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Time and temperature dependent analysis of bleach degradation residues on textile fabrics by presumptive chemical analysis and ion chromatography–mass spectrometry

ABSTRACT

The use of household bleach in assaults against individuals and in crime scene cleanup or concealment often results in bleached clothing items, where dyed areas of the fabric are typically faded. However, there is an absence of a published standardized procedure to presumptively test and quantify bleach degradation residues (chloride and chlorate) on textile fabrics, particularly with respect to the effect of storage conditions prior to testing.

To test the hypothesis that bleach degradation increases with an increase in heat or storage time, 100% cotton single–knit T–shirt and denim twill woven fabrics (sampled as five replicates) were stained with 100 μ L of bleach (Clorox™) and stored in ambient or frozen environments for up to one month. The presumptive tests for hypochlorite ions (active ingredient in bleach) and chlorate ions included pH determination, KI–starch paper test, and orthotolidine and diphenylamine chemical spot tests, while quantification of ion residues was performed using an ion chromatograph coupled with a mass–spectrometer instrument.

Samples stored in a frozen environment produced positive KI–starch paper and orthotolidine color reactions across all storage intervals. The diphenylamine test presented the most consistently positive results, regardless of incubation temperature and storage time. A sensitivity study showed that KI–starch paper and orthotolidine exhibited an approximate 8.25 ppm sensitivity limit for household bleach and diphenylamine exhibited an approximate 82.5 ppm sensitivity limit for household bleach.

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A decrease in storage temperature from 22°C (ambient) to -21°C (frozen) resulted in a statistically significant decrease in chloride ion concentration on single-knit T-shirt fabric for each storage period. Chloride ion concentration increased in both temperature conditions, but the increase in frozen storage was less than samples in ambient storage. Stored frozen, single-knit T-shirt fabric experienced less hypochlorite ion degradation compared to twill denim fabric for up to three weeks of storage. Single-knit T-shirt fabric experienced less hypochlorite ion degradation in ambient storage than twill denim at three and four weeks of storage.

The results of this study show that textile fabric samples stained with bleach should be stored in a frozen environment to minimize the rate of hypochlorite ion degradation. Decreasing degradation rate preserves probative evidence over extended time periods.

Keywords: Bleach Degradation, Diphenylamine, Ion-Chromatography Mass-Spectrometry, Textile Fabric, Storage Conditions, Orthotolidine, KI-Starch Paper

INTRODUCTION

While household bleach is commonly available and beneficial when used properly, its caustic quality allows individuals to harm others quickly and deliberately without large expense. Bleach, sodium hypochlorite in water, begins decomposing immediately into chloride and chlorate ions. The major degradation pathway follows a second order reaction mechanism where two chloride ions and one chlorate ion are produced for every three hypochlorite ions that degrade [1]. The chlorate ion will also undergo degradation into chloride because it participates in oxidation reactions that bleach fabrics and paper products [2]. These processes occur naturally in solution and can continue until all hypochlorite and chlorate ions have degraded. These reactions increase with an increase in heat and light exposure because they serve as energy sources for the reaction [1].

Depending on the extent of ion degradation, the relative concentration of hypochlorite and chlorate ions can be approximated via presumptive chemical tests. The presumptive tests include pH measurement, potassium iodide-starch paper (KI-starch paper), orthotolidine, and diphenylamine chemical spot tests. Chemical spot tests determine the presence of oxidizing agents that may be present in solution [3]. Hypochlorite is indirectly identified by the identification of chlorate and chloride ions. Quantification of chlorate and chloride ion analysis is performed with an instrumental approach that utilizes an ion chromatograph for separation of ions combined with a mass-spectrometer for identification of ions.

When a subject uses bleach to commit a criminal act, there is a high potential for the bleach to stain the victim's clothing, resulting in pink/white stains. A commonly encountered example is chemical assault on another individual. The extent and presence of these stains, along with their location, serve as strong circumstantial evidence to support the conclusion that the subject was in an environment where bleach was used. While these stains may be easily identified via qualitative screening, a standard protocol that includes a visual examination, presumptive screening, and ion confirmation via instrumentation has not been located in the peer-reviewed literature.

The goal of this study was to establish a standardized protocol dedicated to the presumptive detection and quantification of bleach degradation residues from fabric items of evidence. This protocol incorporates multiple presumptive tests that target the oxidizing agents within the solutions. One objective was to identify the most sensitive of these presumptive tests and to determine its limit of sensitivity. Due to the rapid degradation of hypochlorite ions, a second objective was to determine the optimal conditions to store bleach stained fabrics items to limit bleach degradation. Variables such as storage temperature and storage time may affect the results of the analysis performed after storage of the evidence. Backlogs become a prevalent and normal condition in agencies where analysts are inundated with cases. However, given the unstable nature of the hypochlorite ions, long-term storage seems counterintuitive because it may be detrimental to the integrity of the evidence, negatively affecting its probative value. The hypochlorite ions have the potential to continue to degrade beyond their detection limit. Thus, residues found on the stain would not definitively point to bleach as the source of the stain. By identifying the optimal conditions for storage of this evidence, analysts will have knowledge as to how long the evidence can be stored prior to performing an analysis.

MATERIALS AND METHODS

Sample and Bleach Selection

Items of clothing consisting of 100% cotton were chosen as the fabric medium because they represent recurring items of clothing observed in casework. Two different weaves of clothing were selected to observe the effects of weave on bleach degradation. The first clothing item (T-shirt) exhibited the single-knit weave and the second item (denim) displayed the twill fabric weave. The bleach standard used for all experiments was Clorox® Bleach Concentrate (64 fluid ounces) with a sodium hypochlorite concentration of 8.25% (w/v %) as reported on the container label.

Sample Preparation

For each storage temperature and time interval, five swatches of approximately 2" x 2" were cut from both clothing items and placed on white butcher paper. The variable incubation conditions include:

- 0 week - no storage
- 1 week - ambient
- 1 week - frozen
- 2 week - ambient
- 2 week - frozen
- 3 week - ambient
- 3 week - frozen
- 4 week - ambient
- 4 week - frozen

A 100 μ L aliquot of Clorox® bleach was applied to the fabric surface and the resulting stains were dried for approximately 20 minutes (Figure 1). Once dry, the swatches were sealed into individual manila paper coin envelopes. For the samples stored in ambient conditions, individual envelopes were stored in a metal cabinet and arranged so that no two envelopes were in contact with each other (Figure 2 a–b). The temperature of the cabinet was recorded to be 22.3°C. The samples incubated in frozen storage were similarly arranged and placed in a large freezer whose temperature was controlled at -21°C.



Figure 1: Photograph of all 45 T-shirt and denim samples prepared at time zero prior to manila envelope packaging.

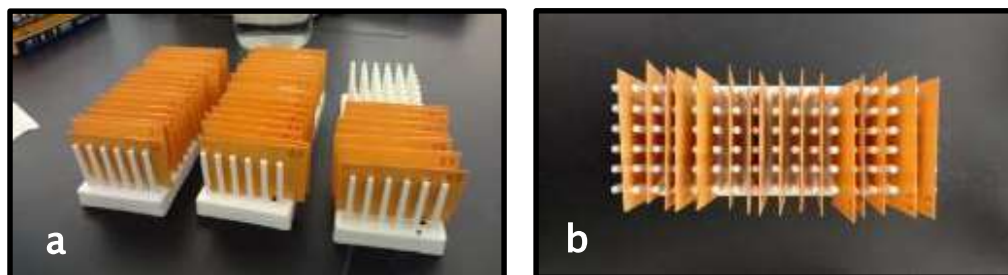


Figure 2: Photograph of packaged samples placed in test tube rack. a: Test tube racks were placed in respective temperature conditions. b: Envelopes did not touch to prevent contamination.

