static discharge
Keep cool

Response
If exposed or concerned: Get medical attention/advice

Inhalation
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Skin
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention

Fire
In case of fire: Use CO2, dry chemical, or foam for extinction

Storage
Store locked up
Store in a well-ventilated place. Keep container tightly closed

Disposal
Dispose of contents/container to an approved waste disposal plant

Hazard  not  otherwise  classified  (HNOC)


3. Composition/Information on Ingredients

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>95-100</td>
</tr>
</tbody>
</table>

4. First-aid measures

General Advice
If symptoms persist, call a physician.

Eye Contact
Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact
Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.

Inhalation
Remove to fresh air. If not breathing, give artificial respiration. Get medical attention if
Ethanol Solution 96%  

Ingestion  
Clean mouth with water and drink afterwards plenty of water.

Most important symptoms and effects  
None reasonably foreseeable. Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting. 

Notes to Physician  
Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media  
Water spray, carbon dioxide (CO2), dry chemical, alcohol-resistant foam. Water mist may be used to cool closed containers.

Unsuitable Extinguishing Media  
Water may be ineffective

Flash Point  
13 - 17 °C / 55.4 - 62.6 °F

Method -  
No information available

Autoignition Temperature  
363 °C / 685.4 °F

Explosion Limits  
Upper 19 vol %
Lower 3.3 vol %

Sensitivity to Mechanical Impact  
No information available

Sensitivity to Static Discharge  
No information available

Specific Hazards Arising from the Chemical  
Flammable. Risk of ignition. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Containers may explode when heated. Thermal decomposition can lead to release of irritating gases and vapors. Keep product and empty container away from heat and sources of ignition. Vapors may form explosive mixtures with air.

Hazardous Combustion Products  
Carbon monoxide (CO). Carbon dioxide (CO₂).

Protective Equipment and Precautions for Firefighters  
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA  

<table>
<thead>
<tr>
<th>Health</th>
<th>Flammability</th>
<th>Instability</th>
<th>Physical hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

6. Accidental release measures

Personal Precautions  
Use personal protective equipment as required. Ensure adequate ventilation. Remove all sources of ignition. Take precautionary measures against static discharges.

Environmental Precautions  
Do not flush into surface water or sanitary sewer system.

Methods for Containment and Clean Up  
Soak up with inert absorbent material. Keep in suitable, closed containers for disposal. Remove all sources of ignition. Use spark-proof tools and explosion-proof equipment.

7. Handling and storage

Handling  
Wear personal protective equipment/face protection. Ensure adequate ventilation. Avoid ingestion and inhalation. Do not get in eyes, on skin, or on clothing. Keep away from open flames, hot surfaces and sources of ignition. Use only non-sparking tools. To avoid ignition of vapors by static electricity discharge, all metal parts of the equipment must be grounded. Take precautionary measures against static discharges.

Storage.  
Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from

## 8. Exposure controls / personal protection

### Exposure Guidelines

<table>
<thead>
<tr>
<th>Component</th>
<th>ACGIH TLV</th>
<th>OSHA PEL</th>
<th>NIOSH IDLH</th>
<th>Mexico OEL (TWA)</th>
</tr>
</thead>
</table>
| Ethyl alcohol   | STEL: 1000 ppm          | (Vacated) TWA: 1000 ppm
                  |                        | (Vacated) TWA: 1900 mg/m³ |
                  |                         | TWA: 1000 ppm        |
                  |                         | TWA: 1900 mg/m³      |

**Legend**

ACGIH - American Conference of Governmental Industrial Hygienists
OSHA – Occupational Safety and Health Administration
NIOSH IDLH: NIOSH - National Institute for Occupational Safety and Health

### Engineering Measures

Ensure adequate ventilation, especially in confined areas. Use explosion-proof electrical/ventilating/lighting equipment. Ensure that eyewash stations and safety showers are close to the workstation location.

### Personal Protective Equipment

#### Eye/face Protection

Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA’s eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

#### Skin and body protection

Wear appropriate protective gloves and clothing to prevent skin exposure.

#### Respiratory Protection

Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

#### Hygiene Measures

When using do not eat, drink or smoke. Provide regular cleaning of equipment, work area and clothing.

