D. Kyle McCandless. ASSESSING METHODOLOGICAL CONTAMINATION IN ORGANIC RESIDUE ANALYSIS USING FT-IR SPECTROSCOPY (Under the Direction of Dr. Laura Mazow), Department of Anthropology, April 2012

Organic residue analysis (ORA) has become a mainstay in modern archaeological and conservation research. Organic residues recovered from archaeological and conservation contexts represent an accumulation of materials by original deposition and post-depositional processes. However, the organic material of interest in the residue can be obscured by contaminants from the environment, and field and laboratory materials. Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique that is becoming increasingly popular in ORA, due to its ease of use, cheap cost, easily repeatable results, and ubiquity in academic laboratories. Infrared spectroscopy has inherent limitations in differentiating components of mixtures. This brings into questions its effectiveness at differentiating target archaeological analytes from contaminants. In this thesis, the question is asked whether materials commonly used in organic residue analysis projects are introducing analytically significant contaminants which have the potential to obscure data generation, analysis, and interpretation. A research project in ORA using FTIR spectroscopy is presented as a case study to address the research question. My research demonstrates that many field and laboratory materials commonly used in ORA sample recovery and laboratory analysis introduce analytically significant contaminants into samples. These include materials for both general use and those which have been manufactured and marketed specifically for use in spectroscopy. ORA projects using IR spectroscopy are cautioned to acknowledge all potential sources of methodological contamination, engage in rigorous control methodologies to independently assess methodological contaminants in their research, and include primary control data in final project publications.

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Using FT-IR Spectroscopy

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Chapter 1
Introduction

Organic residue analysis (ORA) in archaeology refers to the chemical analysis of residual material collected from archaeological contexts and archaeological/historic artifacts. Organic residue analysis has become a mainstay in modern archaeological and conservation sciences. A laundry list of chemical or analytical techniques have been used to characterize organic residues, including Gas Chromatography/Liquid Chromatography-Mass Spectrometry [GC/LC-MS] (e.g., Heron and Evershed 1992; Newmann 1998; Stern et al 2000; Copley 2003; Knappet 2005; Namdar et al 2010; Burton and Levy 2006; Garnar 2008; Barnard 2010; Beck et al 2008; Gregg 2010; Koh 2010; see also Evershed 2008 for a more comprehensive overview of GC-MS in ORA), Nuclear Magnetic Resonance [NMR] (e.g., Ghisalberti 1998; Heron and Evershed 1992; Oudemans et al 2007), Fourier Transform Infrared spectroscopy [FTIR] (e.g., Wilkins 1983; Meilunas et al 1990; Heron and Evershed 1992; Johnson et al 1995; Gillard 1996; Thickett et al 2000; Garside 2003; Ricci 2006; Oudemans 2007; Shillito et al 2009a, 2009b; Sarmiento et al 2010; Luo et al 2012), and Biomolecular Techniques (e.g., Loy and Wood 1989; Loy 1993; Tuross and Dillahey 2011). This list is by no means exhaustive of the multitude of techniques used in ORA. Many of these techniques have been used to characterize residues from a variety of archaeological artifacts such as lithics (e.g., Loy and Wood 1989; Loy 1993; Sobilik 1996; Newmann 1998; Fullerger 2004; Lombard 2004; Galanidou 2006; Tuross and Dillahey 2010), soil (e.g., Davies and Pollard 1988; Shillito et al 2009b;), coprolites (e.g.,Shillito et al 2009a), ceramics (e.g., Heron and Evershed 1992; Dudd and Evershed 1999; Stern et al 2000; Colombini

2005; Knappet et al 2005; Burton and Levy 2006; Eerkens 2007; Oudemans et al 2007; Shishlina 2007; Evans 2008; Garner 2008; Namdar 2010; Shillito et al 2009a; Barnard 2010; Koh and Betancourt 2010), and conservation objects (e.g., Mills and While 1977; Johnson et al 1995, Ricci et al 2006; Thickett et al 2000; Luo et al, in press 2012) in particular paintings and their media (e.g., Meilunas et al 1990; Gillard 1994; Sarmiento et al 2011).

According to Heron and Evershed (2011:239), "Organic residues clearly lack the discernible morphological features that characterize other biological materials that survive in the archaeological record." This suggests that while organic residues form a taxonomic group among themselves, the term "organic residue" is a catch-all for unidentified organic materials that are recovered from archaeological and conservation contexts. Often the information recovered from organic residue analysis is significant enough in its own right, answering primary research questions such as "what were the contents of this vessel" or "what kind of varnish was used on this painting." More often than not, however, the residue analysis data can further be used to address larger research questions concerning trade (e.g., Knappett et al 2005), subsistence (e.g., Copley et al 2003), earliest import of commodities (e.g., Koh and Betancourt 2010), craft production (e.g., Mazow 2008, 2011), ritual contexts (e.g., Burton and Levy 2006), and other artifact-use scenarios (e.g., Heron and Evershed 1993) critical to the interpretation and integration of larger archaeological research questions. In light of the multitude of analytical techniques, and a broad range of applications, it is easy to see how ORA has become common place in modern archaeological research and historic artifact investigations.

Organic residues recovered from archaeological contexts are an accumulation of amorphous components, having been continually subject to site formation and taphonomic processes that significantly modify the residues after deposition (Regert 2007). Residues can be deteriorated by hydraulic and chemical processes, biological activity, or altered by the inclusion of environmental materials (Garner 2008:6). It is the job of the chemical analyst to unravel the components of the recovered residues, separating the original archaeological components, or *analytes*, from later additions. These later additions are commonly referred to as *contaminants* in the sense that they do not represent the target materials and may interfere with target material recovery, analysis, and interpretation if they cannot be analytically isolated.

The analytical technique of primary interest to this research is Fourier Transform Infrared Spectroscopy (FTIR). This technique is becoming increasingly common in modern archaeological research for several reasons. FTIR is relatively inexpensive, easy to use, generates repeatable results, and is commonly available. IR spectroscopy is quite effective at characterizing a broad range of organic materials. These factors have led to a dramatic increase in the use of FTIR in archaeology in the last twenty years. However, IR spectroscopy has one key weakness which must be addressed in organic residue analysis. IR methods are not effective at isolating and identifying individual components in a mixture. This means that IR cannot effectively separate target analytes from contaminants in an archaeological sample without a great deal of background comparative analysis. Most ORA projects work around the weaknesses of FTIR by including other techniques, which can parse individual components of mixtures, in the analytical methodology [most commonly Gas Chromatography/Liquid Chromatography-Mass Spectroscopy [GC/LC-MS]). Projects that do not employ GC/LC-MS as

an analytical method (e.g., Sarmiento et al 2011, Brooks 2010) have a much more difficult task in parsing archaeological residues and contaminants.

In Infrared spectroscopy, differentiating components in a mixture is done by comparing residue data to spectra of known materials. Reference data can be gathered from existing libraries, or generated through analyzing known materials which are targeted as potential matches for the analytes in question. Existing libraries, however, very often do not contain archaeologically relevant spectra. Building reference databases within archaeological programs is time consuming, resource dependent, and still does not promise identification.

Another technique which can isolate methodological contaminants in laboratory analysis is the use of Method Blanks. According to First Environmental Laborartories, Inc. a method blank can be defined by several features; "Method Blank: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank should be carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination resulting from the analytical process" (2012). For example, a typical method blank extraction scenario is one in which an extraction is prepared by placing a sample of material into a flask with a volume of solvent and a second flask is prepared with the same volume of solvent with no sample material added. Both preparations are then processed and analyzed using identical methods. The resulting data are then compared to one another. This allows the researcher to determine the contribution of contaminants from the analytical process itself, and in theory differentiate analytes in the data by removing common elements in both data sets.

FTIR spectra are additive, meaning that signal data from differing components from a sample overlap one another. Method blanks are often employed in spectroscopy so that methodological contaminants can be parsed from the target analytes. Spectroscopy software can "subtract" a method blank spectrum from a sample spectrum to produce a mathematical approximation of the analytes, effectively removing the methodological contaminants from the analysis. However, this subtraction is highly mathematically derived, and can be significantly affected by minor quantitative differences in the sample concentrations and/or atmospheric parameters of analysis. The subtractions *are* helpful for separating methodological contaminants, and interpreting analytes, but spectra resulting from subtraction that are used for identification are often suggestive at best. Preventing contaminants in samples prior to analysis is always preferred, and leads to more sound data and interpretation.

Archaeological residues are exposed to contaminants from three major sources: the environment, human interaction during field work, and in the laboratory. Common environmental contaminants include plastics and plasticizers, which are ubiquitous in the global environment, and organic byproducts of bacterial activity in close proximity to the existing residues (Stern et al 2000:399-400; Garner 2008:6; Solazzo and Erhardt 2007:17). Field and laboratory methodologies employed by researchers can also introduce contaminants such as plastics, plasticizers, and antioxidants (Garner 2008; Koh 2008; Beck et al 2008). These include plastic bags, buckets, etc. used during sample collection, shipping, and storage (Garner 2008:6). Organics from direct human contact have also been noted as methodological contaminants in some residue analysis projects (Garner 2008).

Contamination in organic residue is a general topic of discussion in many publications. However, the specific impact of contaminants, and the research methods employed by researchers to screen for contaminants, are not often presented in the primary literature. Some research publications include a cursory discussion of contaminants that were identified during their analysis (e.g., Heron 1992; Newman 1998; Stern 2000; Oudemans 2007; Garner 2008; Koh 2008, Koh and Betancourt 2010, Namdar 2010). In these publications, plasticizers are the most common contaminant identified. This is most likely due to the fact that plastics and plasticizers are often chemically different from typical archaeological residues and are recently discovered materials of modern origin, and are therefore easily differentiated in many analytical techniques. When plastic and plasticizer contaminants *are* identified during research, analysts (e.g., Newman 1998; Garner 2008; Koh 2008) commonly assume that contaminants have been introduced into residues by either environmental processes or particular methodological materials (i.e. plastic buckets, bags, or human contact). However, the actual sources of the contaminants are not generally verified by independent analytical assessment.

GC-MS can fairly easily identify plastics and plasticizers, but many other techniques cannot (e.g., FTIR, NMR). Environmental contamination will remain both an ever-present concern and an uncontrollable element in organic residue analysis research. The methodological materials used by researchers in the field and laboratory, however, can be controlled. There are a number of methodological materials commonly employed in organic residue analysis (e.g., solvents, bags, aluminum foil, gloves) that are potential sources of contamination. Most publications, however, do not include analytical assessment of these

methodological materials. Some researchers assume aluminum foil is an appropriate wrapping for use in organic residue collection methodologies without assessing the potential for contamination from foil residues (e.g., Koh 2010; Koh and Betancourt 2010). Several researchers identify the solvents used for laboratory analysis as "high grade" or "chromatography grade" (e.g., Koh 2010; Brooks 2010). Researchers assume that high-grade solvents are devoid of contaminants without verification (e.g., Koh 2010; Brooks 2010). It is, however, possible that background controls are being run on these materials within analytical projects, but the data and analysis is not being presented in the final publication. In any case, we are left with two common methodological assumptions explicit or implicit in organic residue analysis publications regarding contamination. The first is that the source of identified plastic contaminants can be safely assumed to have originated from the environment or plastic containers. The second is that there are methodological materials which can be assumed to be free of analytically significant contaminants without independent assessment. It is the goal of this research to assess the validity of these common assumptions. Are common methodological materials used in organic residue analysis projects introducing analytically significant contaminants into samples? If methodological materials are introducing analytically significant contaminants in residue analysis, then control and contamination protocols within ORA projects should become a central theme for discussion in primary literature. In light of these criticisms, it seems likely that the data and interpretations presented in ORA publications needs to be reevaluated. Thus, the present work is of critical significance to the archaeological and conservation communities by providing a case for contamination in methodology, and providing a schema by which other projects may be compared.

To answer the research question, the analytical methods used in a residue analysis project as a case study for evaluating methodological contamination in organic residue analysis were examined. Chapter 2 introduces the case study project in ORA using FTIR spectroscopy. Chapter 3 addresses the theory and methods of FTIR spectroscopy, including a more detailed discussion of its methodological strengths and weaknesses. Chapter 4 covers the complete research experimental methodology including individual experimental background, methods, and analysis. Chapter 5 discusses the experimental results. Chapter 6 answers the research question, and draws conclusions from the research project to address the broader issue of methodological contamination in organic residue analysis.

Chapter 2
The Bathtub Research Project:
a case study for organic residue analysis using FT-IR spectroscopy

The Bathtub Project is an interdepartmental, interdisciplinary research project at East Carolina University that uses FT-IR to analyze archaeological residues. The primary goal of the Bathtub Project is to analyze organic residues from ceramic and stone "bathtubs" to address the function of these vessels in antiquity. The term "Bathtub" describes a group of ceramic or stone ovoid vessels which are commonly fixed with external loop handles and interior drain holes (Figure 1). According to Mazow (2011:4), "*Bathtubs* have been reported from a variety of contexts, including tombs, sanctuaries, and private homes or large official rooms of elite residences." Traditionally, scholars have interpreted these vessels as "bathtubs" based mainly on their physical resemblance to later bathing basins in historic and modern contexts. "Bathtubs" are commonly presumed to have been primarily hygienic or ritualistic in function based on the interpretation of their morphological features rather than empirical data to support the claims. Further, archaeologists have labeled rooms or architectural site features from archaeological contexts as bathrooms or ritual spaces based solely on the presence of "bathtub" vessels and their presumed function (Mazow 2011).

The director of the Bathtub Project, Dr. Laura Mazow in the Department of Anthropology at East Carolina University, has suggested that bathtub vessels may have served a function in industrial activity and craft production. Historically, production of unfinished wool and fabric could be processed in two ways. First, raw wool must be scoured to remove impurities from the

Figure 1-"bathtub" vessel from Tel-Jezrael. Photograph taken by Susanne Grieve for the Bathtub Research Project, Summer 2010, (Israel Antiquities Authority).

material. Scouring is usually done by drenching raw wool in a vessel filled with water, urine, or other liquid detergent mixture and agitated until clean. Second, woven cloth is again washed in a liquid detergent filled basin. This washing of woven wool is known as fulling. Historic records from Roman times suggests that both of these techniques of wool textile processing were carried out in tub shaped vessels such as those commonly labeled as "bathtubs" in pre-Roman contexts (Mazow 2008, 2011). Further, some bathtub vessels have been recovered from site contexts that contained industrial artifacts used in the production of wool textiles such as spindle whirls, loom weights, and structural supports for the housing of wooden drying racks (Mazow 2011). Mazow is seeking chemical evidence in the organic residues that these artifacts may retain for direct empirical evidence with which the role of bathtubs in the textile industry in pre-Roman society may be addressed.

A waxy substance known as lanolin (also referred to as wool wax or wool oil) is the major byproduct of both, wool scouring and fulling. Lanolin is a complex mixture of aliphatic hydrocarbons, alcohols, and sterols. Lanolin has been used in personal hygiene and cosmetic products from antiquity to the modern day. A large portion of the chemical composition of lanolin is organic hydrocarbons. Hydrocarbons are highly resistant to degradation by typical post-depositional and hydraulic processes in soil (Charters et al 1993). Thus, lipids and waxes often survive intact in archaeological contexts. Lanolin and its derivatives are therefore suitable target analytes for modern organic residue analysis (Charters et al 1993).

It is hypothesized that the presence of lanolin or lanolin derivatives on the surface of "bathtub" artifacts would support the interpretation that these vessels were used in wool textile production (Mazow 2008, 2011). In order to test this hypothesis, Mazow and her associate Susanne Grieve, Director of Conservation Studies in the Department of History at East Carolina University, travelled to Cyprus and Israel in the summers of 2010 and 2011 to recover residue samples from "bathtub" vessels.

The sample collection methods employed were limited to non-destructive techniques, and limited by the availability of many volatile sample collection solvents. The samples were recovered using several methods. In most cases the samples were recovered with either foam-tipped plastic or cotton-tipped wooden swabs which had been dipped in an organic solvent such as acetone or petroleum ether. Surface scrapping and solvent baths were also employed to recover residues from some artifacts. The samples were then packed in foil or glass vials, placed in plastic bags, boxed, and shipped to East Carolina University for chemical analysis by FT-IR and other methods. Anthony Kennedy, professor in the Department of Chemistry at East

Carolina University, is aiding in the analysis and interpretation of samples, and all other chemistry related aspects of the project.

While the primary goal of the Bathtub Research Project is to test the hypothesis that "bathtub" artifacts were associated with Bronze and Iron Age wool textile production, a secondary goal of the project is to further develop a non-destructive, yet functional method for future sample recovery and analysis. My thesis research developed out of the laboratory control experiments used to evaluate the field and laboratory methods employed in the Bathtub Project for sample recovery and FT-IR analysis. During the preliminary sample evaluation experiments the significance of methodological contamination in the Bathtub Project became apparent. FT-IR generated spectra of sample residues appeared qualitatively similar to lanolin. However, contaminant materials from swabs and other sampling and storage materials were also influencing the spectral images. The swabs used for sample recovery were then separately extracted and analyzed in a preliminary control experiment. It was determined that the swabs were introducing chemical components in solvent extractions that were both chemically similar in composition to lanolin, and appeared in significant concentration that could obscure residue analytes. Therefore, sample residue analysis could not be confidently interpreted, and lanolin could not be confidently identified, without isolating and removing methodological contaminants through control experiments.