Bleach Residue Extraction

After incubation was completed, all samples were removed from their respective storage environments and the cuttings within the envelopes were placed in individual disposable petri dishes and flooded with approximately 10 mL of sterile Type 1 water. Samples were submerged in this fluid for approximately 20–25 minutes. Liquid was drawn through the fabric at multiple points using a sterile 1 mL volume pipet to dislodge and extract as many chloride and chlorate ion residues as possible. The resulting water extract was used for presumptive and confirmatory testing.

Presumptive Testing

The pH of the solutions was measured using pH test strips (Sigma Chemical Company). The pH measurement was conducted by dropping a portion of the water extract onto a pH test strip and observing any color change. All color changes resulting from these samples were also compared to negative control samples consisting of solely Type 1 water and positive controls consisting of Clorox bleach standard, 8.25% sodium hypochlorite (w/v %).

The presence of oxidizing agents was qualitatively determined using KI–starch paper (Fluka® Analytical). The test was conducted by placing a few drops of the water extract on the KI–starch paper and observing the resulting color change. A dark purple color indicates a high concentration of oxidizing agents and a light purple color indicates low concentration of oxidizers present. Color measurements were determined within the first 10 seconds following the addition of the solution. A positive control of bleach standard and negative control of Type 1 water were used to test the reliability of the paper.

The oxidizing agent concentration was also measured using the orthotolidine and diphenylamine spot tests. Approximately three drops of the water extract were added to a single well of a reaction well plate. A drop of the chemical reagent was added to the same well. For both reagent tests, color reactions were observed and recorded immediately. The orthotolidine will produce varying shades of yellow depending on the concentration of the oxidizing agent. A high concentration will produce a dark orange color. The diphenylamine reagent produces a color change that is murky blue/green for

less concentrated samples and dark blue/black for more concentrated samples. For both chemical spot tests, a positive control of bleach standard and negative control of Type 1 water were used.

Ion Chromatography–Mass Spectrometry Identification and Quantification

Chloride and chlorate ion analysis was performed via ion chromatography–mass spectrometry. The water extract was filtered through a 0.45 μm membrane filter. A 1:10 dilution was prepared by aliquoting 100 μL of the filtrate into individual IC/MS vials and diluting with 900 μL of Type 1 water.

Anion exchange ion chromatography was used to semi-quantitate the chloride and chlorate ions present in the water extract. The ion chromatography instrument used was a Dionex ICS–5000 with MSQ Plus™ spectrometer. Calibration curves and ion measurements from fabric samples were measured as the signal recorded by the conductivity cell detector. Mass spectrometry was used to confirm the identity of chloride and chlorate ions.

Presumptive Test Sensitivity Assays

To test the sensitivity of the KI–starch paper, orthotolidine, and diphenylamine chemical spot tests, a sensitivity assay was constructed by performing serial dilutions of a bleach standard solution and observing color reactions with the addition of the chemical reagents. The concentration of the undiluted bleach solution was 8.25% (w/v %), which corresponds to 82,500 ppm. The following serial dilutions of the bleach standard were prepared: 1:2, 1:10, 1:100, 1:1000, 1:10000, 1:100000, and 1:1000000. These serial dilutions produced expected bleach concentrations of 41,250 ppm, 8250 ppm, 825 ppm, 82.5 ppm, 8.25 ppm, 0.8250 ppm, and 0.08250 ppm.

RESULTS

Presumptive Testing

Substrate samples for both the T–shirt and denim fabrics exhibited average pH values of approximately 4.5 using Type 1 H₂O for extraction. The positive control undiluted bleach standard produced an approximate pH of 12.5 using the pH test strip. The negative control Type 1 water produced a pH measurement of 4.5.

At time zero, the average pH measured 6.6, which is relatively basic compared to the negative control. T–shirt samples incubated at –21°C experienced an overall downward trend in pH as time increased. Figure 3 displays the steeper decline in pH for T–shirt samples stored at 22°C relative to samples stored at –21°C.

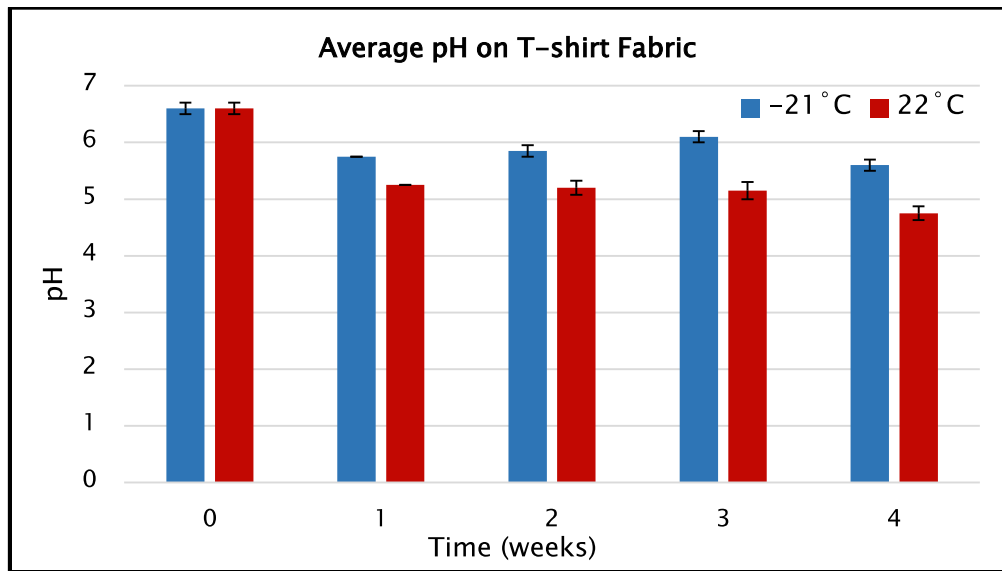


Figure 3: Decreasing pH measurements of bleach stains on T-shirt samples. Error bars represent standard error associated with respective sample means.

For denim samples at time zero, the average pH was 6.4, which is relatively basic compared to the negative control. Denim samples incubated at -21°C experienced an overall downward trend in pH as storage time increased. Similar to T-shirt samples, denim samples incubated at 22°C experienced a faster downward trend in average pH relative to samples stored at -21°C as storage time increased (Figure 4).

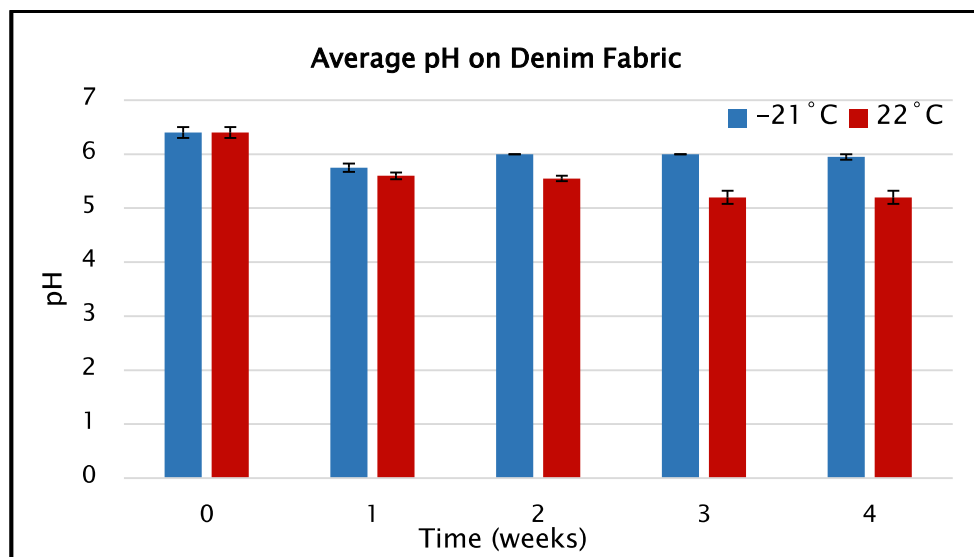


Figure 4: Decreasing pH measurements of bleach stains on denim samples. Error bars represent standard error associated with respective sample means.