## 9. Physical and chemical properties

<table>
<thead>
<tr>
<th>Physical State</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, Colorless</td>
</tr>
<tr>
<td>Odor</td>
<td>sweet, Characteristic</td>
</tr>
<tr>
<td>Odor Threshold</td>
<td>No information available</td>
</tr>
<tr>
<td>pH</td>
<td>No information available</td>
</tr>
<tr>
<td>Melting Point/Range</td>
<td>-114 °C / -173.2 °F</td>
</tr>
<tr>
<td>Boiling Point/Range</td>
<td>78 °C / 172.4 °F</td>
</tr>
<tr>
<td>Flash Point</td>
<td>13 - 17 °C / 55.4 - 62.6 °F</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>No information available</td>
</tr>
<tr>
<td>Flammability (solid,gas)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Flammability or explosive limits</td>
<td>19 vol %</td>
</tr>
<tr>
<td>Upper</td>
<td>3.3 vol %</td>
</tr>
<tr>
<td>Lower</td>
<td>No information available</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>No information available</td>
</tr>
<tr>
<td>Vapor Density</td>
<td>No information available</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>0.80</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Partition coefficient; n-octanol/water</td>
<td>No data available</td>
</tr>
<tr>
<td>Autoignition Temperature</td>
<td>363 °C / 685.4 °F</td>
</tr>
<tr>
<td>Decomposition Temperature</td>
<td>No information available</td>
</tr>
<tr>
<td>Viscosity</td>
<td>No information available</td>
</tr>
</tbody>
</table>
10. Stability and reactivity

Reactive Hazard
None known, based on information available

Stability
Stable under normal conditions.

Conditions to Avoid
Keep away from open flames, hot surfaces and sources of ignition. Incompatible products.

Incompatible Materials
Strong oxidizing agents, Strong acids, Acid anhydrides, Acid chlorides

Hazardous Decomposition Products
Carbon monoxide (CO), Carbon dioxide (CO₂)

Hazardous Polymerization
Hazardous polymerization does not occur.

Hazardous Reactions
None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

<table>
<thead>
<tr>
<th>Component</th>
<th>LD₅₀ Oral</th>
<th>LD₅₀ Dermal</th>
<th>LC₅₀ Inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>LD₅₀ = 10470 mg/kg</td>
<td>Not listed</td>
<td>LC₅₀ = 117-125 mg/l (4h)</td>
</tr>
<tr>
<td></td>
<td>OECD 401 (Rat)</td>
<td></td>
<td>OECD 403 (rat)</td>
</tr>
<tr>
<td></td>
<td>3450 mg/kg (Mouse)</td>
<td></td>
<td>20000 ppm/10H (rat)</td>
</tr>
</tbody>
</table>

Toxicologically Synergistic Products
No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation
Irritating to eyes

Sensitization
No information available

Carcinogenicity
The table below indicates whether each agency has listed any ingredient as a carcinogen.

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>IARC</th>
<th>NTP</th>
<th>ACGIH</th>
<th>OSHA</th>
<th>Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>Not listed</td>
<td>Known</td>
<td>A3</td>
<td>Not listed</td>
<td>A3</td>
</tr>
</tbody>
</table>

IARC (International Agency for Research on Cancer)

NTP: (National Toxicity Program)

ACGIH: (American Conference of Governmental Industrial Hygienists)

OSHA: (Occupational Safety & Health Administration)

Mexico - Occupational Exposure Limits - Carcinogens

Mutagenic Effects
Mutagenic effects have occurred in humans.
Reproductive Effects  No information available.
Developmental Effects  Substances known to cause developmental toxicity in humans.
Teratogenicity  Teratogenic effects have occurred in humans.
STOT - single exposure  None known
STOT - repeated exposure  None known
Aspiration hazard  No information available
Symptoms / effects, both acute and delayed  Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting
Endocrine Disruptor Information  No information available
Other Adverse Effects  Tumorigenic effects have been reported in experimental animals.

12. Ecological information

Ecotoxicity  Contains a substance which is: Toxic to aquatic organisms. The product contains following substances which are hazardous for the environment.