The research presented in this thesis was derived from the experimental controls developed to address methodological contaminants in the Bathtub Project. These control experiments are designed to address three major goals. The first goal is to use FTIR spectroscopy to determine the presence of contaminant materials being introduced during field

and laboratory methodology into the Bathtub Project samples. Second is to build a spectral database of contaminants from methodological materials for comparative analysis in the Bathtub Project. Third is to remove materials and methods which introduce analytically significant contaminants in the existing sample methodology, while maintaining a non-destructive sample recovery methodology.

Chapter 3:
Fourier Transform-Infrared (FT-IR) Spectroscopy

Infrared spectroscopy is an analytical technique based on the property of differing molecules to absorb specific frequencies of electromagnetic radiation based on their particular chemical makeup. Some materials absorb radiation in the infrared spectrum (2.5-40μm) while others do not. When a specific frequency of infrared is absorbed by a particular chemical bond, the absorbed energy causes vibrations in the molecule. This frequency is referred to as the characteristic resonant frequency of that structure (Fifield and Kealey 2000:381). There are several different types or "modes" of vibration that can be caused by excitement from infrared radiation (Figure 2). The strongest vibrational modes, which require the highest amount of energy to excite, are symmetrical and asymmetrical bond stretching. Another set of modes is referred to as the *in-plane* modes. These are the "scissoring" and "rocking" of bonds along a plane relative to a central axis. The final pair of modes is the *out-of-plane* modes. These are "wagging" and "twisting" of molecules around a central axis, swinging in and out of the axial plane.

Infrared Spectrometers measure the frequency and resulting vibrational energy in a sample to determine its chemical components and structure. Fourier Transform-Infrared (FT-IR) spectrometry/spectroscopy is one such method of vibrational analysis. FT-IR spectroscopy is based on the Michelson Interferometer (Figure 3). In a simple Michelson Interferometer a beam of infrared light passes through a beam splitter where it is split into two separate beams.

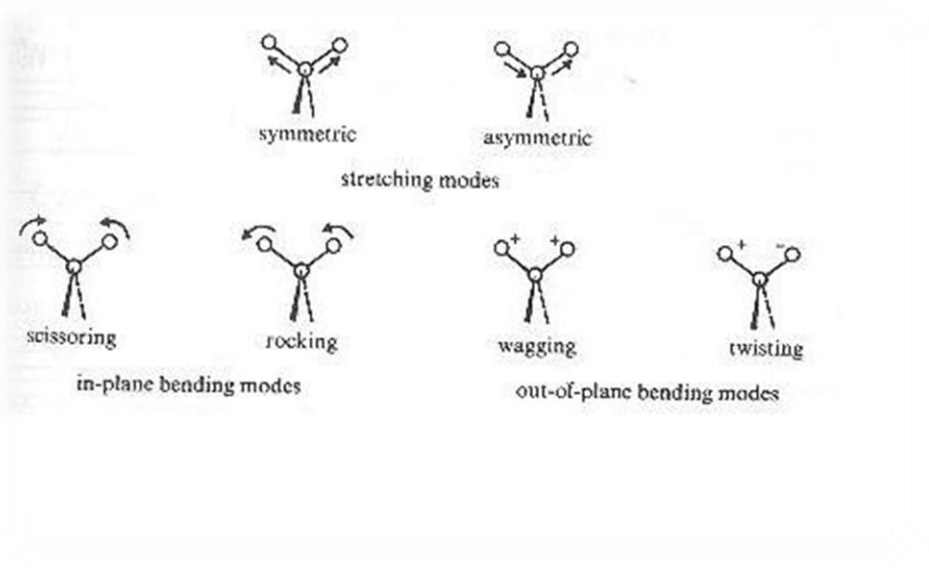

Figure 2-Vibrational Modes (Fifield and Kealey 2000:385).

One beam follows a path to a fixed mirror, the other to a moving mirror. The moving mirror travels from and towards the beam splitter parallel to the source, increasing and decreasing the path length of its beam relative to the fixed mirror path. As the beams reflect off the separate mirrors and recombine at the interferometer, they are out of phase due to the difference in relative path length of each returning wave. The recombined beam then passes through a sample where the wave signal of the beam will be selectively attenuated by the sample's absorption at specific wavelengths. All wavelengths arrive at the detector at once and are recorded as a single datum called an *interferogram*. The *interferogram* is not useful in its native format. The *interferogram* must be converted by another method that is determined by the analytical technique being applied.

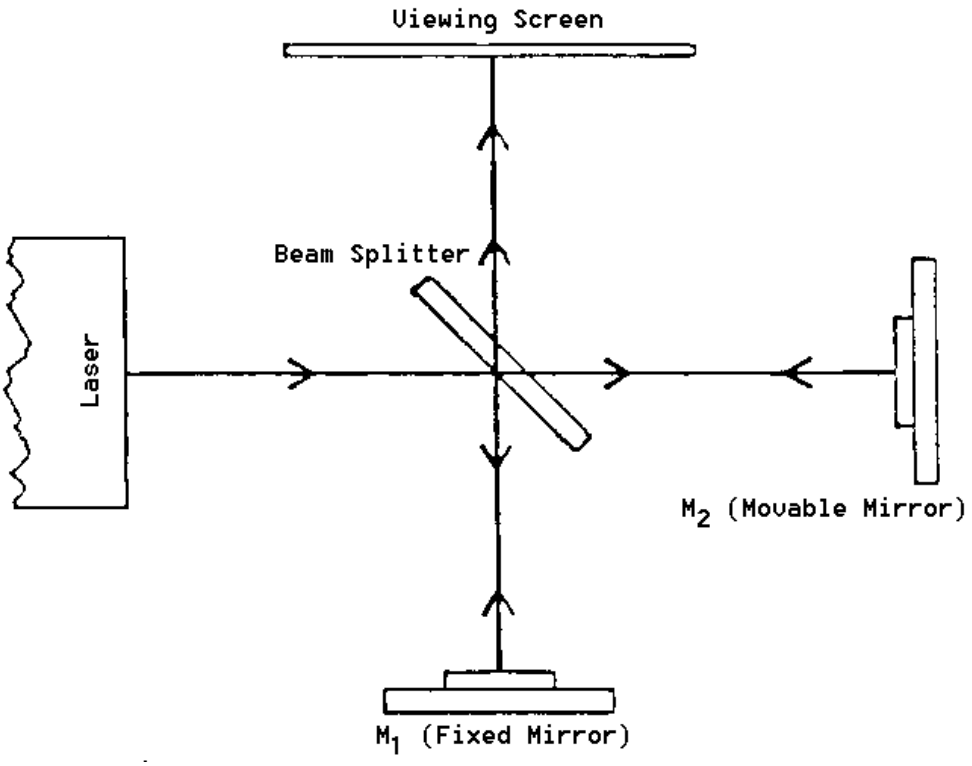

Figure 3-Michelson Interferometer, (http://felix.physics.sunysb.edu/~allen/252/phys51_Michelson.html).

In FT-IR spectroscopy a mathematical data-processing technique called a Fourier Transform, carried out in most cases by a connected computer, converts the raw interferogram into a usable spectrographic image. A spectrum is a graphical representation of sample absorption (Y-axis) vs. frequency (X-axis). The peak position, shape, and peak height ratios reflect the attenuated wave energy and thus the vibrational bond energies of the sampled material. The appearance of an absorption band in a particular region of the spectrum correlates to specific vibrational modes in the molecules. The *spectrum* can be interpreted to determine the bonds present in the sample by which the chemical composition of the sample can be derived. The *spectrum* can also be compared to spectra of known materials to aid in identification and interpretation of the unknown sample material.

There are different spectroscopic sampling methods by which a spectrometer can facilitate the interaction of the infrared light and the sample materials. The most common sampling methods are *direct transmission* and *attenuated total reflectance*. During transmission spectroscopy a sample is presented in a section thin enough for infrared light to transmit through the material along its path. This method requires special preparation of the sample material by sectioning or incorporation into a material that does not absorb IR radiation to facilitate analysis. Thin sections can be mounted for transmission if the sample materials have properties that are conducive to such manipulation. Many solid materials such as minerals that are difficult to thin section must be specially prepared for direct transmission by incorporation in a medium such as potassium-bromide (KBr), which is infrared transparent. KBr pellet creation requires that a sample be ground into a powder, added to potassium-bromide, and compressed into a homogenous disc. This method provides excellent spectra, although sample prep is time consuming and it is difficult to prepare pellets of exactly the same thickness and concentration repeatedly.

Another sampling method is called attenuated total reflectance (ATR). In this method the infrared beam is routed into a crystalline medium in which the beam is totally reflected at the surface of the crystal. The beam then returns to the splitter and is directed towards the detector. While the wave is totally reflected upon contact with the crystal (total internal reflectance), a portion of the initial wave energy is transmitted into the material on the crystal in the form of an evanescent wave, effectively attenuating the source wave (Garside 2003). During ATR analysis the sample is brought directly into contact with the crystal window for characterization. ATR analysis can characterize samples that are intact solids, powders, or

solvent extractions. An intact solid material may be pressed against the crystal for ATR analysis with little to no preparation. However, in some cases a suitable surface for optimal crystal contact is not present, and the material must be ground into a powder that is cast on the crystal. Grinding a sample into a powder prior to analysis provides a greater surface area and homogeneous sample for analysis that increases the likelihood of usable spectra.

A third preparation method is solvent extraction. Solvent extraction is a process of analyte recovery in which a solvent is utilized that will only dissolve components of the sample with particular chemical characteristics. Extraction is an important tool in analysis where the target analyte is incorporated in its original integrated context (such as in soil, or on a stone, fabric, or ceramic surface). One common method of extraction is to soak a portion of the matrix directly in a volume of solvent, where the target analyte can be extracted by the solvent preferentially. In some cases a portion of the matrix is ground into a powder prior to extraction, in others the sample material remains intact. A solvent is chosen which will selectively dissolve a target component of the material while minimizing the removal of the unneeded materials. A sample of the extraction solution is then applied to the ATR crystal and allowed to dry. The residual material which remains on the crystal after the solvent has evaporated can then be characterized by FT-IR. In situations where the sample matrix cannot be destroyed, a sampling implement such as a swab may be used to collect surface samples. This technique is most commonly employed in conservation science to collect sample paints and painting varnishes from the surface of paintings, murals, statues, and other conservation artifacts. A surface sample is recovered by wiping the surface of the material with a utensil that is often dipped in an appropriate solvent to aid in residue collection. This method is often

necessary in situations where the target sample material and analytical laboratory are in separate location, or in cases where the material cannot be excessively damaged or destroyed during analysis, such as rare archaeological or historical artifacts. Collecting samples with swabs or other sampling implements can reduce damage to artifacts. However, though the implement itself can introduce contaminant materials, which can present problems for chemical analysis.

Infrared spectroscopy is most often used to characterize "natural products, polymers, detergents, lubricants, fats, and resins" (Fifield and Kealey 2000). FT-IR analysis is most appropriate for characterizing single component materials of known origin. Materials of unknown composition can be characterized with ease, but the identification of these materials is subject to a great deal of interpretation. Library databases of FT-IR spectra can be compared to unknown samples. This can help identify particular chemical components and structures in sample spectra. Comparison to known substances, however, does not always allow for the identification of samples. Successful identification of unknown materials depends a great deal on the chemical complexity of the sample, the availability of libraries with relevant spectra by which comparative analysis can be employed, and the experience of the chemical analyst.

FT-IR spectroscopy has several methodological strengths. FT-IR is a very sensitive analytical technique which can resolve samples at the nanogram scale (Meilunas et al 1990). The techniques of FT-IR are thus highly effective in characterizing trace amounts of sample material. Nanogram sample size is significantly lower than the minimum required for analysis in related IR techniques. In conservation science, smaller sample size equates to a less destructive invasion of artifact materials, which is preferable in nearly all scenarios. Another

asset of FT-IR analysis is that the results from the technique are highly repeatable. The crystals used in FT-IR analysis allow for direct testing of sample materials with little to no preparation or modification. With a small amount of training the test can be run multiple times without recalibration, which provides repeatable and consistent results.

There are two major weaknesses with FT-IR techniques. First, FT-IR spectroscopy is not well-suited for analyzing mixtures owing to the fact that the resulting spectrum is a combination of all components present in the sample which respond to IR radiation (Fifield and Kealey 2000:378). The resulting spectrum from FT-IR analysis includes absorption from all species in the sample. This means that while certain features of the sample may be differentiated (i.e. C-H bond stretching vs. O-H scissoring), the individual source materials of the varying molecular components cannot be separated or independently identified. Reference to spectra of known substances can allow for the interpretation of mixtures in some cases. In cases with no modern representative database spectra (a common case with archaeological and conservation artifacts), the task of identification is doubly difficult.

The second weakness of FT-IR is its limited use in quantitative analysis. The quantity of a particular feature in a spectrum is dependent not only on the quantity of the component in the sample, but also physical features of the sample medium, such as the refractive index of the extraction fluid (if used), instrumental and atmospheric variability, and the method of crystal casting. Thus, the resulting spectrum can be compared in terms of peak position (wavelength of absorption) or peak height ratios within a spectrum (pseudo-quantitative analysis), though gross quantitative comparison between two sample spectra is problematic and inconsistent at best.

There are several key factors that relate to the use of FTIR spectroscopy in the contexts of archaeology and conservation science, and consequently this research. Due to the strengths of FTIR described above, there has been a dramatic increase in IR techniques in artifact analysis. As mentioned above, the analysis of artifacts often requires that samples be recovered by non-destructive methods. This leaves researchers little choice but to recover samples by means of direct solvent extraction, or sampling implements. Both of these methods have the potential to introduce methodological contaminants that IR spectroscopy cannot effectively parse from target analytes. The limitations of IR spectroscopy and constraints on the destruction of archaeological and conservation materials force researchers to make certain concessions. On the one hand, destructive methods are effective at recovering organic analytes, which provide researchers with the information they seek, though it damages the artifact. On the other hand, non-destructive methods can be employed that leave artifacts intact and may not prove effective in data recovery, though they will likely introduce a significant vector for methodological contamination of samples. The present research evaluates the concerns of contamination when non-destructive methods are used in organic residue analysis research.

Chapter 4
Methodology

Introduction

The purpose of the experimental methodology presented in this research is to determine if contaminants were introduced by the materials and procedures used in the Bathtub Research Project (BRP). The experiments presented herein were designed to mimic the sample collection and analytical methodologies employed by the BRP. Each experiment isolates a sample collection or laboratory material or method. Further, the experiments are organized in such a way that each material, and material interaction, is assessed as it is introduced into the BRP methodology. This allows me to determine the introduction of contaminants as they would appear in the sample collection methodology, rather than as independent variables in isolated contexts. In this way, the results are specifically tailored to answering research questions concerning contamination in the BRP methodology in particular. Table-1 presents the sample collection methodologies used in the BRP and the correlating experiments conducted in this research (T-X) to evaluate each stage in the BRP methodology.

Table 1 - Correlation of BRP methodology and My Research Methodology

Bathtub Project Methodology	Research Methodology
1: A sample swab was selected from either a cotton-tipped wooden swab or a foam-tipped plastic swab for sample collection	• "T-1" evaluates swab contaminants
2: The selected swab was dipped into a sample collection solvent which would increase likelihood of organic residue recovery	• "Solvent Analysis" determines contaminants in raw solvents prior to introduction into methodology • "T-2" and "T-3" evaluate sampling solvent and swab contaminant residues when extracted in petroleum ether • "T-12", and "T-13" evaluate sampling solvent interactions with both swab types independent of petroleum ether extraction
3: The solvent coated swab was wiped over a target area of the bathtub artifact interior to recover residue sample	
4: The sample swab was then wrapped in aluminum foil to protect sample and prevent contamination	• "T-5" and "T-6" evaluate aluminum foil contaminants in methodology
5: The foil wrapped swab was placed in a plastic bag for further protection during storage and shipping	• "T-8" and "T-9" evaluate plastic bag contaminant
6: The samples were removed from the bags and foil in the receiving laboratory where the swabs were placed in extraction solvents for sample extraction.	• "T-0", and "T-11" evaluate vial contaminants introduced into solvents during extraction
7: The extracted residues were analyzed by FTIR spectroscopy	• All experiments were analyzed by FTIR spectroscopy and a functional analytical methodology was developed

In this chapter, I first discuss the general hardware, software, and materials used in the research methodology. Next, I discuss the solvents used in the laboratory which mimic the solvents used by the BRP to collect and extract samples. These are characterized by FTIR to determine, first, the spectroscopic characteristics of the raw solvents, and, second, the presence of spectroscopically visible contaminants in the solvent residues. The BRP and this research use solvents in two different applications, to aid in recovery of samples, and to extract samples in the lab. *Sampling solvent* refers to solvents that are applied to swabs that aid in sample recovery in the field. *Extraction solvent* refers to solvents that are used to preferentially extract the recovered samples from the sample recovery materials that arrive in the laboratory for analysis. Any one solvent can be used as both a Sampling solvent and an extraction solvent depending on the experimental context within which it is used. Lastly, I present the laboratory experiments conducted in this research. Experiment T-0 is designed to determine if the vials used for sample storage and analysis introduce contaminants into sample residues when used to extract samples with petroleum ether. Experiment T-1 evaluates contaminants that are extracted from sample collection swabs when extracted in petroleum ether. Experiments T-2 and T-3 evaluate contaminants extracted in petroleum ether when various sampling solvents are applied to swabs prior to extraction. T-2 and T-3 determine the introduction of analytically significant contaminants introduced into the research methodology by sampling solvent/swab interactions. Experiments T-5 and T-6 evaluate contaminants extracted in petroleum ether when various solvents are applied to sample swabs, and the samples are wrapped in aluminum foil for a week prior to extraction. T-5 and T-6 determine the introduction of analytically significant contaminants introduced into the research

methodology when using aluminum foil as described. Experiments T-8 and T-9 evaluate contaminants extracted in petroleum ether when sampling solvents are applied to swabs, the swabs are wrapped in aluminum foil, and placed in a plastic bag for one week prior to extraction. T-8 and T-9 determine the introduction of analytically significant contaminants introduced into the research methodology when placing samples into plastic bags for storage. Experiment T-11 is designed to determine if the vials used for sample storage and analysis introduce contaminants into sample residues when used to extract samples with acetone, chloroform, ethyl acetate, isopropyl alcohol, methanol, or purified water. Experiments T-12 and T-13 evaluate contaminants introduced by sampling solvent and swab interactions independent of research methodology by extracting swabs in sampling solvents listed in T-11. T-12 and T-13 determine trace contaminants introduced by sampling solvent and swabs that were not visible in previous experiments, and further assess which sampling solvents present appropriate sample/swab interaction characteristics for future BRP sample collection.