The KI–starch paper is used to test for the presence of oxidizing agents in a solution. The positive control of undiluted bleach standard reacted as expected and produced a dark black/purple color that changed to white with time. The negative control of Type 1 water reacted as expected and did not produce a visible color reaction with the paper. At time zero, all T–shirt samples produced dark purple reactions. T–shirt samples incubated at –21°C experienced a decreasing trend in reaction strength as storage time increased. T–shirt samples incubated at 22°C did not produce visible reactions after time zero (Table 1).

Table 1: Summary of KI–starch paper test reactions of T–shirt samples. Very light purple reactions are indicated with (V. Lt. Purple) and no visible reactions are indicated with (No Rxn).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	–21°C	TSHIRT 1	Dark Purple	Light Purple	Light Purple	Light Purple	Light Purple
		TSHIRT 2	Dark Purple	Light Purple	Light Purple	V. Lt. Purple	Light Purple
		TSHIRT 3	Dark Purple	Light Purple	Light Purple	Light Purple	Light Purple
		TSHIRT 4	Dark Purple	Light Purple	Light Purple	Light Purple	Light Purple
		TSHIRT 5	Dark Purple	Light Purple	Light Purple	V. Lt. Purple	Light Purple
	22°C	TSHIRT 1	Dark Purple	No Rxn	No Rxn	No Rxn	No Rxn
		TSHIRT 2	Dark Purple	No Rxn	No Rxn	No Rxn	No Rxn
		TSHIRT 3	Dark Purple	No Rxn	No Rxn	No Rxn	No Rxn
		TSHIRT 4	Dark Purple	No Rxn	No Rxn	No Rxn	No Rxn
		TSHIRT 5	Dark Purple	No Rxn	No Rxn	No Rxn	No Rxn

Denim samples incubated at –21°C experienced a decreasing trend in reaction intensity as storage time increased. At time zero, all denim samples produced purple reactions. One week at –21°C produced a mixture of light and very light purple reactions. Two, three, and four weeks at –21°C produced very light purple or no visible reactions. Denim samples incubated at 22°C did not produce visible reactions after time zero (Table 2).

Table 2: Summary of KI-starch paper test reactions of denim samples. Very light purple reactions are indicated with (V. Lt. Purple) and no visible reactions are indicated with (No Rxn).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	-21°C	DENIM 1	Purple	Light Purple	V. Lt. Purple	No Rxn	V. Lt. Purple
		DENIM 2	Purple	Light Purple	V. Lt. Purple	No Rxn	V. Lt. Purple
		DENIM 3	Purple	V. Lt. Purple	V. Lt. Purple	No Rxn	V. Lt. Purple
		DENIM 4	Purple	V. Lt. Purple	V. Lt. Purple	V. Lt. Purple	V. Lt. Purple
		DENIM 5	Purple	V. Lt. Purple	V. Lt. Purple	V. Lt. Purple	V. Lt. Purple
	22°C	DENIM 1	Purple	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 2	Purple	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 3	Purple	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 4	Purple	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 5	Purple	No Rxn	No Rxn	No Rxn	No Rxn

The orthotolidine chemical spot test screens for the presence of oxidizing agents in a solution. The positive control of undiluted bleach standard reacted as expected and produced a dark orange color reaction. The negative control of Type 1 water reacted as expected and did not produce a visible color reaction. At time zero, all T-shirt samples produced orange color reactions. Table 3 displays consistent reaction intensity for T-shirt samples incubated at -21°C between one week and four weeks of storage. T-shirt samples incubated at 22°C experienced a sharp decline in reaction intensity after initial exposure to bleach (Table 3).

Table 3: Summary of orthotolidine chemical spot test reactions of T-shirt samples. Very light yellow reactions are indicated with (V. Lt. Yellow) and no visible reactions are indicated with (No Rxn).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	-21°C	TSHIRT 1	Orange	Yellow	Yellow	Yellow	Yellow
		TSHIRT 2	Orange	Yellow	Yellow	Yellow	Yellow
		TSHIRT 3	Orange	Yellow	Yellow	Yellow	Yellow
		TSHIRT 4	Orange	Yellow	Yellow	Yellow	Yellow
		TSHIRT 5	Orange	Yellow	Yellow	Yellow	Yellow
	22°C	TSHIRT 1	Orange	V. Lt. Yellow	No Rxn	No Rxn	No Rxn
		TSHIRT 2	Orange	V. Lt. Yellow	No Rxn	No Rxn	No Rxn
		TSHIRT 3	Orange	V. Lt. Yellow	No Rxn	No Rxn	No Rxn
		TSHIRT 4	Orange	V. Lt. Yellow	No Rxn	No Rxn	No Rxn
		TSHIRT 5	Orange	V. Lt. Yellow	No Rxn	No Rxn	No Rxn

At time zero, all denim samples produced orange/yellow color reactions. Table 4 displays that denim samples stored at -21°C experienced a decline in reaction intensity after time

zero, but remained constant between one week and one month of storage. Denim samples incubated at 22°C did not produce visible color reactions at all storage periods after time zero.

Table 4: Summary of orthotolidine chemical spot test reactions of denim samples. No visible reactions are indicated with (No Rxn).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	-21°C	DENIM 1	Orange/Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
		DENIM 2	Orange/Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
		DENIM 3	Orange/Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
		DENIM 4	Orange/Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
		DENIM 5	Orange/Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
	22°C	DENIM 1	Orange/Yellow	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 2	Orange/Yellow	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 3	Orange/Yellow	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 4	Orange/Yellow	No Rxn	No Rxn	No Rxn	No Rxn
		DENIM 5	Orange/Yellow	No Rxn	No Rxn	No Rxn	No Rxn

The diphenylamine chemical spot test screens for the presence of oxidizing agents in a solution. The positive control of undiluted bleach standard reacted as expected and produced a dark black/purple color. The negative control of Type 1 water reacted as expected and did not produce a visible color reaction. At time zero, all T-shirt samples produced opaque blue/green color reactions. As displayed in Table 5, T-shirt samples stored at -21°C and at 22°C experienced reduced but consistent reaction intensity during all subsequent storage intervals.

Table 5: Summary of diphenylamine chemical spot test reactions of T-shirt samples. Opaque blue/green reactions are indicated with (Opaque B/G).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	-21°C	TSHIRT 1	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 2	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 3	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 4	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 5	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
	22°C	TSHIRT 1	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 2	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 3	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 4	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue
		TSHIRT 5	Opaque B/G	Light Blue	Light Blue	Light Blue	Light Blue

Denim samples stored at -21°C experienced a decline in reaction intensity between time zero and one week of storage, but then remained constant between two to four weeks of storage. At time zero, all denim samples produced light blue reactions. One week at -21°C produced only very light blue reactions. Two, three, and four weeks at -21°C produced light blue color reactions. Denim samples incubated at 22°C experienced an overall decline in reaction intensity between time zero and four weeks of storage. After time zero, a mixture of light blue and very light blue color reactions were observed for all storage intervals (Table 6).

Table 6: Summary of diphenylamine chemical spot test reactions of denim samples. Very light blue reactions are indicated with (V. Lt. Blue).