<table>
<thead>
<tr>
<th>Component</th>
<th>Freshwater Algae</th>
<th>Freshwater Fish</th>
<th>Microtox</th>
<th>Water Flea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>EC50 (72h) = 275 mg/l</td>
<td>Fathead minnow (Pimephales promelas) LC50 = 14200 mg/l/96h</td>
<td>Photobacterium phosphoreum: EC50 = 34634 mg/L/30 min Photobacterium phosphoreum: EC50 = 35470 mg/L/5 min</td>
<td>EC50 = 9268 mg/L/48h EC50 = 10800 mg/L/24h</td>
</tr>
</tbody>
</table>

Persistence and Degradability  Persistence is unlikely based on information available.
Bioaccumulation/Accumulation  No information available.
Mobility  Will likely be mobile in the environment due to its volatility.

<table>
<thead>
<tr>
<th>Component</th>
<th>log Pow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>-0.32</td>
</tr>
</tbody>
</table>

13. Disposal considerations

Waste Disposal Methods  Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT
<table>
<thead>
<tr>
<th>UN-No</th>
<th>Proper Shipping Name</th>
<th>Hazard Class</th>
<th>Packing Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN1170</td>
<td>ETHANOL</td>
<td>3</td>
<td>II</td>
</tr>
</tbody>
</table>

TDG
<table>
<thead>
<tr>
<th>UN-No</th>
<th>Proper Shipping Name</th>
<th>Hazard Class</th>
<th>Packing Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN1170</td>
<td>ETHANOL</td>
<td>3</td>
<td>II</td>
</tr>
</tbody>
</table>

IATA
<table>
<thead>
<tr>
<th>UN-No</th>
<th>Proper Shipping Name</th>
<th>Hazard Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN1170</td>
<td>ETHANOL</td>
<td>3</td>
</tr>
</tbody>
</table>
### 15. Regulatory information

#### United States of America Inventory

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>TSCA</th>
<th>TSCA Inventory notification - Active-inactive</th>
<th>TSCA - EPA Regulatory Flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>X</td>
<td>ACTIVE</td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- **TSCA** US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)
- X - Listed
- 'X' - Not Listed

**TSCA 12(b) - Notices of Export**  Not applicable

#### International Inventories

Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>DSL</th>
<th>NDSL</th>
<th>EINECS</th>
<th>PICCS</th>
<th>ENCS</th>
<th>ISHL</th>
<th>AICS</th>
<th>IECSC</th>
<th>KECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>X</td>
<td>-</td>
<td>200-578-6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>KE-13217</td>
</tr>
</tbody>
</table>

KECL - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

#### U.S. Federal Regulations

**SARA 313**  Not applicable

**SARA 311/312 Hazard Categories**  See section 2 for more information

**CWA (Clean Water Act)**  Not applicable

**Clean Air Act**  Not applicable

**OSHA - Occupational Safety and Health Administration**  Not applicable

**CERCLA**  Not applicable

**California Proposition 65**  This product contains the following Proposition 65 chemicals. Ethyl alcohol is only a considered a Proposition 65 developmental hazard when it is ingested as an alcoholic beverage.

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>California Prop. 65</th>
<th>Prop 65 NSRL</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>Development (alcoholic beverages only) Carcinogen</td>
<td>-</td>
<td>Developmental Carcinogen</td>
</tr>
</tbody>
</table>

#### U.S. State Right-to-Know Regulations

<table>
<thead>
<tr>
<th>Component</th>
<th>Massachusetts</th>
<th>New Jersey</th>
<th>Pennsylvania</th>
<th>Illinois</th>
<th>Rhode Island</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Ethanol Solution 96%  

Revision Date 24-Dec-2021

U.S. Department of Transportation
Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade  Serious risk, Grade 3

Authorisation/Restrictions according to EU REACH

Safety, health and environmental regulations/legislation specific for the substance or mixture

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>OECD HPV</th>
<th>Persistent Organic Pollutant</th>
<th>Ozone Depletion Potential</th>
<th>Restriction of Hazardous Substances (RoHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>Listed</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol</td>
<td>64-17-5</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Annex I - Y42</td>
</tr>
</tbody>
</table>

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 21-May-2009
Revision Date 24-Dec-2021
Print Date 24-Dec-2021
Revision Summary
This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). SDS sections updated: 2.

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of SDS
1. Identification

**Product Name**: Sodium hydroxide

**Cat No.**: BP359-500; BP359-212

**CAS No**

1310-73-2  

**Synonyms**

Caustic soda

**Recommended Use**

Laboratory chemicals.

**Uses advised against**

Food, drug, pesticide or biocidal product use.