General

Hardware and Software

All FT-IR analysis was run on a ThermoFisher Nicolet 6700 Spectroscope. This instrument uses an everglo® infrared source, a potassium bromide (KBr) beam splitter, and a DGTS deuterated triglycine sulfate detector. Unless otherwise stated, attenuated total reflectance (ATR) analyses were run on a SmartArk accessory with a 45degree multi-bounce zinc-selenide (ZnSe) internal

reflectance element (IRE). Some background volatile solvent characterizations were analyzed on a Golden Gate-DiamondATR accessory with a single bounce diamond IRE. All spectra were collected at a resolution of 4cm-1. Omnic 8 software was used for analysis and processing of all spectra. After initial data collection, spectra were processed to correct for atmosphere, then ATR corrected, and finally automatically baseline corrected. Atmosphere correction compensated for minor atmospheric fluctuations that appear on spectra. All spectra in this thesis are presented in absorption mode.

Solvents

All solvents used in this research represent solvents that were used in the BRP to collect field samples or to extract samples during laboratory analysis. Each experiment outlines the list of solvents used in it, and how these solvents were employed (e.g., as sampling solvents or extraction solvents). Ethyl acetate, chloroform, petroleum ether, and methanol were all spectrophotometric grade for use in chemical analysis. Petroleum ether was packaged in a metal jug with a plastic pop-out spout, while the other three high-grade solvents were contained in standard, dark brown glass bottles. Acetone, isopropyl alcohol, and purified water were store-bought brands packaged in standard Low Density Poly Ethylene (LDPE) retail bottles. A list of complete manufacturer information for solvents can be found in Appendix A.

Glassware and Miscellaneous Materials

All Erlenmeyer Flasks and beakers were boro-silicate glass newly purchased for this project. New glass transfer pipets were used to apply solvents to the crystal and dispense solvents for cleaning. These pipets were not washed prior to use and were discarded after a single transfer of raw solvent or sample material. When a solvent is referred to as "raw", it implies solvent portioned directly from the manufacturer's container without alteration or contact with experimental materials. Nitrile gloves were worn during the entire research project, and they were changed between handling different solvents or sample containers to prevent cross-contamination. Laboratory wipes were used in conjunction with solvents to clean the crystal between analyses.

Several flask stopper materials were qualitatively assessed prior to the experimental setup. Rubber and cork stoppers were rejected based on possible introduction of polymer or organic contaminants during the extraction process. Glass stoppers were considered to be prohibitively expensive. Aluminum foil was used as a cap for flasks during extraction because it was cost effective and inert. Aluminum foil was already being used, however, as an independent variable within the experimental design. This double use of aluminum foil introduced the possibility for cross-contamination of samples and misinterpretation of data. To prevent cross-contamination, the aluminum foil used as a cap was washed three times with petroleum ether prior to use. Foil used to wrap sample swabs in several experiments was not cleaned prior to use so that the presence of contaminants on foil could be assessed during experimentation. Also, foil was folded in such a way that the "matte" side was the side of contact when employed as a cap, and the "shiny" side was in contact with the sample swabs

during the preparation phase of T-2/3/5/6/8/9. These procedures ensured consistency and some measure of distinction between the two uses of foil in the experiments.

A method blank was prepared in order to assess contamination introduced when solvent washed foil was used as a flask cap. The method blank was prepared and analyzed as follows. A new, clean 250mL flask was filled with 75mL of petroleum ether, capped with a solvent cleaned aluminum foil, and placed on a stir plate for one week. A sample of the solvent was applied to the zinc selenide (ZnSe) crystal and dried. The residue was then analyzed by FTIR, and the resulting spectrum was compared to the dry residue of raw petroleum ether. Comparison of the spectra suggests that the foil did not introduce contaminants into sample extractions when employed as a cap (Figure 4). Based on this assessment, solvent cleaned aluminum foil was considered contaminant free and used to seal flasks throughout the experimental process.

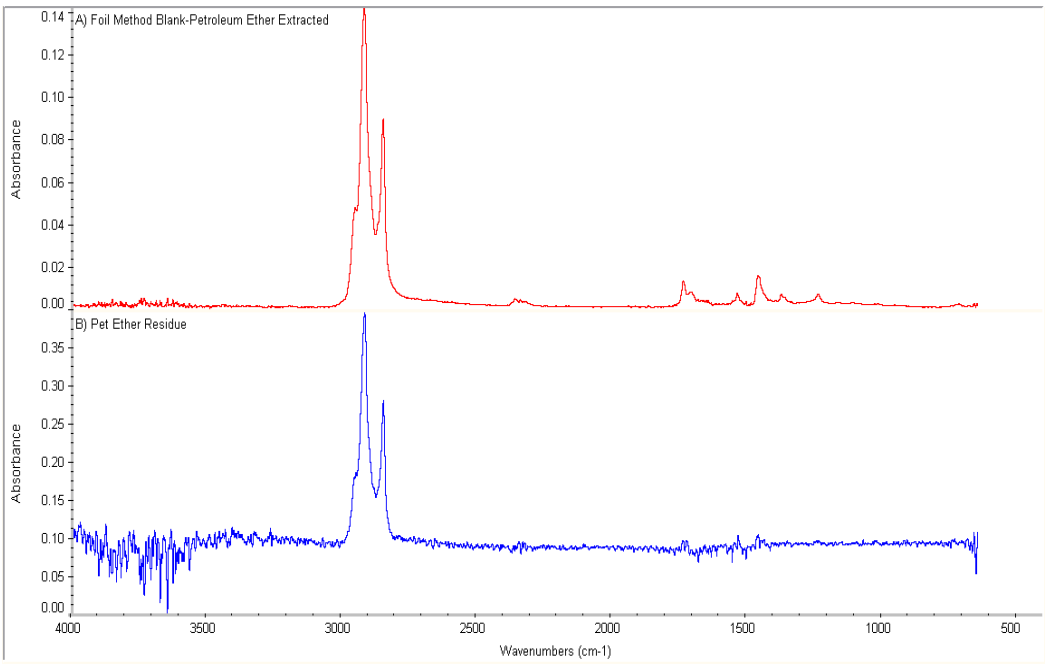

Figure 4 - (A) Foil-capped extraction blank and (B) Raw petroleum ether dry residue spectra.

Notes on Analysis

A standardized analytical protocol was developed for these experiments to minimize variability in results and to allow for consistent comparative analysis of data. Crystals were cleaned with raw solvents and allowed to dry for at least ten minutes prior to background characterization. When water was analyzed on the crystal, acetone was used for cleaning between casts. Fifty drops of each sample was applied to the crystal with a clean, new pipet. All samples were allowed to dry on the crystal for a minimum of thirty minutes and up to forty eight hours to allow for complete dissipation of solvent materials prior to analysis. In some cases where concentration of samples in extraction was expected to be particularly low, an increase in the

number of scans was employed regardless of solvent volatility in order to increase the signal to noise ratio (S/N) in the final spectrum.

Background Solvent Analysis

Prior to experimentation all solvents intended for use in subsequent analyses were characterized by FT-IR. Both the aqueous solvents and dry residues of solvents were tested (Figures 5-11). Several wet solvents required the use of a diamond ATR optic with a volatile cap for proper characterization. These solvents dissipate in a rapid or unstable manner which prevents characterization on an open optic such as the ZnSe used in all other FTIR analyses. Spectra generated by Diamond-ATR are not directly comparable to those generated on ZnSe-ATR which can complicate comparative analysis. However, characterization by Diamond-ATR does allow for a general comparison of the characteristics of the samples spectra taken with ZnSe-ATR.

Diamond Methodology

Five drops of solvent were applied to the diamond crystal. A “volatiles cap” was used to cover samples and prevent rapid dissipation. Sample spectra were acquired in 32 scans.

Zinc Selenide [ZnSe] Methodology

Ten to fifteen drops of each solvent were applied to the crystal for wet sample analysis. Wet samples were analyzed quickly, using thirty two scans, to allow proper characterization prior to

dissipation. For dry residues, fifty drops of solvent were applied to the crystal and allowed to dry for varying durations depending on the volatility of the individual solvent, ranging from fifteen minutes with Chloroform to 24 hours for purified water. Dry sample spectra were acquired in 64 scans.

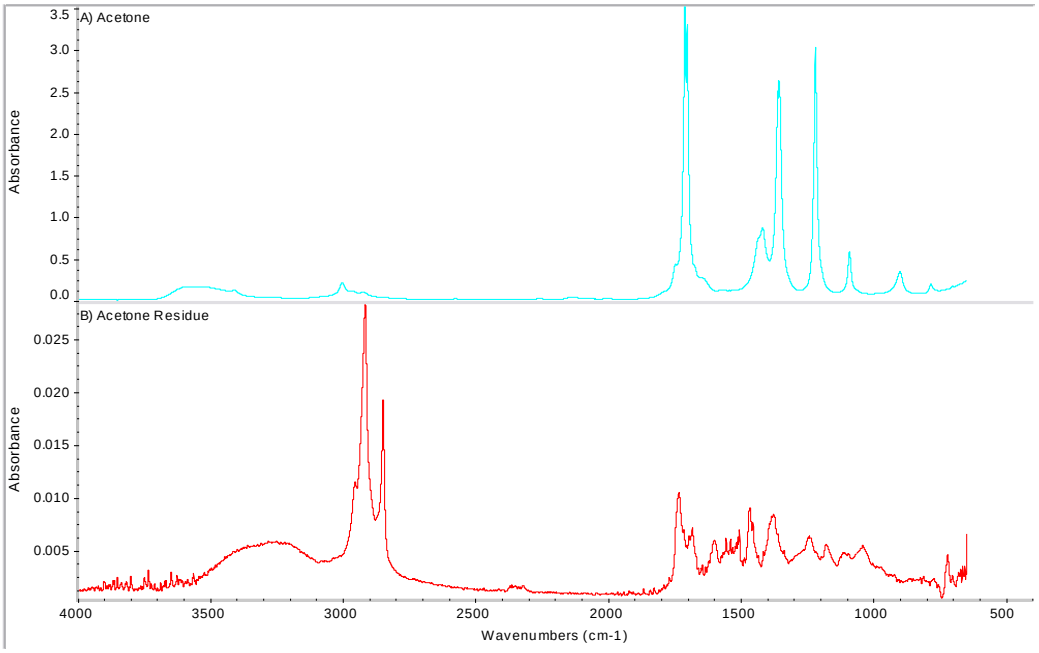

Figure 5 - (A) Spectrum of acetone on Zinc Selenide crystal and (B) Dry residue of acetone on Zinc Selenide crystal demonstrating a high level of contaminants. The contaminant is composed of alcohols (broad peak in 3600-3000cm^{-1}), hydrocarbons (3000-2800cm^{-1}), and carbonyl (1600cm^{-1}) groups, and is most likely a plastic polymer, an organically derived wax or oil, or a combination of components.

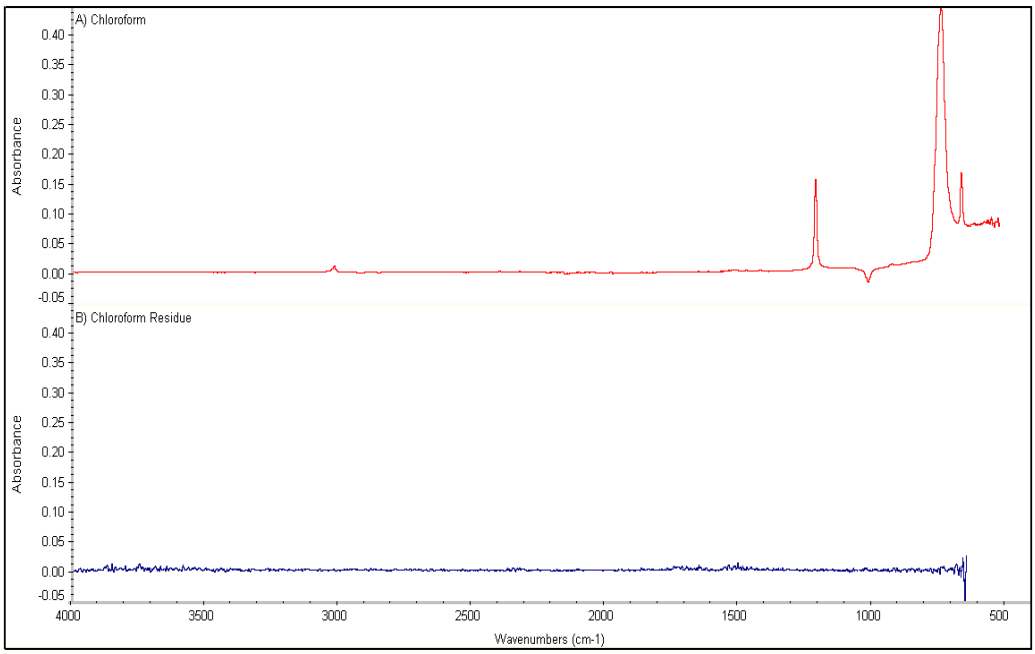

Figure 6 - (A) Chloroform on diamond crystal and (B) Dry residue of chloroform on Zinc Selenide crystal showing only atmospheric contributions and no apparent contamination.

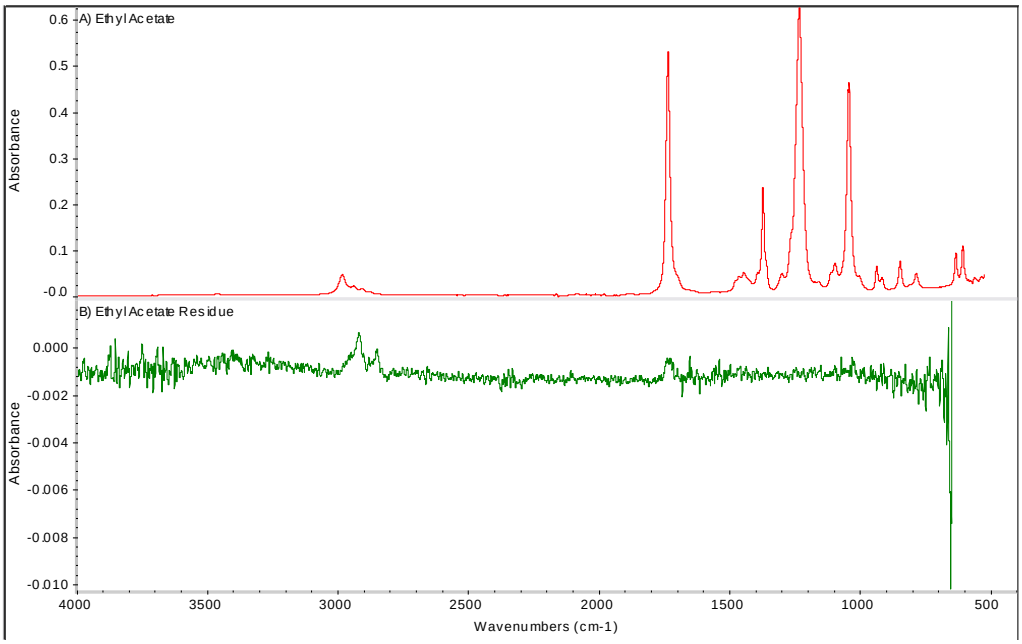

Figure 7 - (A) Spectrum of ethyl acetate on a diamond crystal and (B) Dry residue of ethyl acetate on Zinc Selenide crystal showing possible mild contamination. (B) has very slight peak formations in the hydrocarbon region (3000-2800cm^{-1}) and possibly at the carbonyl position (1600cm^{-1}), which may be a sign of plastic, or lipid contamination.