		INCUBATION PERIOD					
		0 WEEK	1 WEEK	2 WEEK	3 WEEK	4 WEEK	
INCUBATION TEMPERATURE	-21°C	DENIM 1	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 2	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 3	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 4	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 5	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
	22°C	DENIM 1	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 2	Light Blue	V. Lt. Blue	Light Blue	Light Blue	Light Blue
		DENIM 3	Light Blue	Light Blue	Light Blue	V. Lt. Blue	Light Blue
		DENIM 4	Light Blue	Light Blue	Light Blue	V. Lt. Blue	V. Lt. Blue
		DENIM 5	Light Blue	Light Blue	Light Blue	V. Lt. Blue	V. Lt. Blue

Ion Chromatography–Mass Spectrometry Identification and Quantitation

As observed in Figure 5, T-shirt samples stored in frozen and ambient environments experienced overall increases in average chloride ion concentration over the four-week storage period. Samples stored at -21°C experienced less of an increase of chloride ion concentration compared to the increase observed for samples stored at 22°C ; this difference is statistically significant. When comparing sample averages of the same storage period but of different storage temperatures, sample sets stored in frozen incubation for one, two, three, and four weeks were significantly less degraded than those samples stored in ambient conditions.

T-shirt samples stored in frozen and ambient conditions experienced decreases in chlorate ion concentration from zero to one week of storage but remained relatively constant thereafter (Figure 6).

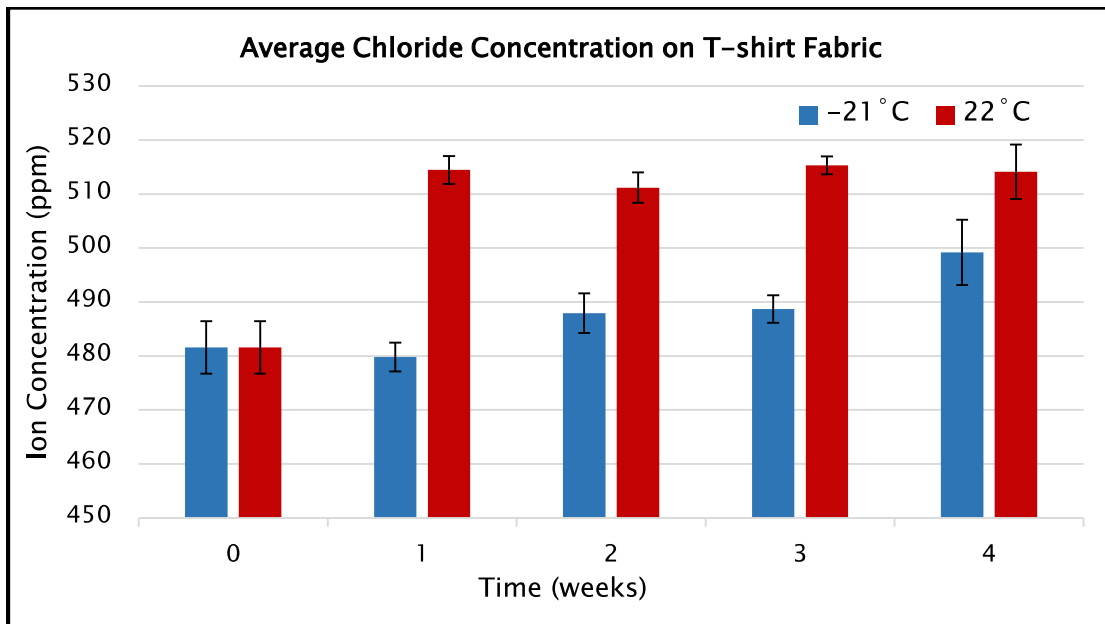


Figure 5: Increasing chloride ion concentration on T-shirt samples stored in frozen and ambient environments. Error bars represent standard error associated with respective sample means.

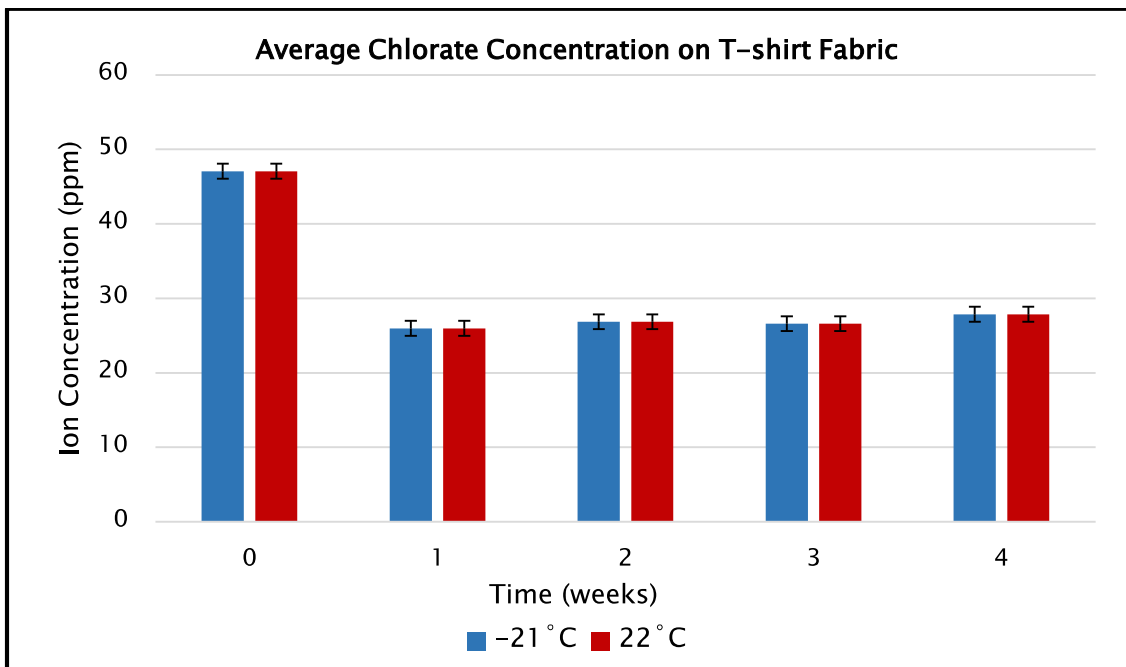


Figure 6: Decreasing chlorate ion concentration on T-shirt samples stored in frozen and ambient environments. Error bars represent standard error associated with respective sample means.

Denim samples stored in frozen and ambient environments experienced overall increases in average chloride ion concentration over the four-week storage period. Denim samples stored at -21°C experienced less of an increase of chloride ion concentration as compared

to the increase observed for samples stored at 22°C; this difference is statistically significant. When comparing sample means of the same storage period but different storage temperatures, chloride ion concentrations of samples stored at -21°C for three and four weeks were significantly less than for those stored at 22°C. The steeper increase in chloride ion concentration over time for T-shirt and denim samples stored in ambient conditions is observed in Figures 5 and 7, respectively.

Denim samples stored in frozen and ambient conditions experienced decreases in chlorate ion concentration from zero to one week of storage but remained relatively constant thereafter (Figure 8).