---

**Details of the supplier of the safety data sheet**

**Company**  
Fisher Scientific Company  
One Reagent Lane  
Fair Lawn, NJ 07410  
Tel: (201) 796-7100

**Emergency Telephone Number**

CHEMTREC®, Inside the USA: 800-424-9300  
CHEMTREC®, Outside the USA: 001-703-527-3887

---

2. Hazard(s) identification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

<table>
<thead>
<tr>
<th>Hazard Classifications</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosive to metals</td>
<td>Category 1</td>
</tr>
<tr>
<td>Skin Corrosion/Irritation</td>
<td>Category 1 A</td>
</tr>
<tr>
<td>Serious Eye Damage/Eye Irritation</td>
<td>Category 1</td>
</tr>
<tr>
<td>Specific target organ toxicity (single exposure)</td>
<td>Category 3</td>
</tr>
<tr>
<td>Target Organs - Respiratory system.</td>
<td></td>
</tr>
</tbody>
</table>

---

**Label Elements**

**Signal Word**

Danger

**Hazard Statements**

May be corrosive to metals  
Causes severe skin burns and eye damage  
May cause respiratory irritation
Sodium hydroxide

Precautionary Statements
Prevention
Do not breathe dust/fume/gas/mist/vapors/spray
Wear protective gloves/protective clothing/eye protection/face protection
Use only outdoors or in a well-ventilated area
Keep only in original container
Response
Immediately call a POISON CENTER or doctor/physician
Inhalation
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Skin
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
Ingestion
IF SWALLOWED: Rinse mouth. DO NOT induce vomiting
Spills
Absorb spillage to prevent material damage
Storage
Store locked up
Store in a well-ventilated place. Keep container tightly closed
Store in corrosive resistant polypropylene container with a resistant inliner
Store in a dry place
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>100</td>
</tr>
</tbody>
</table>

4. First-aid measures

General Advice
Immediate medical attention is required. Show this safety data sheet to the doctor in attendance.

Eye Contact
Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required. Keep eye wide open while rinsing.

Skin Contact
Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. Call a physician immediately.

Inhalation
Remove to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth method if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. Call
Sodium hydroxide

Revision Date 07-Sep-2023

a physician or poison control center immediately.

Ingestion
Do NOT induce vomiting. Immediate medical attention is required. Never give anything by mouth to an unconscious person. Drink plenty of water.

Most important symptoms and effects
Causes burns by all exposure routes. Product is a corrosive material. Use of gastric lavage or emesis is contraindicated. Possible perforation of stomach or esophagus should be investigated. Ingestion causes severe swelling, severe damage to the delicate tissue and danger of perforation.

Notes to Physician
Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media
Not combustible. Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

Unsuitable Extinguishing Media
Do not use a solid water stream as it may scatter and spread fire.

Flash Point Method -
No information available

Autoignition Temperature
No information available

Explosion Limits
No data available

Upper
No data available

Lower
No data available

Sensitivity to Mechanical Impact
No information available

Sensitivity to Static Discharge
No information available

Specific Hazards Arising from the Chemical
The product causes burns of eyes, skin and mucous membranes. Reacts violently with water. Contact with metals may evolve flammable hydrogen gas.

Hazardous Combustion Products
Hydrogen. Sodium oxides.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear. Thermal decomposition can lead to release of irritating gases and vapors.

NFPA

Health 3
Flammability 0
Instability 1
Physical hazards N/A

6. Accidental release measures

Personal Precautions
Use personal protective equipment as required. Evacuate personnel to safe areas. Avoid contact with skin, eyes or clothing.

Environmental Precautions
Do not allow material to contaminate ground water system. Should not be released into the environment. Do not flush into surface water or sanitary sewer system. See Section 12 for additional Ecological Information.

Methods for Containement and Clean Up
Avoid dust formation. Sweep up and shovel into suitable containers for disposal.

7. Handling and storage

Handling
Wear personal protective equipment/facemask. Use only under a chemical fume hood. Do not get in eyes, on skin, or on clothing. Do not breathe dust. Do not ingest. If swallowed then seek immediate medical assistance.