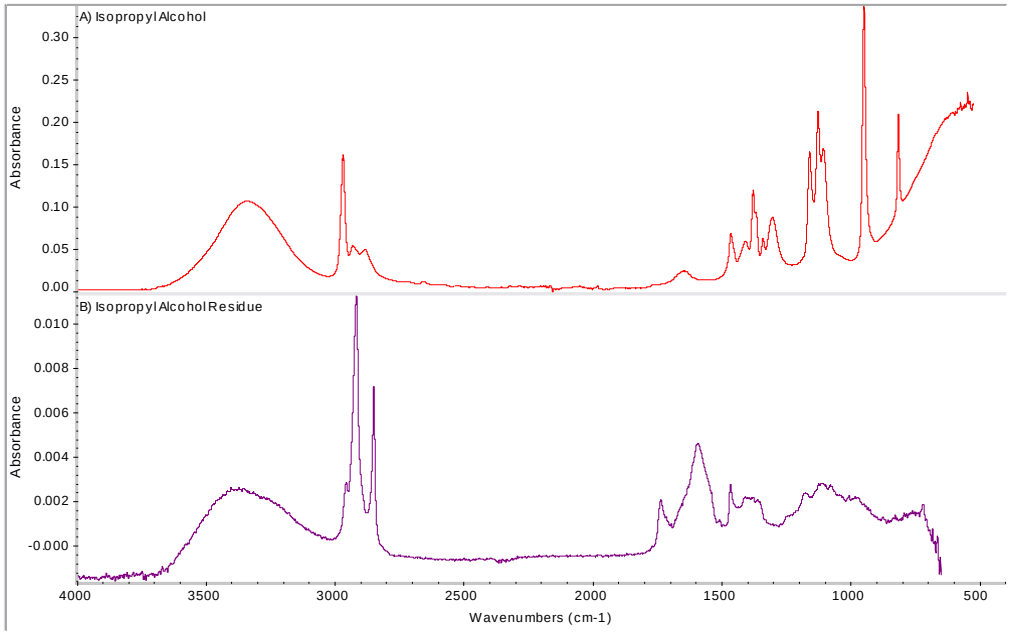

Figure 8 - (A) Isopropyl alcohol on a diamond crystal and (B) Dry residue of Isopropyl alcohol on a Zinc Selenide crystal demonstrating a high amount of contamination. The contaminant in B) is composed of alcohols (broad peak in 3600-3000cm^{-1}), hydrocarbons (3000-2800cm^{-1}), and carbonyl (1600cm^{-1}) groups, and is most likely a plastic polymer, an organically derived wax or oil, or a combination of components.

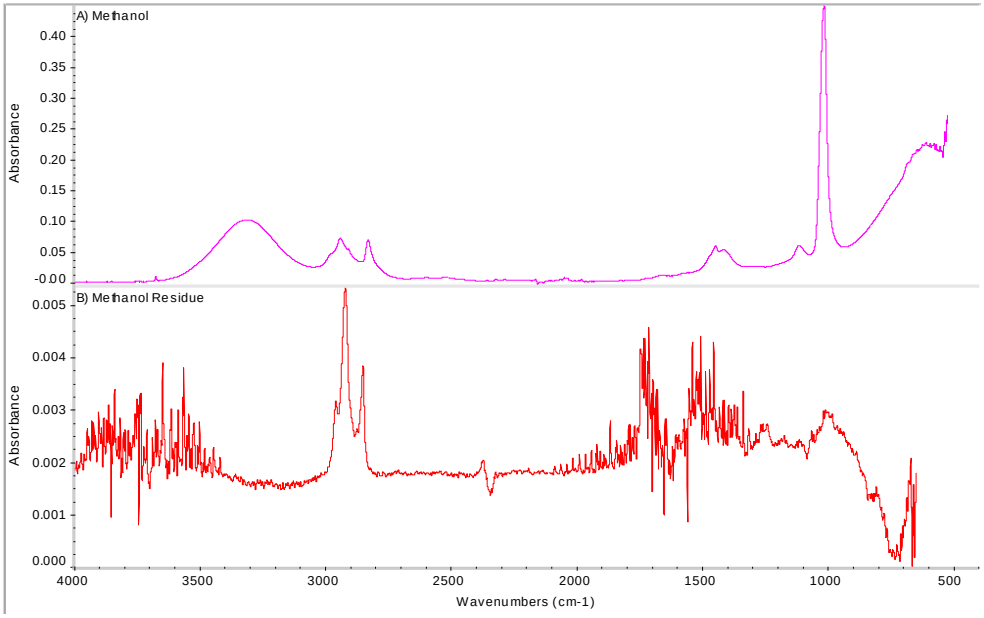

Figure 9 - (A) Methanol on diamond crystal and (B) Dry residue of methanol on a Zinc Selenide crystal demonstrating significant atmospheric noise and apparent hydrocarbon (3000-2800cm^{-1}) contaminants. The hydrocarbons are most likely plastics, or organically derived wax or oil contamination.

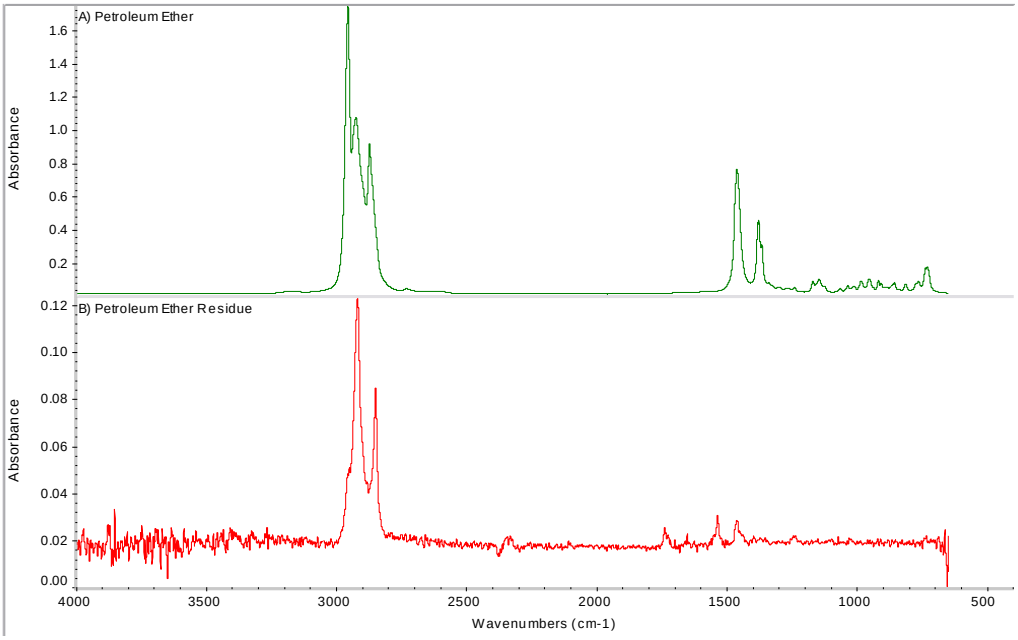

Figure 10 - (A) Petroleum ether on Zinc Selenide crystal and (B) Dry residue of petroleum ether on Zinc Selenide crystal demonstrating apparent atmospheric noise, hydrocarbon (3000-2800cm^{-1}), and carbonyl (1600cm^{-1}) contaminants. The hydrocarbons and carbonyls contaminants in B) are most likely from plastics, or organically derived wax or oil contamination.

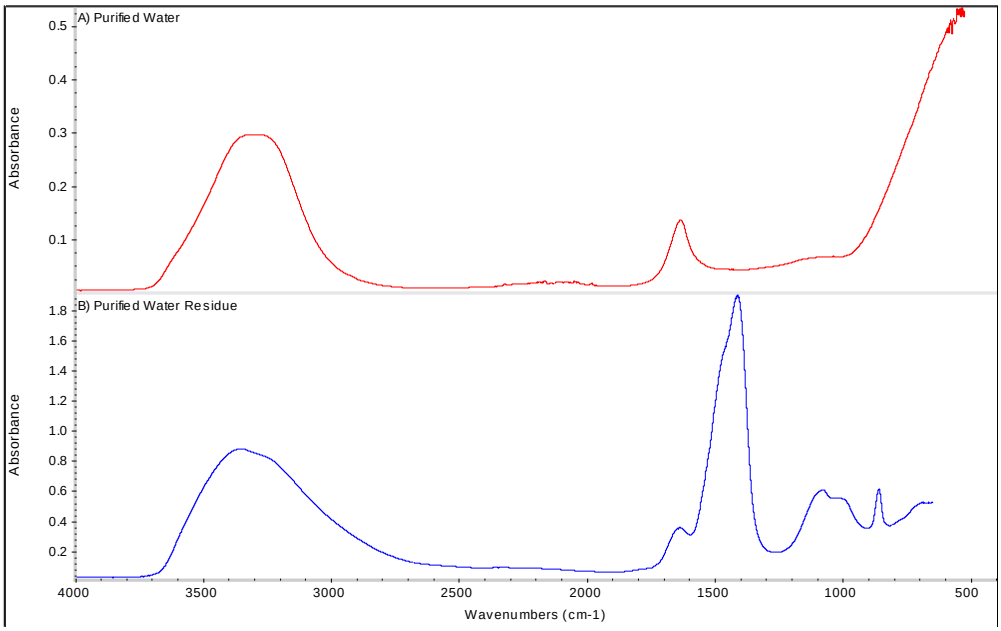

Figure 11 - (A) Purified water on a diamond crystal and (B) Dry residue of purified water on Zinc Selenid crystal demonstrating significant contaminants in the alcohol region (3600-3000cm^{-1}), the carbonyl position (1600cm^{-1}), and the fingerprint region (1500-500cm^{-1}). This suggests variable dissolved contaminants in water residue. Although the sample was dried for 24 hours, specta (B) may contain water which had not completely evaporated.

Experiment T-0

Introduction

20mL scintillation vials are common containers used in laboratory settings, as they are cheap, sturdy, and highly effective for general use in research of all types. 20mL scintillation vials have been used during the Bathtub Research Project for direct experimental purposes and for storage of many of the sample materials. The vials used are made of boro-silicate glass, which should be inert and non-reactive with most common solvents used in residue analysis. Though the vial glass itself should not introduce contaminants during contact with solvents, there are several ways in which contaminants could have been introduced into or onto the vials during manufacture and shipping. The vials used during this research are packed open in a cardboard tray which is then wrapped in plastic. This method of packaging may allow for exposure of the vial interior to atmosphere during packaging and the plastic wrapping during storage and shipping. The lids for use with these vials are made of a hard plastic material and could contribute plastics or plasticizers into the sampling solvents which were stored in the vials. All caps for a tray of vials (100) are shipped in a single separate plastic bag, which is another possible source of contamination in association with the caps. When utilizing vials and caps packaged in such a way, one cannot rule out, without running appropriate experimental controls, the possibility of plastics and other contaminant materials in or on the vials that could be introduced into samples. The purpose of T-0 was to test for contaminants which could obscure analysis and interpretation of spectral data, which may be introduced into experimental samples by the 20mL vials used during the Bathtub Research Project.

Experimental Methodology

Materials

20mL scinitillation vials w/caps X 3, 30mL Petroleum Ether, 100mL glass beaker X 1

Preparation

Three unwashed 20mL scintillation vials were filled with approximately 10mL of Petroleum Ether and capped with the included white plastic lids. The Petroleum Ether had been poured from the original manufacturer's container into a clean, new 100mL glass beaker that had been washed and rinsed with Petroleum Ether minutes prior. Three separate vials were prepared for analysis in order to generate redundant data which would rule out potential one-off results. The three vials were then labeled X, Y, and Z. All three vials were placed on a shelf for one week prior to the first analysis to simulate sample storage practices that would be used throughout the experimental process.

Hypothesis 1

The vials or caps will contribute contaminants into the stored solvent which will be detectable by analysis with FT-IR.

Null-Hypothesis 1

The vials or caps will not introduce any contaminants that are visible by FT-IR.

Hypothesis 2

Visibility of the contaminants will increase as a factor of contact time.

Null-Hypothesis 2

The visibility of contaminants will not be affected by contact time.

Analysis

A sample from each vial was tested using FT-IR each week for one month. The ZnSe crystal was cleaned with petroleum ether (40-60°) and allowed to dry ten minutes prior to background spectrum characterization. A background spectrum was acquired using 32 scans. Fifty drops of a sample were then applied to the crystal and allowed to dry for five minutes. A sample spectrum was acquired using 32 scans. The residue spectrum was then recorded for later analysis. This process was repeated in all recurring sample analyses for vials X, Y, and Z.

Discussion

Through comparison of the petroleum ether spectrum generated in our background controls with the T-0 spectra, it was determined that insufficient drying of the solvent caused the resulting residue spectra to contain peaks that represented petroleum ether which had not fully evaporated from the crystal prior to analysis. The samples had not completely dried in the time allotted which caused corruption of the spectra with solvent. Therefore, comparative and quantitative analysis with spectra collected during weeks one through three could not be performed. Hypothesis-2, therefore, could not be confirmed or denied. After the third week, drying times were lengthened to a 20 minute minimum to correct the issue.

On the fourth week sample spectra were generated that did not appear to be corrupted by insufficient drying time. These spectra contained peaks that were consistent with those of

the background spectra of the Petroleum Ether residue (Figure 12). The peaks in the sample spectrum did not differ in any qualitatively or quantitatively significant way from the reference spectrum of the petroleum ether residue. There appeared to be possible differences in the shoulder at 2950cm^{-1} which may suggest the introduction of hydrocarbon components in to the extraction from the vial. However, the control residue spectrum for petroleum ether was analyzed after sixteen hours of drying in a vented fume hood, while the vials X, Y, and Z samples were dried for only twenty minutes. Thus, the slight shoulder is most likely a portion of the petroleum ether that had not fully dissipated due to incomplete drying time. Therefore, it was concluded that the scintillation vials had not introduced any contaminants into the solvent that were visible by FT-IR analysis. Thus, Null-hypothesis-1 was confirmed.

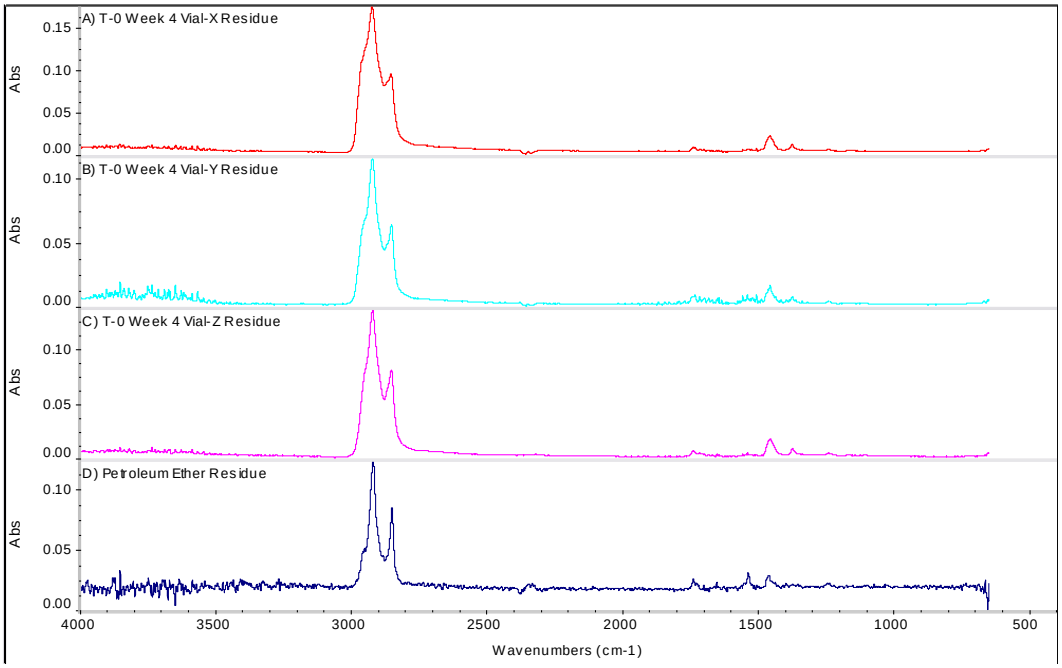

Figure 12 – (A-C) Spectra of vial X, Y, Z content after four weeks exposure and (D) Background spectrum of the petroleum ether residue identified in background controls.

It was decided that T-0 did not need to be retested in order to generate data that would allow evaluation of Hypothesis-2. The lack of visible contaminant residues from the scintillation vials or caps during week four analysis suggested that there would not have been any residues for comparative analysis in earlier weeks either. Vials X, Y, and Z have been stored with the original solvent contents still intact. They may be analyzed in the future to determine if longer contact times lead to spectroscopically visible levels of contamination in stored samples.