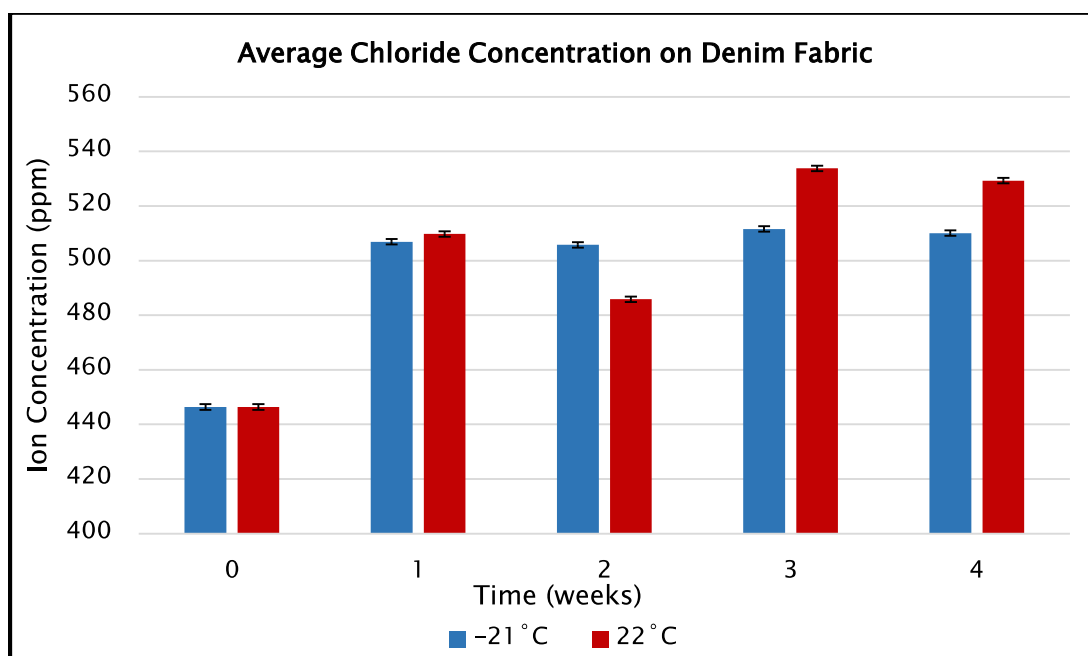


Figure 7: Increasing chloride ion concentration on denim samples stored in frozen and ambient environments. Error bars represent standard error associated with respective sample means.

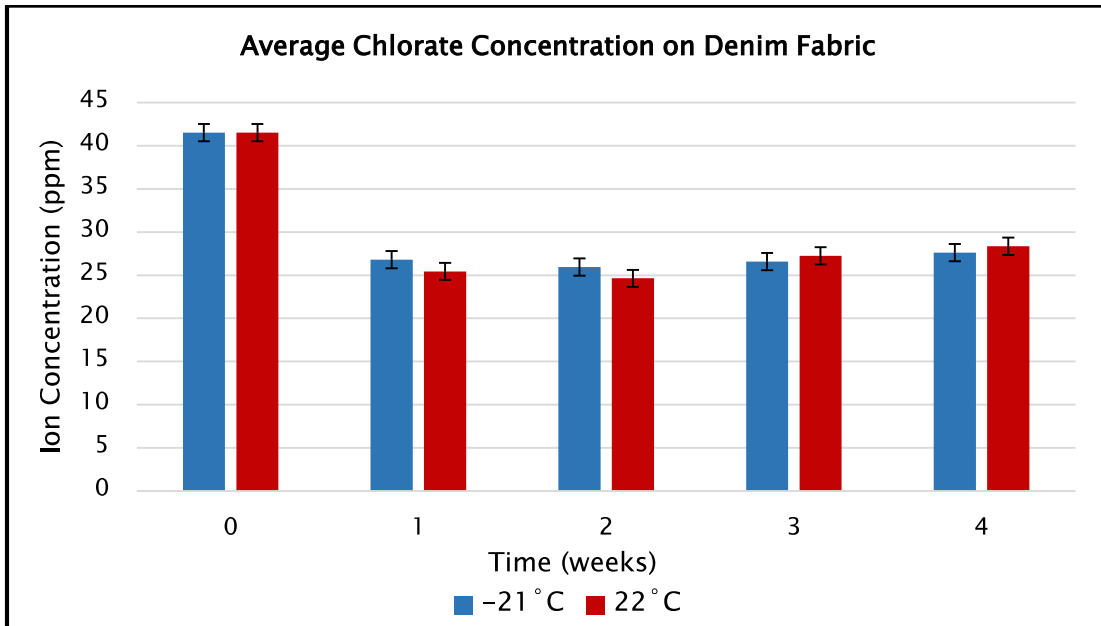


Figure 8. Decreasing chlorate ion concentration on denim samples stored in frozen and ambient environments. Error bars represent standard error associated with respective sample means.

Shown in Figure 9, the chloride ion concentration of the single-knit T-shirt fabric samples was statistically lower than for the twill woven denim fabric at one, two, and three weeks of frozen storage, and higher at time zero. The difference in average chloride ion concentration observed after four weeks of frozen storage was not statistically significant.

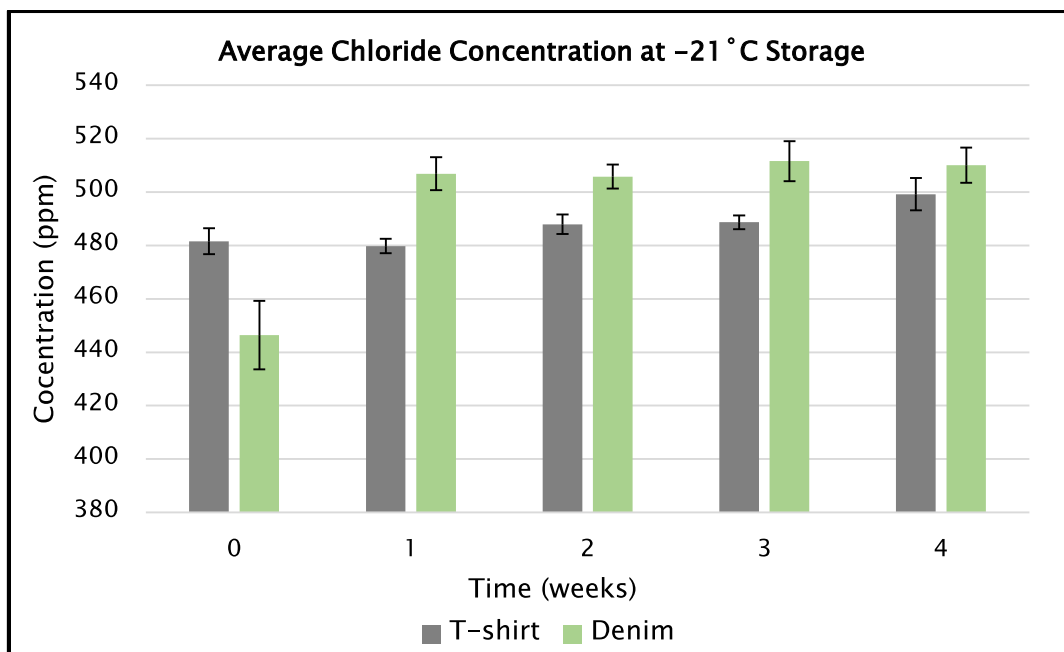


Figure 9: Increasing chloride ion concentration on T-shirt and denim samples stored in a frozen environment. Error bars represent standard error associated with respective sample means.

For samples stored in ambient conditions, the chloride ion concentration extracted from single-knit T-shirt fabric was lower compared with twill woven denim fabric at three and four weeks of storage. This difference is statistically significant (Figure 10).