Storage.
Keep containers tightly closed in a dry, cool and well-ventilated place. Corrosives area.
Sodium hydroxide


8. Exposure controls / personal protection

<table>
<thead>
<tr>
<th>Component</th>
<th>ACGIH TLV</th>
<th>OSHA PEL</th>
<th>NIOSH</th>
<th>Mexico OEL (TWA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>Ceiling: 2 mg/m³</td>
<td>Ceiling: 2 mg/m³</td>
<td>IDLH: 10 mg/m³</td>
<td>Ceiling: 2 mg/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TWA: 2 mg/m³</td>
<td>Ceiling: 2 mg/m³</td>
<td></td>
</tr>
</tbody>
</table>

Legend

ACGIH - American Conference of Governmental Industrial Hygienists
OSHA - Occupational Safety and Health Administration
NIOSH - National Institute for Occupational Safety and Health

Engineering Measures

Use only under a chemical fume hood. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal Protective Equipment

Eye/face Protection
Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Tight sealing safety goggles. Face protection shield.

Skin and body protection
Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection
Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Recommended Filter type:
Particulates filter conforming to EN 143.

Hygiene Measures
Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

<table>
<thead>
<tr>
<th>Physical State</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White</td>
</tr>
<tr>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>Odor Threshold</td>
<td>No information available</td>
</tr>
<tr>
<td>pH</td>
<td>14 (5 %)</td>
</tr>
<tr>
<td>Melting Point/Range</td>
<td>318 °C / 604.4 °F</td>
</tr>
<tr>
<td>Boiling Point/Range</td>
<td>1390 °C / 2534 °F @ 760 mmHg</td>
</tr>
<tr>
<td>Flash Point</td>
<td>No information available</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Flammability (solid, gas)</td>
<td>Not flammable</td>
</tr>
<tr>
<td>Flammability or explosive limits</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>No data available</td>
</tr>
<tr>
<td>Lower</td>
<td>No data available</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>1 mbar @ 700 °C</td>
</tr>
<tr>
<td>Vapor Density</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>No information available</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>2.13 g/cm³</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Partition coefficient; n-octanol/water</td>
<td>No data available</td>
</tr>
<tr>
<td>Autoignition Temperature</td>
<td>No information available</td>
</tr>
<tr>
<td>Decomposition Temperature</td>
<td>No information available</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>H Na O</td>
</tr>
</tbody>
</table>
Sodium hydroxide

Molecular Weight 40

10. Stability and reactivity

Reactive Hazard Yes
Stability Stable under normal conditions.
Conditions to Avoid Incompatible products. Excess heat.
Incompatible Materials Strong oxidizing agents, Acids, Metals, Water
Hazardous Decomposition Products Hydrogen, Sodium oxides
Hazardous Polymerization Hazardous polymerization does not occur.
Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information
Component Information

<table>
<thead>
<tr>
<th>Component</th>
<th>LD50 Oral (Rat)</th>
<th>LD50 Dermal (Rabbit)</th>
<th>LC50 Inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>140 - 340 mg/kg</td>
<td>1530 mg/kg</td>
<td>Not listed</td>
</tr>
</tbody>
</table>

Toxicologically Synergistic Products
Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation Causes severe burns by all exposure routes
Sensitization No information available
Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>IARC</th>
<th>NTP</th>
<th>ACGIH</th>
<th>OSHA</th>
<th>Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
<td>Not listed</td>
</tr>
</tbody>
</table>

Mutagenic Effects No information available
Reproductive Effects No information available.
Developmental Effects No information available.
Teratogenicity No information available.
STOT - single exposure Respiratory system
STOT - repeated exposure None known
Aspiration hazard No information available

Symptoms / effects, both acute and delayed Product is a corrosive material. Use of gastric lavage or emesis is contraindicated. Possible perforation of stomach or esophagus should be investigated: Ingestion causes severe swelling, severe damage to the delicate tissue and danger of perforation

Endocrine Disruptor Information No information available
Other Adverse Effects The toxicological properties have not been fully investigated.
12. Ecological information

Ecotoxicity
Do not empty into drains. Large amounts will affect pH and harm aquatic organisms.

<table>
<thead>
<tr>
<th>Component</th>
<th>Freshwater Algae</th>
<th>Freshwater Fish</th>
<th>Microtox</th>
<th>Water Flea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>Not listed</td>
<td>LC50 = 45.4 mg/L, 96h static (Oncorhynchus mykiss)</td>
<td>Not listed</td>
<td>Not listed</td>
</tr>
</tbody>
</table>

Persistence and Degradability
Soluble in water Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation
No information available.