Experiment T-1

Introduction

There are two swab types used in bathtub project which were possible sources of contaminant materials. During the bathtub project these swab were used to take samples directly from the surface of "bathtub" artifacts. These swabs came in direct contact with the sample materials, and were the medium upon which the samples resided for long periods of time during shipping and storage. This prolonged exposure of the swabs to recovered samples introduced a significant possibility for contamination of the samples during shipping and storage.

Solvent extraction which was necessary to recover the sample materials form the swabs was potentially extracting undesired contaminants from the swabs during sample extraction. Although the BRP was operating under the assumption that neither swab type would interact

with petroleum ether, this had not been independently verified within the Bathtub Project methodology. The first type of swab used was a cotton-tipped wooden swab (Swab-C). According to S. Grieve (as per personal correspondence) a methylcellulose adhesive is used to hold the cotton tip to the wooden shaft on the swabs used by the BRP and this reseearch. The second type of swab is a foam-tipped plastic swab (Swab-F). The shaft of these swabs is a mixture of polyethylene and polypropylene, while the foam tip is a mixture of several plastic polymers. I was unable to determine the components of the adhesive used in Swab-F, although the adhesives used in this kind of application are commonly paraffin waxes and/or plastic mixtures. Both swab types are packaged and shipped in polyethylene bags from the manufacturer. Experiment T-1 was designed to determine whether Swab-C and F were introducing spectroscopically visible contaminants into the samples during petroleum ether extraction that could interfere with our data analysis

Experimental Methodology

Materials

Swab-C X 10, Swab-F X 10, 200mL Petroleum Ether, 250mL Erlenmeyer Flask (Glass) X 2, 20mL scintillation Vial w/Caps X 2, 100mL glass Beaker X 1

Preparation

Ten Swab-F were placed into a clean, new 250mL Erlenmeyer flask. Ten Swab-C were placed in another. 100mL of petroleum ether was added to each of the Flasks. Each flask was capped with solvent cleaned foil and sealed with tape. Garner (2008) demonstrated that foil can introduce contaminants which are derived from the release agents used on foil during manufacture in residue analysis (see below for further discussion). As discussed earlier, in

order to isolate any variables the foil may be adding into the experimentation, the foil caps were always folded “matte” side out and cleaned with petroleum ether prior to use. The flasks were labeled and set on a stir plate to increase chemical interaction in the flasks. The plate was set on 80rpm rotation rate. After one week the contents of each flask were concentrated to ~15mL by volume. Compressed air was blown into the opening of the flask for around thirty minutes to increase the rate of evaporation during the concentration process. The concentrated extractions were then transferred into separate 20mL scintillation vials, labeled, and stored.

Hypothesis

One, or both, of the extractions will produce spectroscopically visible contamination residues when the swabs are extracted by petroleum ether.

Null-Hypothesis

Neither of the extractions will produce spectroscopically visible contamination residues when the swabs are extracted by petroleum ether.

Analysis

A sample from each vial was analyzed by FT-IR. The crystal was cleaned with petroleum ether and dried for ten minutes. A background spectrum was acquired using 32 scans. Fifty drops of sample from Swab-C extraction were applied to the crystal and allowed to dry for twenty five minutes. The sample spectrum was acquired using 32 scans. The resulting residue spectrum was generated and saved for analysis. The process was repeated for the Swab-F concentration sample.

Discussion

Both swab types introduced spectroscopically visible contaminant residues when extracted by petroleum ether, thus confirming the T-1 hypothesis (Figure 13). The spectrum of the residue from Swab-C had many spectral characteristics in common with the petroleum ether residual contaminant spectrum (compare Figure 13: A and C). Peak position and peak height ratio of the aliphatic hydrocarbon stretching peaks suggest that the contaminant is chemically quite similar in both petroleum ether and Swab-C residues. It seemed possible that either the Swab-C extraction spectrum represented the residues from raw petroleum ether, or that the swab had introduced new contaminant materials, or possibly both. However, initial comparison between the raw petroleum ether residue spectrum and the T-1 Swab-C spectrum suggested that there was a much higher concentration of contaminant materials in the Swab-C extraction than would be expected had we simply concentrated raw petroleum ether from 100mL to 15mL. Therefore, a method blank was prepared and analyzed to determine the source of these contaminants.

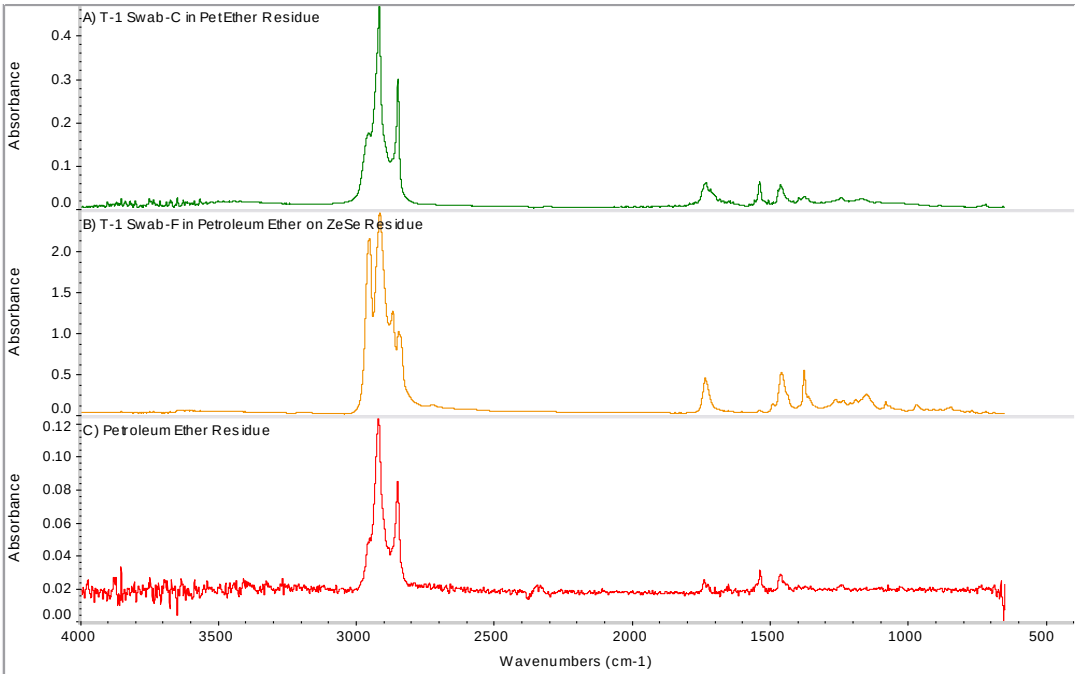

Figure 13 – (A) Method Blank spectrum, (B) Swab-C spectrum, and (C) Swab-F extraction residue spectra.

The method blank consisted of 100mL of Petroleum Ether added to a clean, new 250mL Erlenmeyer flask, capped with clean aluminum foil, and stirred on a stir plate for one week at 80rpm. The method blank did produce slightly higher quantities of contaminants that that of raw petroleum ether (due to the effects of concentration), though the concentration was nearly an order-of-magnitude lower than the Swab-C extraction (compare Figure 13: A and C). The residue from the Swab-C extraction also demonstrated peak features at 1700cm^{-1} (C=O ester bonds), and several in the fingerprint region (1500-500cm^{-1}) that were inconsistent with the raw petroleum ether residue spectrum. It was concluded that Swab-C was introducing contaminant materials that were different yet qualitatively similar to the contaminants in raw petroleum ether. The methylcellulose adhesive used in Swab-C production is a likely suspect for the hydrocarbon and carbonyl contaminants in the residue spectrum. Although the

petroleum ether is spectrophotometric grade, the manufacturer's container in which it is stored and shipped has a plastic (most likely Low Density Polyethylene-LDPE) spout. This spout is in direct contact with the contents and is the most likely source of the hydrocarbon polymer detected in the petroleum ether residue. The spectral interpretation and library database comparison for both spectra supports these interpretations.

The Swab-F extraction spectrum was visibly different from both the method blank and Swab-C spectra. There were distinct peaks in the 2800-3000cm^{-1} region that suggest a different hydrocarbon structure than was seen in the previous two samples (compare Figure 13: C with, A and B). Features in the fingerprint region (1500-500cm^{-1}) of the Swab-F spectrum were also somewhat different than both the method blank and Swab-C extraction spectra. Library database searches suggest the Swab-F contaminant material most closely matches a mixture of polyethylene, polypropylene, and polydiene. These materials are consistent with the polypropylene, polyethylene mixture known to make up the shaft of the swab, and most likely a major constituent of the foam tipped material as well.

Spectral comparison suggests that Swab-F extraction contains a much higher concentration of contaminant materials than Swab-C (compare Figure 13: A-C absorption). This however is not necessarily suggestive of a difference between the swabs' reactivity with petroleum ether. The plastic swabs (Swab-F) are much more massive than cotton swabs (Swab-C). In this experiment, swabs were quantified by count rather than by weight, which may explain the higher quantity of contaminant in Swab-F extractions; there was simply more material with which to interact relative to the volume of solvent.

Experiments T-2/T-3

Introduction

The Bathtub Research Project is developing a non-destructive methodology for recovering samples from *in situ* vessels. The non-destructive methods included using several types of swabs that were coated with solvents prior to sampling. The addition of an organic solvent to the swab was believed to increase the likelihood of organic residue recovery from target vessel surfaces while reducing the amount of sample material required for analysis, thus minimizing the study's impact on the vessel surface. The solvents used during the project include ethyl acetate, methanol, acetone, chloroform, and purified water. Both Swab-F and Swab-C types were used in conjunction with all, or some, of these solvents during sample collection. The interaction of these swabs and the Sampling solvents was presumed to be null or minimal based on the manufacturer specifications. However, any amount of chemical interaction between the solvents and swabs could introduce unknown contaminants into the samples which would interfere with sample analysis and interpretation, and therefore must be experimentally verified. Experiments T-2 and T-3 were designed to test for the introduction of contaminants into sample extractions due to sampling solvent and Swab-C/F interactions.

Experimental Methodology

T-2 Materials

Swab-C X 70, 250 mL Flask X 7, 100mL Beaker X 7, 20mL Scintillation Vials w/ caps X 7, Aluminum Foil X 5 feet, 700mL of Petroleum Ether, 20mL each of Acetone, Chloroform, Ethyl Acetate, Isopropyl Alcohol, Methanol, purified Water

T-3 Materials

Swab-C X 70, 250 mL Flask X 7, 100mL Beaker X 7, 20mL Scintillation Vials w/ caps X 7, Aluminum Foil X 5 feet, 700mL of Petroleum Ether, 20mL each of Acetone, Chloroform, Ethyl Acetate, Isopropyl Alcohol, Methanol, purified Water.

T-2/T-3 Preparation

One clean, new beaker was rinsed with raw Acetone to remove any contaminants from shipping and storage. The beaker was then filled with 20mL of raw acetone. Ten Swab-C were dipped into the acetone for a duration of 30 seconds. The swabs were allowed to dry for one minute, and then placed in a clean, new 250mL Erlenmeyer flask to simulate field methods. 100mL of petroleum ether was then added to the flask. Solvent cleaned aluminum was used to cap flask during extraction to prevent evaporation and sample contamination. The flask was placed on a stir plate and stirred at 80rpm. This process was repeated with each of the five remaining solvents. All six flasks remained on the stir plate for one week. After one week, each flask was removed from the stir plate and uncovered. Compressed air was blown into each flask to increase the rate of solvent evaporation. The content of each flask was concentrated to ~15mL by volume. Each concentrated extraction was then transferred into separate 20mL scintillation vials, labeled, and stored. The complete process was repeated for experiment T-3 substituting Swab-F for Swab-C.

T-2/T-3 Hypothesis

One, or more, extraction(s) will produce spectroscopically visible contamination that differs from T-1 contaminants when solvents are applied to Swab-C/F prior to extraction in petroleum ether.

T-2/T-3 Null Hypothesis

None of the extractions will produce spectroscopically visible contamination that differs from T-1 extraction contaminants when solvents are applied to Swab-C/F prior to extraction in petroleum ether.

T-2/T-3 Analysis

A sample from each vial was individually analyzed by FTIR. The crystal was cleaned with petroleum ether and allowed to dry for five minutes prior to each background characterization. All background spectra were acquired using 32 scans. Fifty drops of sample material were applied to the crystal and allowed to dry for thirty minutes prior to each analysis. The sample spectra were acquired using 32 scans and recorded for later comparative analysis.

Discussion

T-2 (Swab-C)

Spectra generated in T-2 demonstrate that the application of solvents onto Swab-C prior to extraction in petroleum ether did not introduce contaminants qualitatively different than the extraction of Swab-C in T-1, thus confirming the T-2 Null-Hypothesis (Figure 14). However, it could still have been possible that chemical interactions had in fact produced contaminant materials that were simply not present in a quantity high enough to be visible relative to the

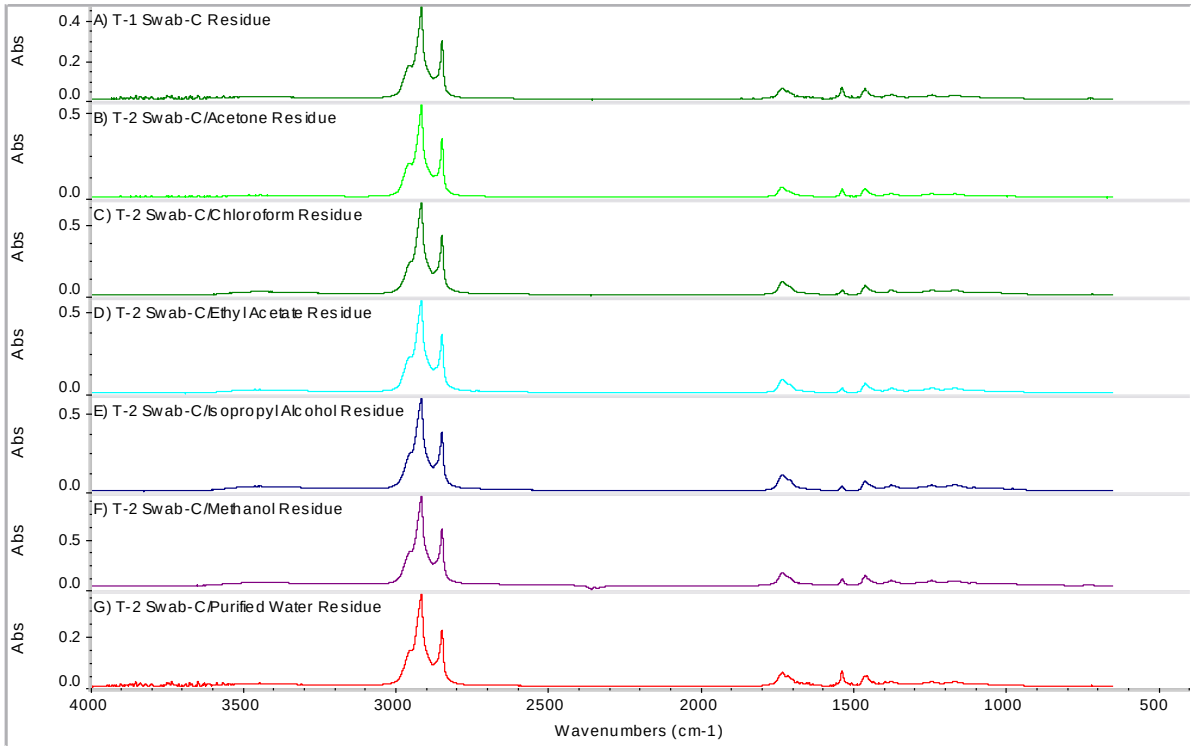

Figure 14 – (A) T-1 Swab-C Spectrum and (B-G) T-2 Spectra

larger amount of Swab-C contaminants in the sample. Therefore, the Swab-C spectrum from T-1 was subtracted from the T-2 spectra as an attempt to identify residues from solvent/swab interaction that may have been obscured by swab contamination. The resulting spectra did not produce visible features, therefore, it can be concluded that no analytically significant contaminants had been introduced into the test samples by solvent/swab interaction.

T-3 (Swab-F)

Spectra generated in T-3 demonstrate that the application of solvents onto Swab-F prior to extraction in petroleum ether did not introduce contaminants qualitatively different than the extraction of Swab-F in T-1, thus confirming the T-3 Null-Hypothesis (compare Figure 15: A and

B-H). Noticeable, however, were slight increases in the concentration of contaminants in some T-3 extractions relative to T-1-Swab-F (Figure 15: B, and D-G). This suggests that the applied solvents may have increased the solubility of Swab-F components or increased the rate of chemical interaction between the solvent and swab. The Swab-F dipped in chloroform exhibited significant polymer swelling, and suggested a significant chemical interaction between the two materials. The chloroform applied swab however showed no indication of significant chemical change relative to other Swab-F extractions in T-3. In sum, across the board in T-2 and T-3, the application of sampling solvents to the swabs did not qualitatively alter the spectral characterization of swab residues from petroleum ether extractions.