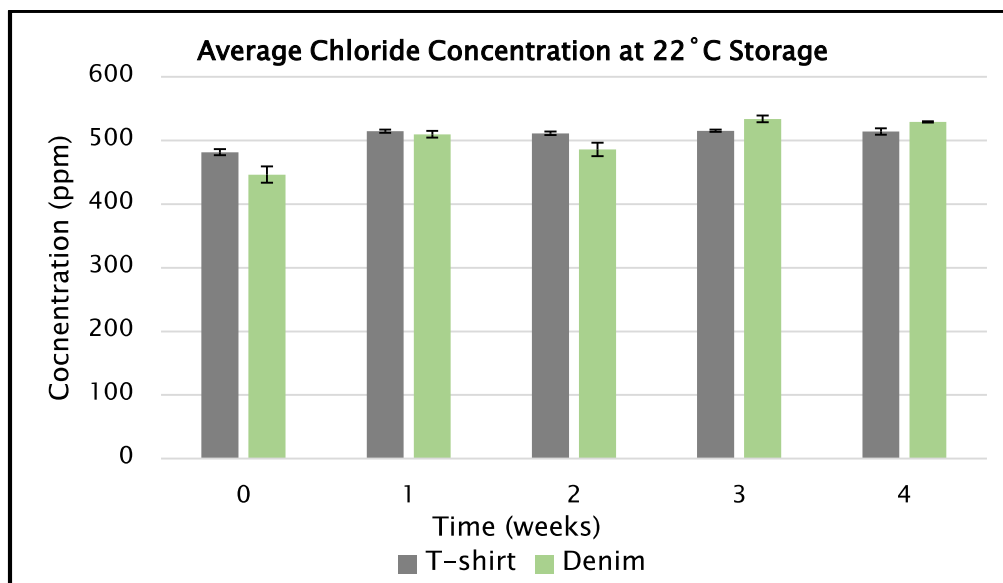


Figure 10: Increasing chloride ion concentration on T-shirt and denim samples stored in an ambient environment. Error bars represent standard error associated with respective sample means.

Presumptive Test Sensitivity Assays

Upon addition of the serial dilutions to the potassium iodide–starch paper, the resulting reactions were observed and photographed (Figure 11). The positive control of undiluted bleach standard produced an initial dark black/purple color that changed to white with time. The negative control of Type 1 water did not produce a visible color reaction with the paper. The 1:2 and 1:10 dilutions produced similar reactions to the positive control as the interior of the stain changed to white. Overall, the reaction intensity decreased as the dilutions increased. The last dilution where a visible color reaction was observed is the 1:10000 dilution. No color reactions were observed for the 1:100000 and 1:1000000 dilutions.

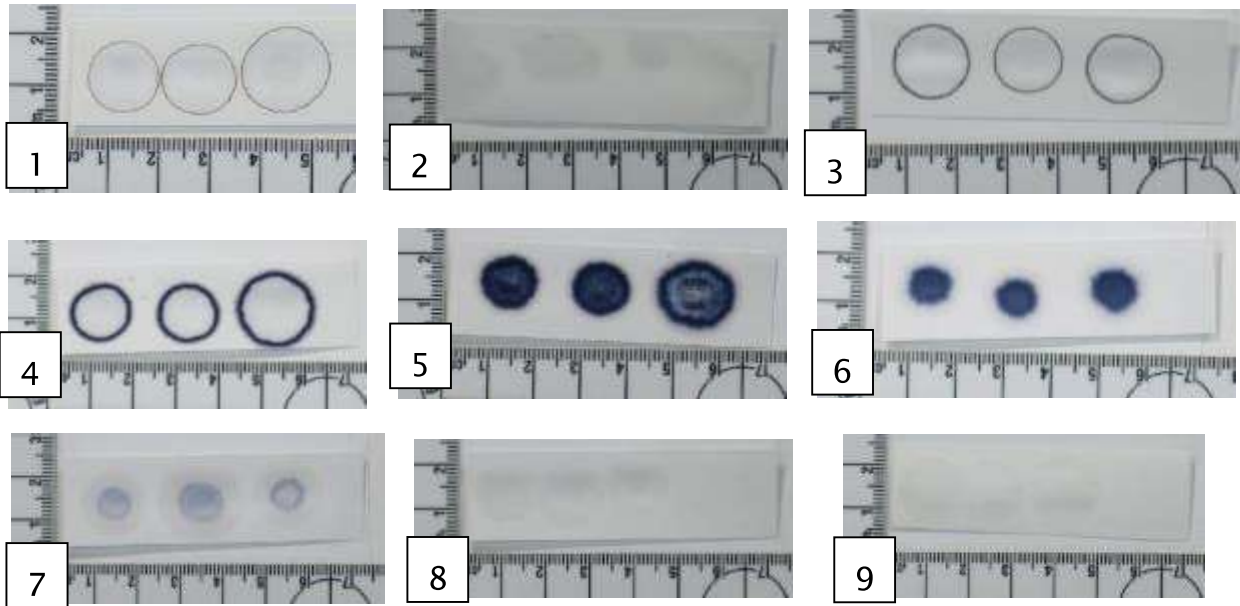


Figure 11: Photographs of reactions of potassium iodide–starch paper sensitivity assay. 1: Positive Control. 2: Negative Control. 3: 1:2 Dilution – 41,250 ppm. 4: 1:10 Dilution – 8,250 ppm. 5: 1:100 Dilution – 825 ppm. 6: 1:1000 – 82.5 ppm. 7: 1:10000 – 8.25 ppm. 8: 1:100000 – 0.825 ppm. 9: 1:1000000 – 0.0825 ppm.

Upon addition of the orthotolidine reagent, the positive control of undiluted bleach standard produced a dark orange color and the negative control of Type 1 water did not produce a visible color reaction. Overall, the reaction intensity decreased as the dilutions increased. The last dilution where a visible color reaction was observed is the 1:10000 dilution. No color reactions were observed for the 1:100000 and 1:1000000 dilutions (Figure 12).

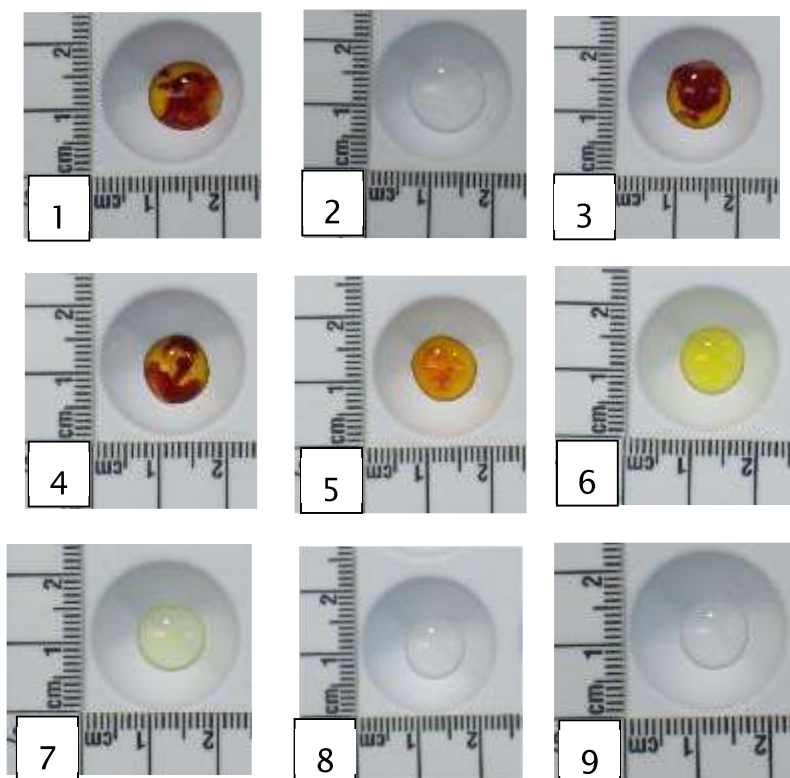


Figure 12: Photographs of reactions of orthotolidine sensitivity assay. 1: Positive Control. 2: Negative Control. 3: 1:2 Dilution - 41,250 ppm. 4: 1:10 Dilution - 8,250 ppm. 5: 1:100 Dilution - 825 ppm. 6: 1:1000 - 82.5 ppm. 7: 1:10000 - 8.25 ppm. 8: 1:100000 - 0.825 ppm. 9: 1:1000000 - 0.0825 ppm.