Mobility
Will likely be mobile in the environment due to its water solubility.

13. Disposal considerations

Waste Disposal Methods
Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT
- UN-No: UN1823
- Proper Shipping Name: SODIUM HYDROXIDE, SOLID
- Hazard Class: 8
- Packing Group: II

TDG
- UN-No: UN1823
- Proper Shipping Name: SODIUM HYDROXIDE, SOLID
- Hazard Class: 8
- Packing Group: II

IATA
- UN-No: UN1823
- Proper Shipping Name: Sodium hydroxide, solid
- Hazard Class: 8
- Packing Group: II

IMDG/IMO
- UN-No: UN1823
- Proper Shipping Name: Sodium hydroxide, solid
- Hazard Class: 8
- Packing Group: II

15. Regulatory information

United States of America Inventory

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>TSCA</th>
<th>TSCA Inventory notification - Active-Inactive</th>
<th>TSCA - EPA Regulatory Flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>X</td>
<td>ACTIVE</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend:
- TSCA US EPA (TSCA) - Toxic Substances Control Act (40 CFR Part 710)
- X - Listed
- - - Not Listed

- TSCA - Per 40 CFR 751, Regulation of Certain Chemical Substances & Mixtures, Under TSCA Section 6(h) (PBT)
  - Not applicable

- TSCA 12(b) - Notices of Export
  - Not applicable
**Sodium hydroxide**

**Revision Date** 07-Sep-2023

**International Inventories**
Canada (DSU/NDSSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>DSL</th>
<th>NDSL</th>
<th>EINECS</th>
<th>PICCS</th>
<th>ENCS</th>
<th>ISHL</th>
<th>AICS</th>
<th>IECSC</th>
<th>KECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>X</td>
<td>-</td>
<td>215-185-5</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>KE-31487</td>
</tr>
</tbody>
</table>

**KECL** - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

**U.S. Federal Regulations**

**SARA 313**
Not applicable

**SARA 311/312 Hazard Categories**
See section 2 for more information

**CWA (Clean Water Act)**

<table>
<thead>
<tr>
<th>Component</th>
<th>CWA - Hazardous Substances</th>
<th>CWA - Reportable Quantities</th>
<th>CWA - Toxic Pollutants</th>
<th>CWA - Priority Pollutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>X</td>
<td>1000 lb</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Clean Air Act**
Not applicable

**OSHA - Occupational Safety and Health Administration**
Not applicable

**CERCLA**
This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)

<table>
<thead>
<tr>
<th>Component</th>
<th>Hazardous Substances RQs</th>
<th>CERCLA EHS RQs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1000 lb</td>
<td>-</td>
</tr>
</tbody>
</table>

**California Proposition 65**
This product does not contain any Proposition 65 chemicals.

**U.S. State Right-to-Know Regulations**

<table>
<thead>
<tr>
<th>Component</th>
<th>Massachusetts</th>
<th>New Jersey</th>
<th>Pennsylvania</th>
<th>Illinois</th>
<th>Rhode Island</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>X</td>
</tr>
</tbody>
</table>

**U.S. Department of Transportation**

<table>
<thead>
<tr>
<th>Reportable Quantity (RQ):</th>
<th>DOT Marine Pollutant</th>
<th>DOT Severe Marine Pollutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**U.S. Department of Homeland Security**
This product does not contain any DHS chemicals.

**Other International Regulations**

**Mexico - Grade**
No information available

**Authorisation/Restrictions according to EU REACH**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>-</td>
<td>Use restricted. See item</td>
<td></td>
</tr>
</tbody>
</table>
Sodium hydroxide


Safety, health and environmental regulations/legislation specific for the substance or mixture

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS No</th>
<th>OECD HPV</th>
<th>Persistent Organic Pollutant</th>
<th>Ozone Depletion Potential</th>
<th>Restriction of Hazardous Substances (RoHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>Listed</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Contains component(s) that meet a ‘definition’ of per & poly fluoroalkyl substance (PFAS)?
Not applicable

Other International Regulations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Annex I - Y35</td>
</tr>
</tbody>
</table>

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 16-Jun-2009
Revision Date 07-Sep-2023
Print Date 07-Sep-2023
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS