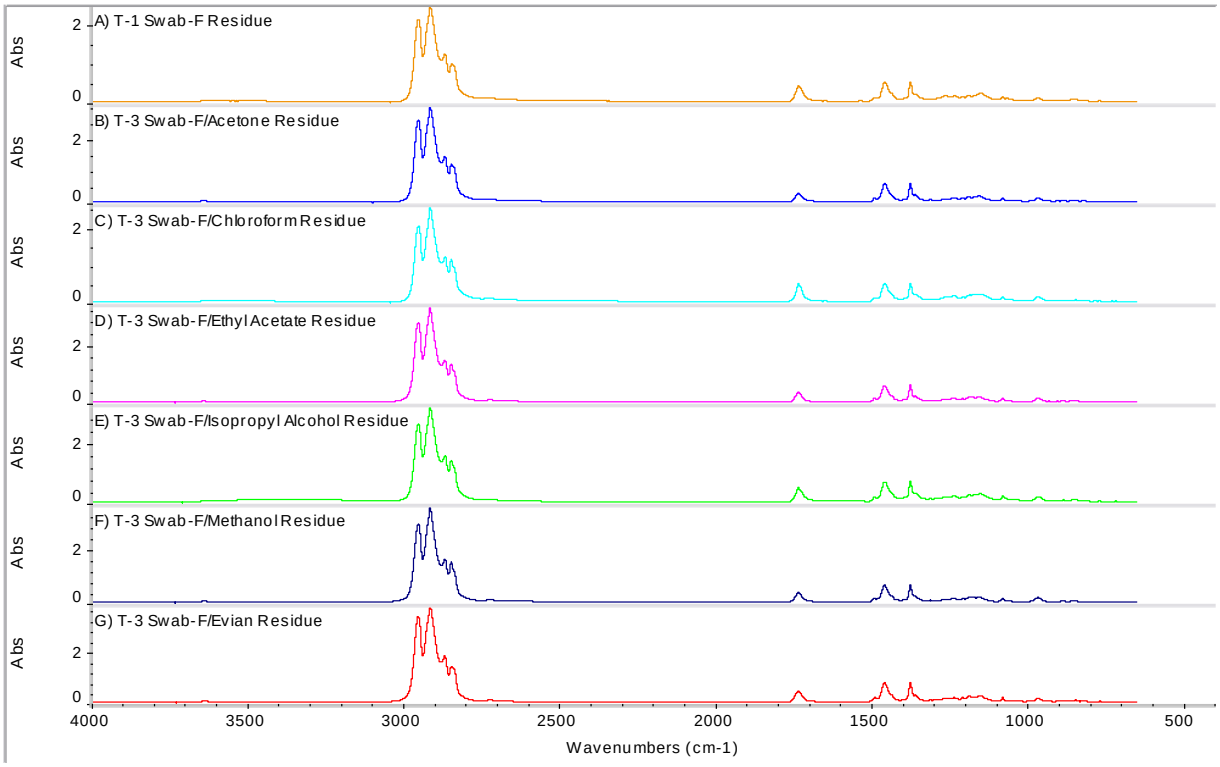

Figure 15 - (A) T-1 Swab-F spectrum and (B-G) T-3 spectra.

Therefore, I concluded that the application of solvents to both swabs prior to extraction in petroleum ether did not introduce spectroscopically visible contaminants into samples.

Experiment T-4 (CANCELLED)

Discussion

Two types of foam-tipped plastic swabs (flat and round tipped) were used during the sample collection for the Bathtub Research Project. Originally, I planned to run a full set of experiments with both types of plastic swabs. However, to avoid redundancy it was decided that only one of the two swab types would be analyzed. In order to validate the use of one swab to proxy for both types used in the original sample material, I had to verify the chemical makeup of both swab types. The distributor and manufacturer of these swabs confirmed that the two types should be chemically identical. In addition, preliminary controls were run by the Bathtub Research Project in which both swab types were separately extracted in petroleum ether. The resulting spectra suggested that the two swab types are chemically similar. Both swabs did produce spectroscopically visible contaminants when extracted in petroleum ether. Due to time, resource, and budget constraints round foam-tipped swabs have been removed from the experimental schedule. Experiments T-4, T-7, and the original T-10 have been omitted from this research. The title T-10 was used for a later set of experiments not to be confused with the cancelled experiments discussed hereunder.

Experiments T-5/T-6

Introduction

In most organic residue analysis projects, collected samples are wrapped in aluminum foil for protection and to prevent inadvertent contamination from shipping and handling (e.g., Shishlina 2007; Koh 2010; Koh and Betancourt 2010; Garner 2010). Aluminum foil is used due to its strength, ease of use, and relatively nonreactive properties (i.e. does not react with solvents, samples, or other storage materials used in organic residue analysis). While archaeologists consider aluminum foil to be free of contaminating properties and completely safe for residue analysis applications (e.g., Shishlina 2007, Koh 2010), Garner (2010) has raised concerns with the manufacturing of foil that put such assumptions in questions. According to Garner (2010:6), aluminum foil often contains surface traces of machine oil used as a release agent during its manufacture. He therefore suggests that projects assess the use of aluminum foil and any contaminant oils it may introduce into analysis. All samples that have been taken to date in the Bathtub Project have been stored and shipped in aluminum foil. In light of Garner's reticence in trusting foil without analytical assessment, I tested the impact of aluminum foil when used in the methodologies employed during the Bathtub Project. Experiments T-5 and T-6 were designed to test for contaminant residues introduced into analysis when storing sample swabs in aluminum foil prior to extraction in petroleum ether.

Experimental Methodology

T-5 Materials

Swab-C X 70, 100mL Beaker X 7, 250mL Erlenmeyer flask X 7, Aluminum Foil X $5ft^2$, 750mL Petroleum Ether, 20mL scintillation vial w/caps X 7, 20mL each of Acetone, Chloroform, Ethyl Acetate, Methanol, Isopropyl Alcohol, and Purified Water.

T-6 Materials

Swab-F X 70, 100mL Beaker X 7, 250mL Erlenmeyer flask X 7, Aluminum Foil X $5ft^2$, 750mL Petroleum Ether, 20mL scintillation vial w/caps X 7, 20mL each of Acetone, Chloroform, Ethyl Acetate, Methanol, Isopropyl Alcohol, and Purified Water.

T-5/T-6 Preparation

Ten Swab-C were dipped in acetone for thirty seconds and then air dried for one minute. After one minute the swabs were wrapped in aluminum foil. The foil pack was then labeled, and stored in a fume hood. The process was repeated for each of the solvents being tested. A second set of prepared swabs were also created using Swab-F. After one week each group was unwrapped and placed in separate clean, new 250mL Erlenmeyer flasks. 100mL of petroleum ether was added to each flask for extraction. The flasks were capped with solvent washed aluminum foil. All flasks were placed on a stir plate and stirred at 80rpm. After one week the flasks were removed from the plate and uncovered. Solvent was evaporated under a steady stream of air to increase the rate of solvent evaporation. The contents of each vial were concentrated to ~15mL by volume and transferred into clean, new 20mL scintillation vials for storage.

T-5/T-6 Hypothesis

One, or more, extraction residues will produce spectrographically visible contamination that differs from the T-2/T-3 contaminants when solvents are applied to Swab-C/F, wrapped in aluminum foil, and extracted in petroleum ether.

T-5/T-6 Null Hypothesis

None of the extraction residues will produce spectrographically visible contamination that differs from the T-2/T-3 contaminants when solvents are applied to Swab-C/F, wrapped in aluminum foil, and extracted in petroleum ether.

T-5/T-6 Analysis

A sample from each of the vials was individually analyzed by FTIR. The crystal was cleaned with petroleum ether and allowed to dry for five minutes prior to each background characterization. A background spectrum was acquired using 32 scans. Fifty drops of sample material were applied to the crystal and allowed to dry for thirty minutes prior to each analysis. The sample spectra were acquired using 32 scans, and recorded for later comparative analysis. The T-5 and T-6 spectra were then compared to the T-2 and T-3 spectra respectively to determine whether new contaminants had been introduced by the addition of aluminum foil into the methodology.

Discussion

T-5 (Swab-C)

Spectra from T-5 did not demonstrate any qualitative or quantitative differences in peak form or position relative to T-2 spectra, confirming the Null Hypothesis (compare Figure 16: A and B-G). Comparative analysis between T-2 and T-5 spectra suggests that there were no

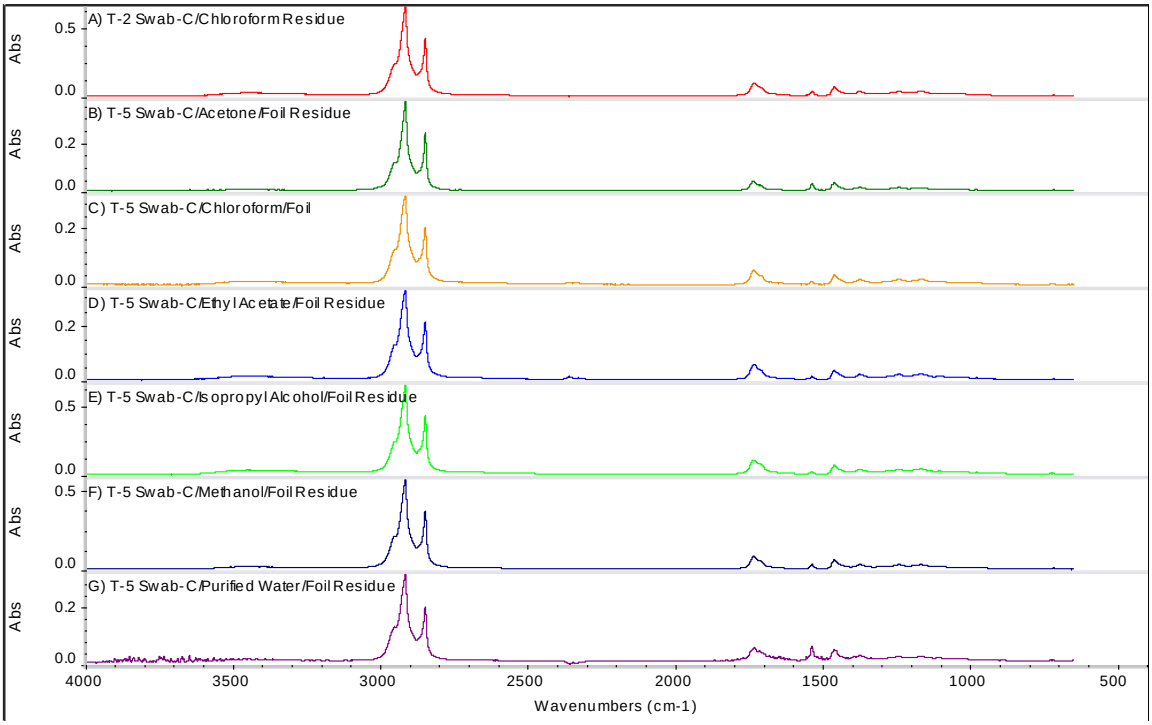

Figure 16 – (A) T-2 Swab-C chloroform representing all T-1 spectra and (B-G) T-5 spectra.

contaminants introduced into the sample materials which were spectroscopically visible within the test methodologies employed when storing sample swabs in aluminum foil.

T-6 (Swab-F)

Spectra generated in experiment T-6 did not demonstrate any qualitative or quantitative differences in peak form or position relative to the T-3 spectra, confirming the Null Hypothesis (compare Figure 17: A and B-G). Comparative analysis between T-3 and T-6 spectra suggests that there were no contaminants, which were spectroscopically visible within the test methodologies employed, introduced into the sample materials when storing sample swabs in aluminum foil.

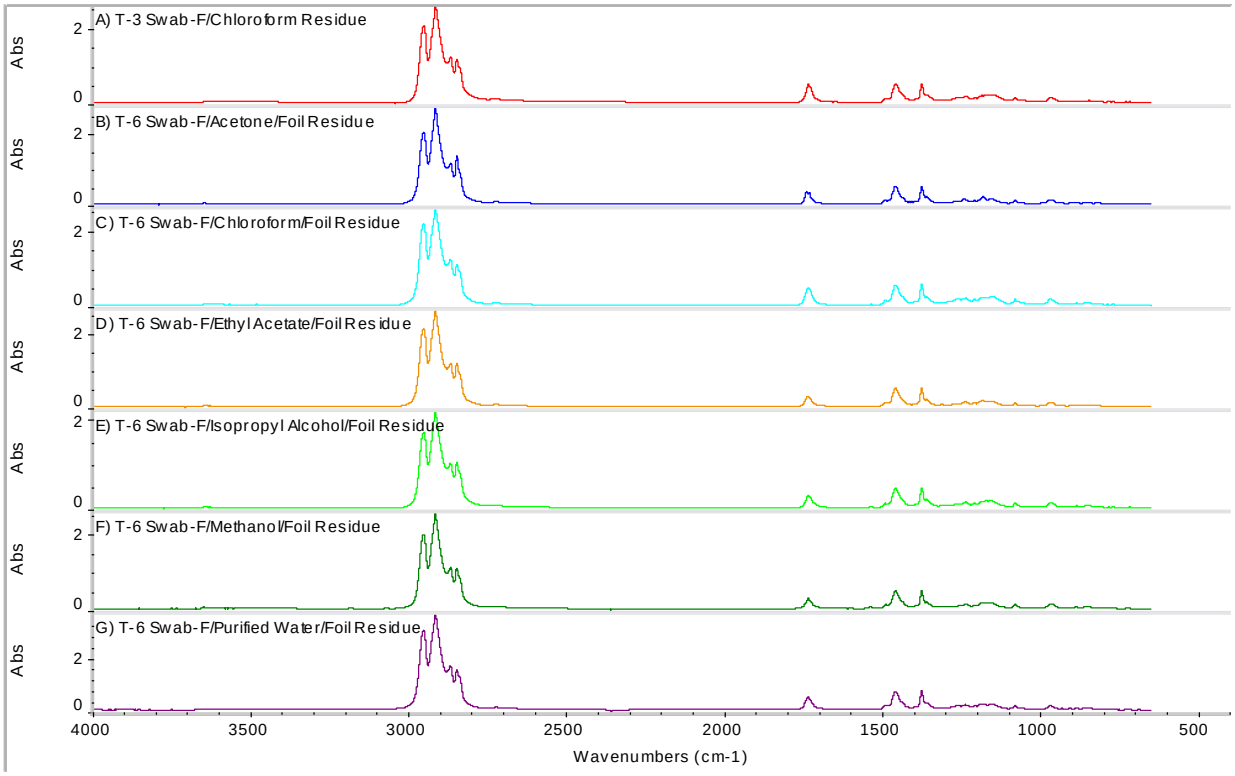

Figure 17 - (A) T-3 Swab-F chloroform representing all T-3 spectra, and (B-G) T-6 spectra.

Experiment T-7 (Cancelled **See T-4**)

Experiments T-8/T-9

Introduction

Many archaeologists have discussed the potential of contaminants being introduced into ORA from storing sample materials in plastic bags (e.g., Garner 2008; Koh 2010). Plastics can easily infiltrate sample materials, introducing phthalates, other plasticizers and hydrocarbon polymers that can obscure chemical analysis (Garner 2008, Koh 2010). Plasticizers are ubiquitous in the modern environment and should be expected to appear during residue analysis to some degree (Garner 2008). However, field and laboratory methodologies are also sources for plastic contamination in residue analysis research. Researchers can control this to some degree by limiting contact with plastic materials.

In the Bathtub Research Project, plastic bags were used extensively for the storage and shipping of sample materials. Their potential for introducing contaminants based on the methodologies employed must be addressed. Experiments T-8 and T-9 were designed to determine the introduction of contaminants by the use of plastic bags in the Bathtub Research Project.

Experimental Methodology

T-8 Materials

Swab-C X 70, 250mL flask w/stopper X 7, 100mL Beakers X 7, Aluminum Foil,
Plastic Bag X 7, 20mL scintillating vial w/cap X 7, 600mL Petroleum Ether, 25mL each; Methanol, Ethyl Acetate, Acetone, Chloroform, Isopropyl Alcohol, Acetone, Purified water

T-9 Materials

Swab-F X 70, 250mL flask w/stopper X 7, 100mL Beakers X 7, Aluminum Foil,
Plastic Bag X 7, 20mL scintillating vial w/cap X 7, 600mL Petroleum Ether, 25mL each; Methanol, Ethyl Acetate, Acetone, Chloroform, Isopropyl Alcohol, Acetone, Purified water

T-8/T-9 Preparation

Ten Swab-C were dipped in acetone for thirty seconds and then air dried for one minute. After one minute the swabs were wrapped in aluminum foil. The foil pack was then labeled, placed in a plastic bag, and stored in a fume hood. The process was repeated for each of the solvents being tested. A second set of prepared swabs were also created using Swab-F. After one week each group of swabs was unwrapped and placed in separate clean, new 250mL Erlenmeyer flasks. 100mL of petroleum ether was added to each flask for extraction. The flasks were capped with solvent washed aluminum foil. All flasks were placed on a stir plate and stirred at 80rpm. After one week the flasks were removed from the plate and uncovered. Compressed air was blown into each flask to increase evaporation. The contents of each vial were concentrated to ~15mL by volume and transferred into clean, new 20mL scintillation vials for storage.