Upon addition of the diphenylamine reagent, the positive control produced a dark purple/black color reaction. The negative control produced no visible reaction. Reaction intensity decreased as the dilution increased. The last dilution where a visible blue color reaction occurred was the 1:1000 dilution. This faint blue color reaction is observed in Figure 13, Panel 6. A faint orange color is observed in the interior of the 1:1000 color reaction. This faint orange color is also observed in the 1:10000 color reaction. A decreased orange color reaction was observed for the 1:100000 dilution. No visible color reaction was observed for the 1:1000000 dilution.

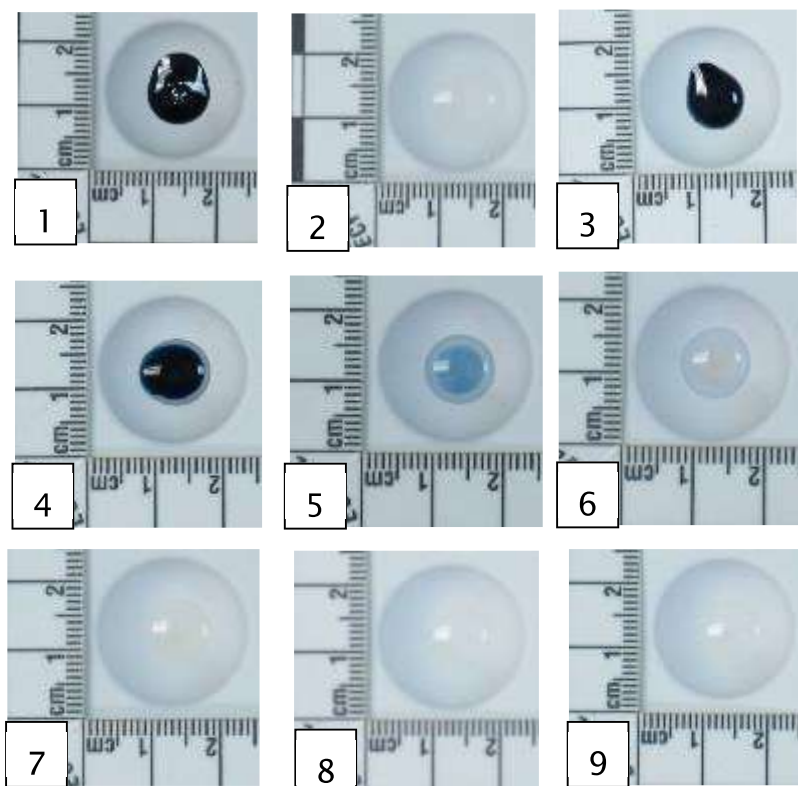


Figure 13: Photographs of reactions of diphenylamine sensitivity assay. 1: Positive Control. 2: Negative Control. 3: 1:2 Dilution - 41,250 ppm. 4: 1:10 Dilution - 8,250 ppm. 5: 1:100 Dilution - 825 ppm. 6: 1:1000 - 82.5 ppm. 7: 1:10000 - 8.25 ppm. 8: 1:100000 - 0.825 ppm. 9: 1:1000000 - 0.0825 ppm.

DISCUSSION

By decreasing the storage temperature, the degradation rate of hypochlorite ions to chloride ions decreased. T-shirt and denim samples stored at -21°C experienced slower declines in overall pH as compared with samples stored at 22°C . As displayed in Tables 1 - 4, the presence of visible color reactions with potassium iodide-starch paper and orthotolidine for samples stored frozen demonstrates that colder storage environments contribute to slower hypochlorite and chlorate ion degradation. The absence of a color reaction for samples stored in ambient conditions indicates that a warmer temperature led to greater degradation of oxidizing agents. As degradation continued, hypochlorite and chlorate ion concentrations fell below the sensitivity limit of these screening tests. The consistent reaction intensity observed with the diphenylamine test on T-shirt and denim samples at both storage temperatures indicates that this screening test is more sensitive than the other presumptive techniques included in this study.

The presumptive test results were supported by the ion quantification levels detected, further demonstrating that freezing reduces the degradation rate. For the T-shirt samples, there was a smaller increase in chloride ion concentration over the four-week

period for samples stored at -21°C compared with those stored at 22°C . This difference in chloride ion formation can be observed in Figures 5 and 7. This indicates a long-term trend that colder temperatures inhibit chloride ion production because of reduced thermal degradation. There is a statistically significant difference in chloride ion concentration at each week, which indicates that temperature has an effect on bleach degradation and is consistent regardless of the storage interval. Similar to T-shirt samples, frozen storage contributed to a reduced chloride ion formation on denim samples over the four-week study because of reduced thermal degradation. However, this overall trend was only observed in samples stored for three and four weeks. The similar chloride ion concentrations observed following storage for one and two weeks may be a result of inadequate time for detection of meaningful differences. As time proceeded, the effect of storage temperature on chloride ion production was supported through statistical testing.

Overall, while chlorate ion concentration decreased from zero to one week of storage time and remained relatively constant from one to four weeks, frozen storage did not significantly affect chlorate ion concentration. However, the participation of chlorate ions in oxidation reactions with the fabric substrate may account for the overall decrease in concentration over time, as contrasted with the increase in chloride ion concentration over the same time period [2]. The transient nature of the chlorate ion concentration indicates that chloride ion concentration is more informative when measuring the effect of temperature on bleach degradation.

As storage time increased from 0 to 1 week, chlorate ion concentrations decreased (remained relatively constant from 1 to 4 weeks). This caused the pH for each fabric at both temperature conditions to approach a more neutral pH (see Figures 3 and 4). This occurs because degradation will occur naturally over time, so it is predictable that degradation cannot be stopped completely. The KI-starch paper test best demonstrates hypochlorite and chlorate ion degradation as a factor of time because fabric samples stored in frozen conditions produced strong to weak reactions as time proceeded. Stronger and intermediate reactions indicate a high concentration of oxidizing agents because less degradation and oxidation has occurred. The weak reactions are a result of low oxidizing agent concentrations because more degradation has occurred over a longer period of time. The orthotolidine and diphenylamine tests display the effect of storage temperature on bleach degradation only in the initial decline of reaction intensity between time zero and one week of storage. The decrease in reaction intensity demonstrates that hypochlorite and chlorate ion degradation start occurring immediately and can be observed after only one week of storage. The inconsistent reaction intensity observed for denim samples stored in frozen storage may be a result of sample variation that caused degradation to occur at non-uniform rates for samples stored in the same conditions.

Regardless of storage temperature, both fabric types experienced statistically significant increases in chloride ion concentration from 0 through 4 weeks of storage time and

statistically significant decreases in chlorate ion concentration from 0 to 1 week of storage time. The observation that chloride ion concentration increased on T-shirt and denim fabric regardless of storage temperature indicates that degradation will occur with time. Decreases in chlorate ion concentration for both fabrics between time zero and one week of storage demonstrates that chlorate ions are consumed by oxidation reactions with the fabric. Hypochlorite ion degradation may be better estimated by chloride production rather than chlorate ion production. However, the presence of chlorate ions is still required to conclude a stain originated from a chlorine bleach product.

Of the two fabrics included in this study, the single-knit T-shirt fabric showed less bleach degradation in comparison with denim. A plausible explanation for this is that the weave of the T-shirt had a smaller surface area per square inch compared with the densely woven denim fabric.

Presumptive tests for the presence of hypochlorite and chlorate consistently indicated higher concentrations of oxidation agents on T-shirt samples. The KI-starch paper test and orthotolidine chemical spot test produced stronger color reactions over the entire four-week study for T-shirt samples, indicating that these samples possessed a higher concentration of hypochlorite and chlorate. This further supports the theory that the smaller surface area per square inch of the T-shirt weave led to a higher concentration of oxidizers present to react with the presumptive tests. Likewise, the greater surface area in the denim swatch could increase the rate of degradation of these oxidizers, thus leading to weaker color reactions.

Quantified ion levels show that hypochlorite and chlorate degradation occurred at a higher rate for twill woven denim because it displayed higher levels of chloride ions, the primary degradation product of bleach. This difference in degradation can be observed in chloride ion levels for samples stored at -21°C between one and three weeks, as well as at 22°C at three and four weeks (Figures 9 and 10, respectively). The concentration of chloride ions was determined to be significantly lower on the T-shirt samples compared with the denim samples. The lower chloride ion levels on the T-shirt samples are possibly a result of fewer degradation reactions occurring on the fabric substrate because of its smaller surface area. Therefore, the bleach staining on the paper packaging, differences in presumptive color reaction intensity, and the quantified chloride ion levels suggests the possibility that single-knit T-shirt fabric exhibits less bleach degradation relative to twill woven denim because of a difference in fiber surface area of the samples swatches.

Presumptive Test Sensitivity Assays

The sensitivity study showed that KI-starch paper and orthotolidine exhibited an approximate 8.25 ppm sensitivity limit for household bleach and diphenylamine exhibited an approximate 82.5 ppm sensitivity limit for household bleach.

The white interior exhibited by the positive control, 1:2 dilution, and 1:10 dilution for the KI–starch paper test demonstrates that the concentration of hypochlorite and chlorate was high enough to bleach any color reaction that occurred. However, the periphery boundaries of the reactions were dark purple, which indicates that a color reaction did occur. The purple colors observed on the 1:100, 1000, and 10000 dilutions indicate that oxidizers were present, but not in sufficient quantity to cause bleaching on the paper. The results of the KI–starch paper indicate that there is an apparent limitation to this presumptive test with solutions of high concentration. As a purple color reaction was bleached away by the presence of excess hypochlorite and chlorate, one may mistake a very high concentration of bleach as a negative reaction.

The orange color reactions from the orthotolidine spot test exhibited expected behavior. The reaction intensity decreased as the dilution increased. The final dilution that exhibited a positive color reaction was the 1:10000 dilution (8.25 ppm) as it displayed a light orange/yellow color.

Finally, the diphenylamine test results present a unique dilemma in regard to sensitivity limits. The test produces an opaque dark blue/black color reaction that was observed in the positive control and 1:2 and 1:10 dilutions. As expected, the reaction intensity decreased as dilutions increased. The 1:1000 solution experienced a weak positive reaction as the blue color reaction ceased to exhibit true opaqueness. Within this blue color was a faint orange color. The 1:10000 solution exhibited this orange color solely (Figure 13, Panel 7). Comparison with the negative control provides a slight contrast in the colors of the two solutions.

The original sensitivity limit of the diphenylamine reagent for sodium hypochlorite as reported by the United States Food and Drug Administration (USFDA) in a 2006 study was 2.0 ppm [3]. This sensitivity limit was achieved by the addition of 100 μ L of diphenylamine reagent to hypochlorite standards of incrementally lower concentrations. The paper identifies a positive reaction as one that produces “an immediate deep blue color” [3]. Using the USFDA criteria, the sensitivity limit for this study would be established at approximately 825 ppm as an opaque blue/green color was produced from this reaction (Figure 13, Panel 5). However, a distinct color reaction (faint blue) was observed in the more dilute solution (Figure 13, Panel 6). Using broader criteria to conclude a positive reaction for the presence of oxidizers, this sensitivity assay established a lower sensitivity limit of 82.5 ppm. The disparity in sensitivity limits may be a result of differences in the amount of diphenylamine reagent added for testing. For the study presented here, the procedure included the addition of one drop of diphenylamine reagent to three drops of bleach solution. The USFDA study utilized 100 μ L of diphenylamine reagent to an unspecified amount of the hypochlorite standard. The higher volume of diphenylamine reagent used in the USFDA study may have contributed to a lower concentration being detected, resulting in a lower sensitivity limit.

During the sensitivity assay, the findings that the KI–starch paper and orthotolidine tests were more sensitive than the diphenylamine test contrasts with the findings that the diphenylamine test performed better for fabric samples stored over the one–month study. One would expect the diphenylamine test to exhibit the highest sensitivity as it provided consistently positive results across all storage periods for both fabric types. While KI–starch paper and orthotolidine displayed higher sensitivity, diphenylamine’s consistent color reactions on textile fabrics leads to the conclusion that diphenylamine should be used as a screening technique for oxidizing agents during practical casework.

As a result of the rapid degradation of hypochlorite ions, the concentration values calculated for all solutions represent ideal concentrations without considering the effects of degradation. It is expected that some degradation occurred within the bleach container prior to initiating the study presented here. Therefore, all concentrations are expected to be lower than the calculated values, thus decreasing the recorded sensitivity limit for the diphenylamine test. The 82.5 and 8.25 ppm concentrations represent the highest possible sensitivity limit (lowest concentration value) for each presumptive test but does not exclude the possibility that the sensitivity limit is actually lower (concentration is of a higher value).

CONCLUSIONS

This study demonstrates that a standardized procedure dedicated to the extraction and analysis of bleach residue on textile fabrics can be tested and formalized. Because hypochlorite degrades to chloride and chlorate ions, presumptive screening and ion quantitation was focused on detecting these decomposition products as an indirect test for the presence of bleach product. In addition, this study identified optimal temperature conditions to store bleach stained fabric evidence to minimize hypochlorite degradation and prolong evidence integrity.

A decrease in storage temperature from 22 °C to –21 °C contributes to a slower decline in measured pH of bleach stains and a slower decline in color intensity with the KI–starch paper test and orthotolidine spot test. However, reaction intensity decreased for both storage conditions as storage time proceeded, indicating that hypochlorite continues to degrade even in a frozen environment. On fabric samples, the diphenylamine test was the most sensitive presumptive screening technique for the presence of oxidizers because it produced consistent positive color reactions regardless of storage conditions. However, during the sensitivity study, the KI–starch paper and orthotolidine tests exhibited a sensitivity limit for household bleach of approximately 8.25 ppm while the diphenylamine presumptive test exhibited a sensitivity limit of approximately 82.5 ppm for household bleach.

The trends observed by presumptive testing were supported by semi–quantitative ion levels determined by the ion chromatograph–mass spectrometer. Decreasing storage

temperature from 22°C to –21°C resulted in a statistically significant decrease in chloride formation on single-knit T-shirt fabric for each storage period and denim fabric for the three and four-week storage periods. For samples stored at –21°C, chloride ion concentration increased less than for those samples stored at 22°C. This difference in chloride ion production for samples stored at –21°C and at 22°C over the four-week study was statistically significant. In contrast, chlorate ion concentration decreased as storage length increased at both temperatures. Finally, stronger presumptive color reactions and statistically significant differences in chloride ion levels indicate that the single-knit T-shirt fabric experienced less hypochlorite ion degradation compared to the twill woven denim fabric in this study. This may be due to the greater surface area of the denim fabric contributing to an increase in the oxidation reaction rate of hypochlorite and chlorate ions.

This study formalized a standard protocol dedicated to the indirect detection of dried bleach decomposition products via presumptive screening for hypochlorite ions and chloride and chlorate identification and quantitation. Upon contact with fabric, dried bleach solution will produce a faded color on the fabric, indicating that hypochlorite ions may be present. Presumptive testing via the diphenylamine test provides further support as oxidizers will produce a strong color reaction. Finally, the hypochlorite decomposition products, chloride and chlorate, are identified and quantitated using IC-MS instrumentation. The combination of a positive visual examination, presumptive color reaction, and meaningful concentration of chloride and chlorate ions indicates the presence of bleach product on fabric. The conclusions drawn from this study indicate that bleach-stained textile fabric samples should be stored in a frozen environment prior to analysis or analysis should be performed as soon as possible upon arrival to the laboratory to minimize the amount of hypochlorite ion degradation.

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