T-8/T-9 Hypothesis

One, or more, extraction residues will produce spectrographically visible contamination that differs from T-2/T-3 spectra when Sampling solvents are applied to Swab-C/F, which had been wrapped in aluminum foil, placed in a plastic bag, and extracted in petroleum ether.

T-8/T-9 Null Hypothesis

None of the extraction residues will produce spectrographically visible contamination that differs from T-2/T-3 spectra when Sampling solvents are applied to Swab-C/F, which had been wrapped in aluminum foil, placed in a plastic bag, and extracted in petroleum ether

T-8/T-9 Analysis

A sample from each of the vials was individually analyzed by FTIR. The crystal was cleaned with petroleum ether and allowed to dry for five minutes prior to each background characterization. A background spectrum was acquired using 32 scans. Fifty drops of sample material were applied to the crystal and allowed to dry for thirty minutes prior to each analysis. The sample spectra were acquired using 32 scans, and recorded for later comparative analysis. The T-8 and T-9 spectra were then compared to the T-2 and T-3 spectra respectively to determine whether new contaminants had been introduced by the addition of plastic bags into the methodology.

Discussion

T-8

Spectra generated in experiment T-8 did not demonstrate any qualitative or quantitative differences in peak form or position relative to the T-2/5 spectra, confirming the Null Hypothesis (compare Figure 18: A, B, and C-H). Comparative analysis between T-2, T-5, and T-8 spectra suggests that there were no spectroscopically visible contaminants introduced into the sample materials when storing sample aluminum foil wrapped swabs in plastic bags.

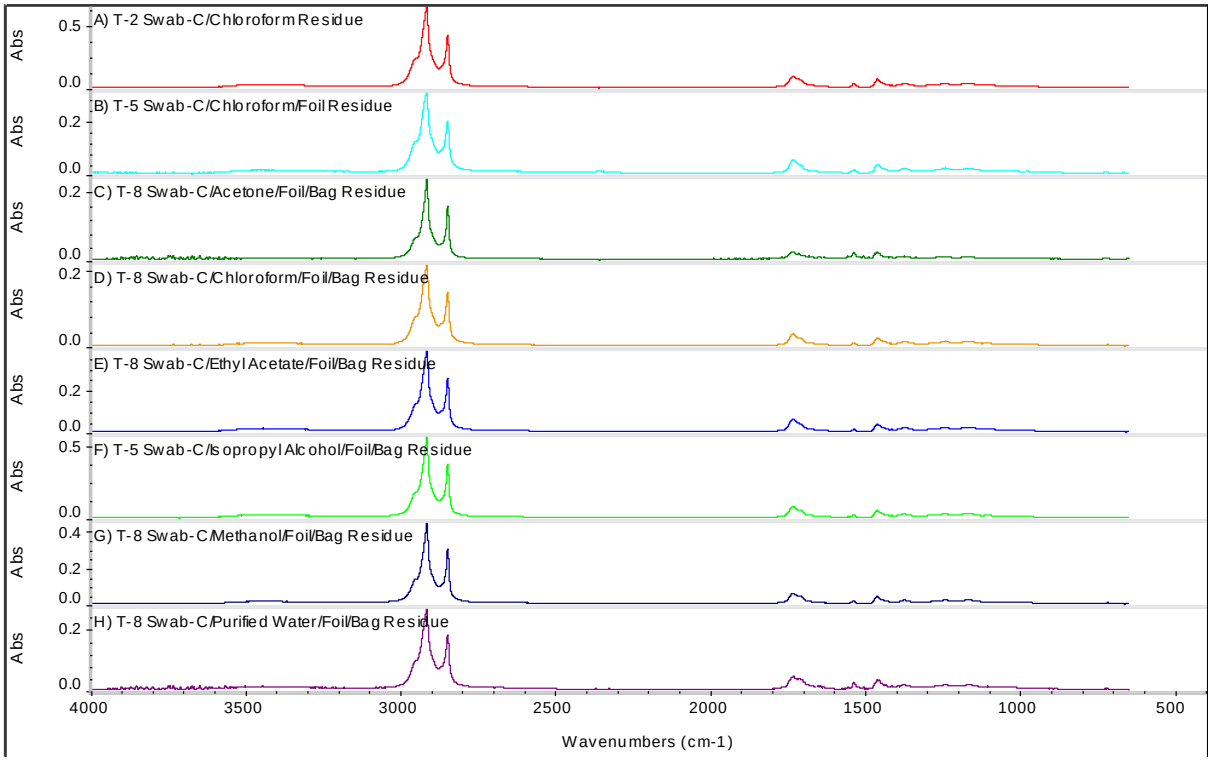

Figure 18 – (A) T-2 Swab-F chloroform representing all T-2 spectra, (B) T-5 Swab-F/Foil chloroform representing all T-5 spectra, and (C-H) T-8 spectra.

T-9

Spectra generated in experiment T-9 did not demonstrate any qualitative or quantitative differences in peak form or position relative to T-3/6 spectra, thus confirming the Null Hypothesis (compare Figure 19: A, B, and C-H). Comparative analysis between T-3, T-6, and T-9 spectra suggests that there were no spectroscopically visible contaminants introduced into the sample materials when storing sample aluminum foil wrapped swabs in plastic bags.

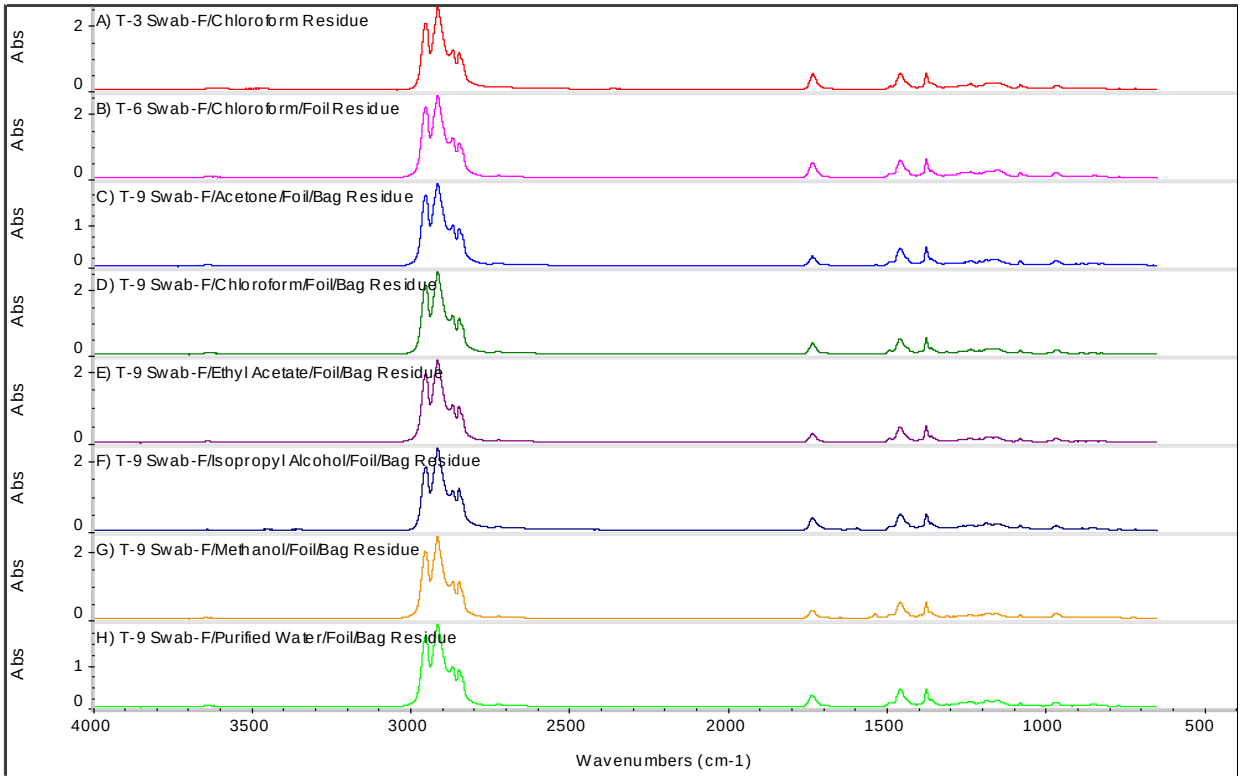

Figure 19 - (A) T-3 Swab-F chloroform representing all T-3 spectra, (B) T-6 Swab-F/Foil chloroform representing all T-6 spectra, and (C-H) T-9 spectra.

Experiments T-10 (Cancelled **See T-4**)

Experiment T-11

Introduction

Experiment T-0 determined that petroleum ether did not extract spectroscopically visible contaminants from 20mL scintillation vials. However, it was not known if the Sampling solvent would extract contaminant materials from 20mL vials when sampling solvents where used to extract swabs. Experiments T-12 and T-13 would assess the byproducts of solvent and swab interactions during sample storage. In this experiment, Swab-C and Swab-F are independently extracted by each sampling solvent to determine the chemical byproducts of solvent/swab interaction in experiments T-12 and T-13 respectively. Contaminants caused by sampling solvent and scintillation vial interaction during extraction required independent verification. Experiment T-11 is designed to determine whether contaminants are produced when Sampling solvents are used to extract samples in 20mL scintillation vials. This experiment is conceptually similar to T-0, although T-11 evaluates a broader set of solvents and their interaction with vials.

Experimental Methodology

Materials

Scintillation Vials w/caps x 6, 15mL each: Acetone, Chloroform, Ethyl Acetate, Isopropyl Alcohol, Methanol, Purified Water.

Preparation

15mL of each raw solvent were added to separate clean, new 20mL scintillation vials and capped. The vials were labeled and placed in storage for one week prior to analysis.

Hypothesis

The vials and/or caps will contribute spectroscopically visible contaminants into the solvents extractions contained within.

Null-Hypothesis

The vials and/or caps will not contribute spectroscopically visible contaminants into the solvent extractions contained within.

Analysis

A sample from each of the vials was individually analyzed by FT-IR. The crystal was cleaned with a portion of the solvent being tested and allowed to dry for ten minutes prior to background characterization. A background spectrum was acquired using 128 scans. Fifty drops from each vial were separately applied to the crystal and allowed to sit in a fume hood until dry. Individual dry times varied based on differing solvent volatility to ensure complete dissipation of solvents prior to data collection. The sample spectrum was acquired using 128 scans. The resulting spectrum was recorded for later comparative analysis. The set of residue spectra produced were then compared to solvent background spectra presented earlier to determine if spectroscopically visible contaminants had been introduced into extractions from solvent/vial interaction.

Discussion

In all cases the T-11 spectra matched their correlating background solvent spectra characterized during solvent background controls, thus confirming the Null-Hypothesis (compare A and B spectra of each figure respectively, Figures 20-25). Comparative analysis of peak position and form (Figures 20-25) between T-11 and raw solvent background spectra suggest that there were no spectroscopically visible contaminants extracted into any of the solvents when stored in scintillation vials. Due to the minute concentration of analytes in both background solvent and T-11 samples, the spectra are partially obscured by fluctuations in atmospheric gas concentrations during FT-IR analysis. However, the major peak features and formations that can be discerned allow for comparative qualitative analysis of the spectra. Notations for particular atmospheric features are included for each figure individually.

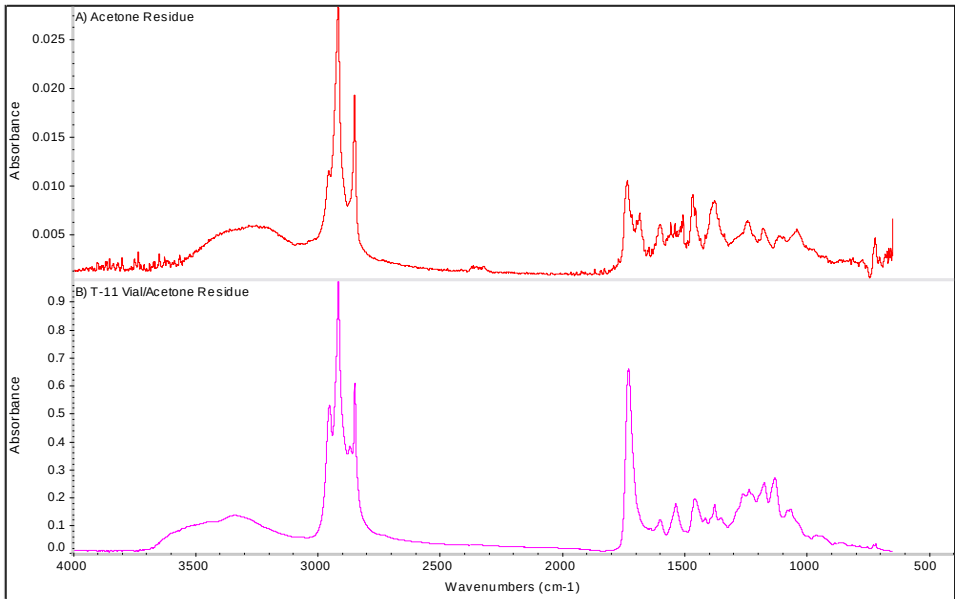

Figure 20 - Comparison of (A) raw acetone residue with (B) T-11 acetone extraction in 20mL scintillation vial; Note significant atmospheric noise resulting in jagged features in the 4000-3300cm^{-1} and 2000-1300cm^{-1} regions. Atmospheric noise interferes with the peak features in the fingerprint region (1500-500cm^{-1}) of the T-11 spectrum.

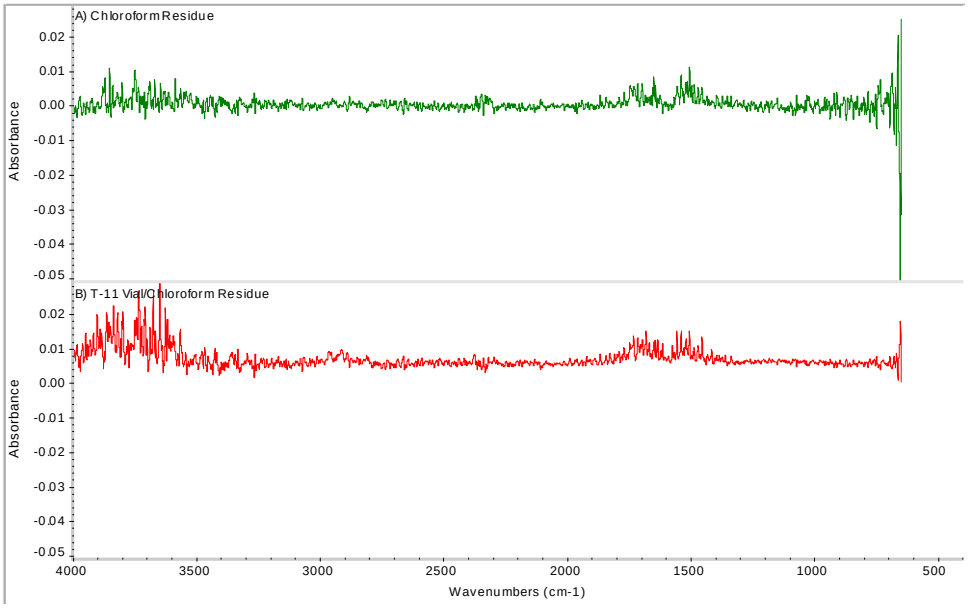

Figure 21 - Comparison of (A) raw chloroform residue with (B) T-11 chloroform in 20mL Scintillation Vial; Note atmospheric noise in both images. No peak features in either spectrum.

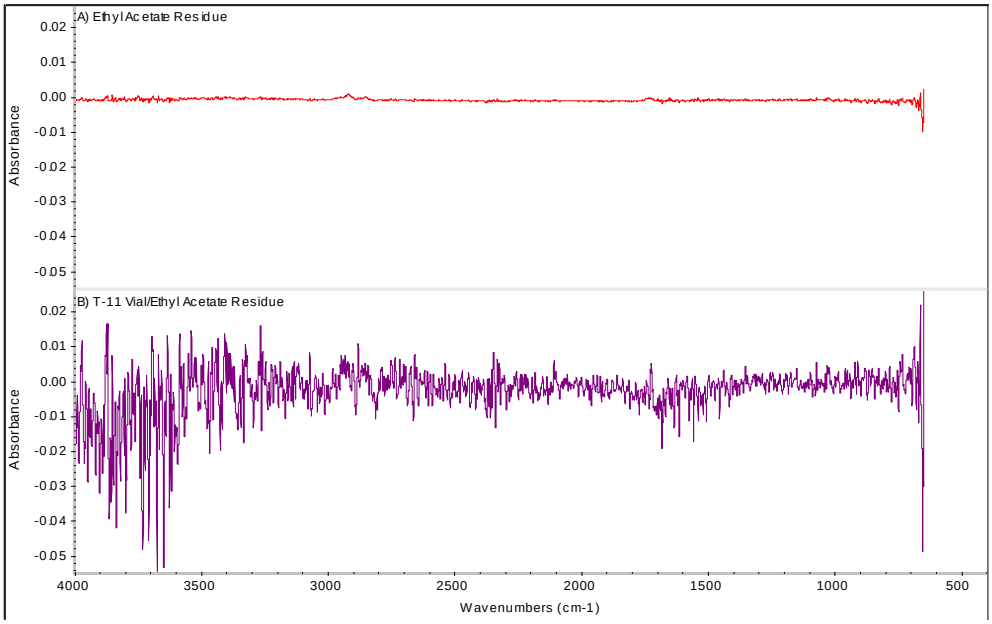

Figure 22 - Comparison of (A) raw ethyl acetate residue and B) T-11 ethyl acetate in 20mL scintillation Vial; Note atmospheric noise in T-11 spectrum. No peak features in either Spectrum.

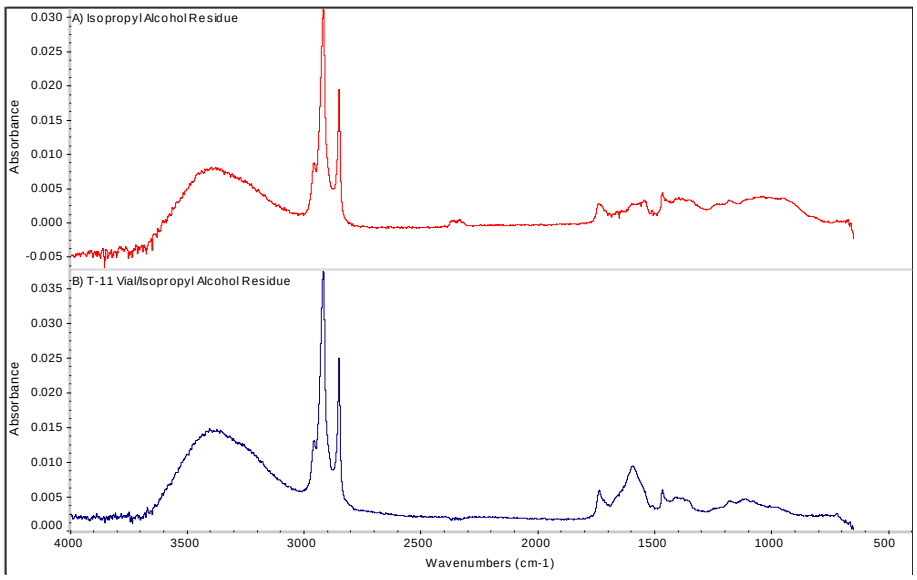

Figure 23 – Comparison of (A) raw isopropyl alcohol residue and B) T-11 isopropyl alcohol in 20mL scintillation vial; Note atmospheric noise in the 4000-3300cm^{-1} range which distorts the alcohol peak in the 3500-3000cm^{-1} region. Peak position and features are otherwise similar in both spectra.

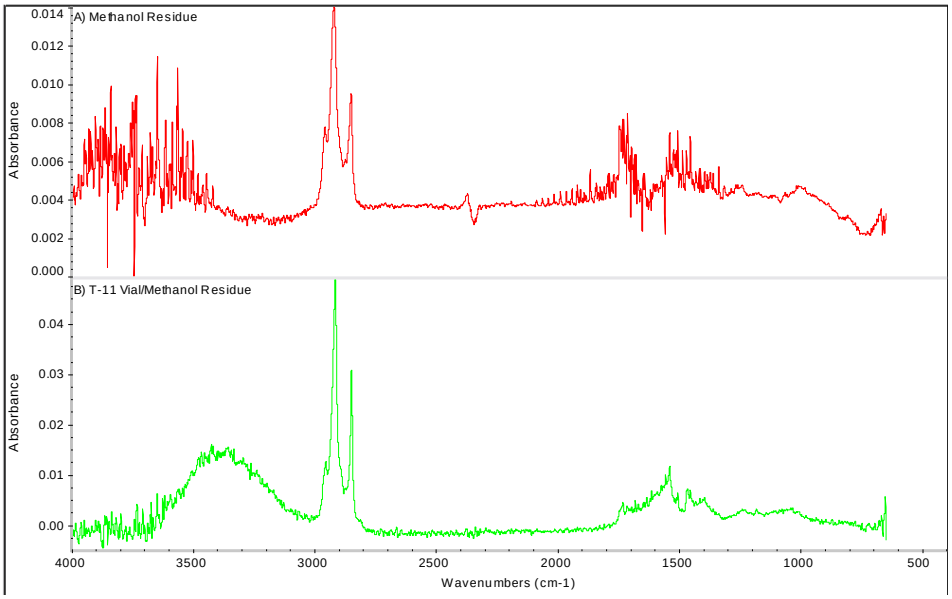

Figure 24 – Comparison of (A) raw methanol residue and (B) T-11 methanol in 20mL scintillation vial; Note heavy atmospheric noise interference in the 4000-3300cm^{-1} and 2000-1200cm^{-1} ranges which obscure visibility of peak features.

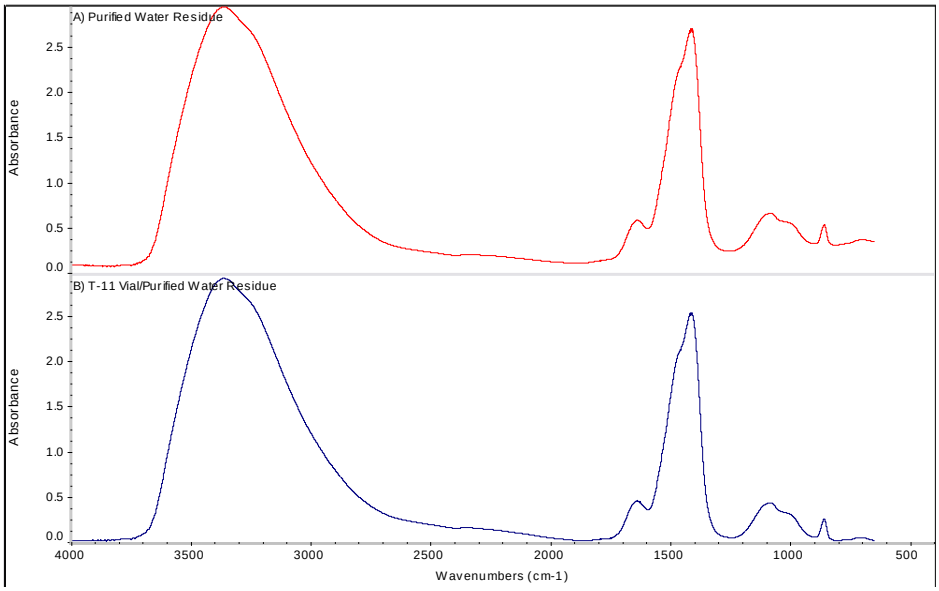

Figure 25 - Comparison of (A) raw purified water residue and B) T-11 purified water in 20mL scintillation vial; Note no atmospheric noise interference in these spectra due to large concentration of residual contaminants relative to other solvents tested.

Experiments T-12/T-13

Introduction

Throughout the earlier experiments the significant quantity of contaminants from Swab-C and Swab-F being introduced during the sample methodologies was disconcerting to the BRP members. This concern focused on the possibility that the relatively high quantity of contaminants when extracting Swab-C/F in petroleum ether was obscuring the visibility of trace contaminants which may have been produced by sampling solvent and swab interactions. Therefore it was decided that the interaction and subsequent chemical byproducts of both swab types and Sampling solvents would need to be independently verified and characterized

by FT-IR. Sampling solvents were chosen by the Bathtub Research Project team due to each solvent's ability to dissolve organic waxes and other organic compounds to some degree. The most favorable solvents, however, would be those that could dissolve lanolin and lanolin byproducts, while producing the least spectroscopically visible residues when extracting Swab-C/F. This would allow the project to recover organic residues while limiting the amount of contaminants being introduced into the sample from sampling solvent and swab interaction during collection. T-12 and T-13 were designed to evaluate the contaminants produced when sample Swabs-C/F are extracted in the Sampling solvents and to determine which solvents are best suited for sample recovery in the project methodology.

Experimental Methodology

T-12 Materials

Swab-C x 6, 20mL scintillation vials w/caps x 6, 100mL beaker x 6, 15mL each of: Acetone, Chloroform, Ethyl Acetate, Isopropyl Alcohol, Methanol, Purified Water.

T-13 Materials

Swab-F x 6, 20mL scintillation vials w/caps x 6, 100mL beaker x 6, 15mL each of: Acetone, Chloroform, Ethyl Acetate, Isopropyl Alcohol, Methanol, Purified Water.

T-12/T-13 Preparation

15mL of each solvent was added to a separate clean, new 20mL scintillation vial. Rather than quantify swabs by count, as in earlier tests, during T-12 and T-13 approximately the same relative mass of each swab type were added per vial. This would allow for quantitative

comparison between the spectra of the two swab types. In T-12 seven Swab-C were added to each vial, and in T-13 three Swab-F were added to each vial. Both swab types were broken or cut into pieces to allow them to fit in 20mL scintillation vials for extraction. Each vial was then capped, labeled, and placed into a cabinet for storage during extraction.

T-12/13 Hypothesis

One or more extractions of Swab-C/F in sampling solvents will produce spectroscopically visible contaminants.

T-12/13 Null Hypothesis

None of the extractions of Swab-C/F in sampling solvents will produce spectroscopically visible contaminants.

Analysis

A sample from each of the vials was individually analyzed using FT-IR. The crystal was cleaned with the solvent being tested and allowed to dry for ten minutes. A background spectrum was acquired using 128 scans. Fifty drops from each vial were separately applied to the crystal and allowed to dry for between thirty and one hundred and twenty minutes prior to analysis. Individual dry times were lengthened based on lower volatility of solvents to ensure complete dissipation prior to data collection. The spectrum was acquired using 128 scans. The resulting residue spectrum was recorded for later analysis.

Discussion

T-12 (Swab-C)

Several of the extractions produced spectra which contained contaminants from Swab-C, thus confirming the T-12 Hypothesis (Figure 26). Peak forms and peak positions of T-12 spectra were then qualitatively compared in order to determine which solvents demonstrate the least amount of chemical interaction with Swab-C (Compare all spectra in Figure 26). methanol and isopropyl alcohol extracted the greatest amount of alcohol components evident in the broad band peaks from 3300-3000cm^{-1} and in peak forms within the fingerprint region around 1000cm^{-1} (Figure 26: E and F), suggesting methanol and isopropyl alcohol would not be preferred solvents for sample collection or extraction. Chloroform, ethyl acetate, and acetone did not extract as much alcohol components from Swab-C or from the peaks around 1000cm^{-1} as did methanol and isopropyl alcohol. Chloroform, ethyl acetate, and acetone did, however, extract slightly more components in the fingerprint region (1500-500cm^{-1}) than petroleum ether, though still less than either methanol or isopropyl alcohol (Figure 26: B, C and D). Chloroform, ethyl acetate, and acetone are therefore comparable to petroleum ether for use in sample collection methodology based on extraction of Swab-C components.

Ethyl acetate, acetone, and petroleum ether produced spectroscopically visible contaminants during background controls, while chloroform did not. Chloroform was thus determined to be the more favorable sample and extraction solvent for use in the Bathtub Research Project methodology when using Swab-C as a sample media. Ethyl acetate, acetone, and petroleum ether would be acceptable sample collection solvents in organic residue

recovery if chloroform where not available, assuming a source for solvents can be procured which does not contain plastic contaminants.

Purified water produced a result which introduced an unforeseen consequence when used as an extraction solvent. After only a one week extraction period, the contents of the Swab-C/purified water extraction had turned brown. Also, what appeared to be a cotton-ball type growth was suspended in the water. It was hypothesized that the suspended growth was fungal due to morphological characteristics, and that the brown discoloration was most likely a byproduct of metabolic or other biological processes. A sample of the vial contents were plated on fungal augar by the microbiology laboratory staff at ECU. The growth was confirmed to be a fungus of undetermined species. All other solvents used during field and laboratory methodologies are antimicrobial in the concentration employed. Water, however, introduced a probiotic environment in which microbes had begun to flourish. This suggests that although water may be a favorable solvent due to its relatively low cost and toxicity, water would be an unfavorable solvent due to the capacity of biological agencies to corrupt organic residue samples. The Swab-C in purified water test was rerun with a three day extraction time as an attempt to prevent significant microbial growth prior to analysis. There were no visible signs of biological activity in the vial after three days and sample analysis was resumed. The spectrum generated from the retest suggests that purified water contains many chemical components which produce spectroscopically visible features that may obscure sample analysis. Further, purified water was not effective at recovering hydrocarbon components, therefore it would also not be a preferred Sampling solvent (Figure 26: G). Water may, however, be a suitable solvent for cleaning the surface of organic residues prior to sample collection. Water used in

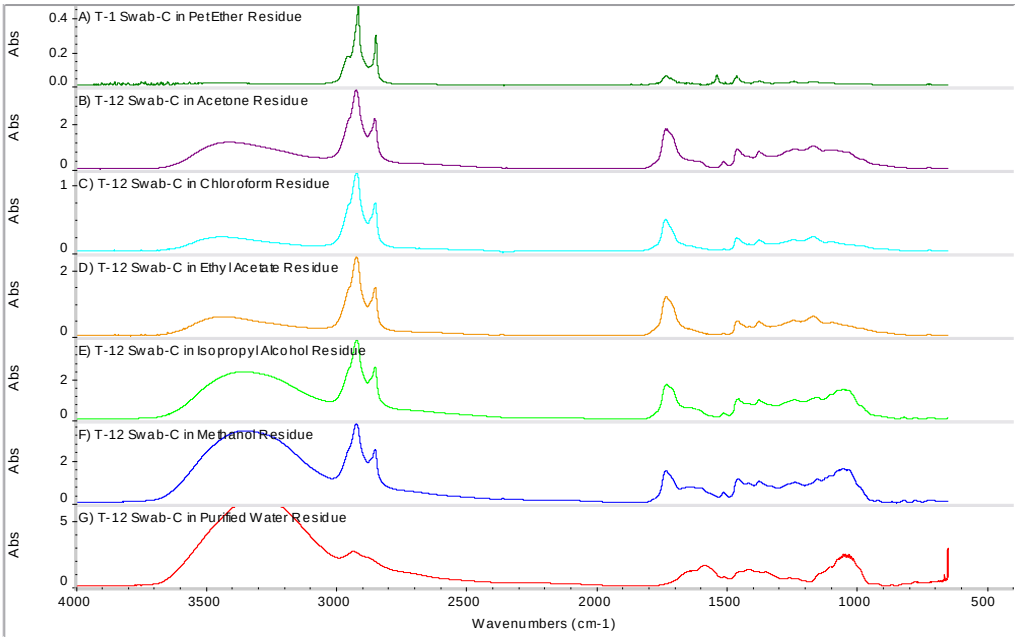

Figure 26 - Comparison of (A) T-1 spectrum of Swab-C in petroleum ether residue and (B-G) T-12 Swab-C residue spectra.

this way could remove hydrophilic/nonpolar surface contaminants without removing organics, which are primarily nonpolar hydrocarbons.

T-13 (Swab-F)

Several of the extractions produced spectra which contained contaminants from Swab-F, thus confirming the T-12 Hypothesis (Figure 27). Peak forms, peak heights, and peak positions of T-12 spectra were then qualitatively compared in order to determine which solvents demonstrate the least amount of chemical interaction with Swab-F (Compare all spectra in Figure 27). As with earlier characterizations, the T-13 purified water residues were wildly different than other T-13 solvent extractions. Water did not extract any hydrocarbons, or carbonyl group components around the 1700cm^{-1} range, which correlate to other extractions of Swab-F. The

alcohol (3500-3100cm^{-1}) and the fingerprint (1500-500cm^{-1}) regions are completely obscured by the purified water residue (Figure 27: G). These features suggest that purified water is not an appropriate solvent for sample collection or extraction with Swab-F.

Methanol, ethyl acetate, acetone, and isopropyl alcohol spectra all demonstrate a higher quantity of carbonyl extraction (1700cm^{-1}) and more features in the fingerprint region (1500-500cm^{-1}) than Petroleum Ether (Figure 27: B, D, E F). These four solvents also extract a lower relative amount of hydrocarbons (3000-2800cm^{-1}) than chloroform or petroleum ether. While this could suggest that these four solvents would introduce the least amount of hydrocarbon contaminants when sampling or extracting with Swab-F, it also may suggest that these solvents would be less likely to recover lanolin, which is mostly hydrocarbon chains. Further controls with sampling solvents are required to determine the efficacy of these solvents at extracting lanolin. Ethyl acetate is the better of the four solvents for use with Swab-F due to the lack of contaminants in the raw solvent characterized during background controls, and the relatively low reactivity with Swab-F evident in the T-13 spectra (Figure 27: D). Acetone, methanol, and isopropyl alcohol could be used with Swab-F based on T-13 (Figure 27: B, E, and F). The contaminants found in the solvent background spectra, however, make them less favorable for use in residue analysis in general, regardless of their low reactivity with Swab-F.

Chloroform interacted the most strongly with Swab-F during experimental preparation. The swabs visibly swelled when brought in contact with Chloroform. This phenomenon was also seen during T-3/6/9 preparation when chloroform was applied to Swab-F. It is no surprise then that the spectrum of chloroform demonstrated higher amounts of extracted contaminants

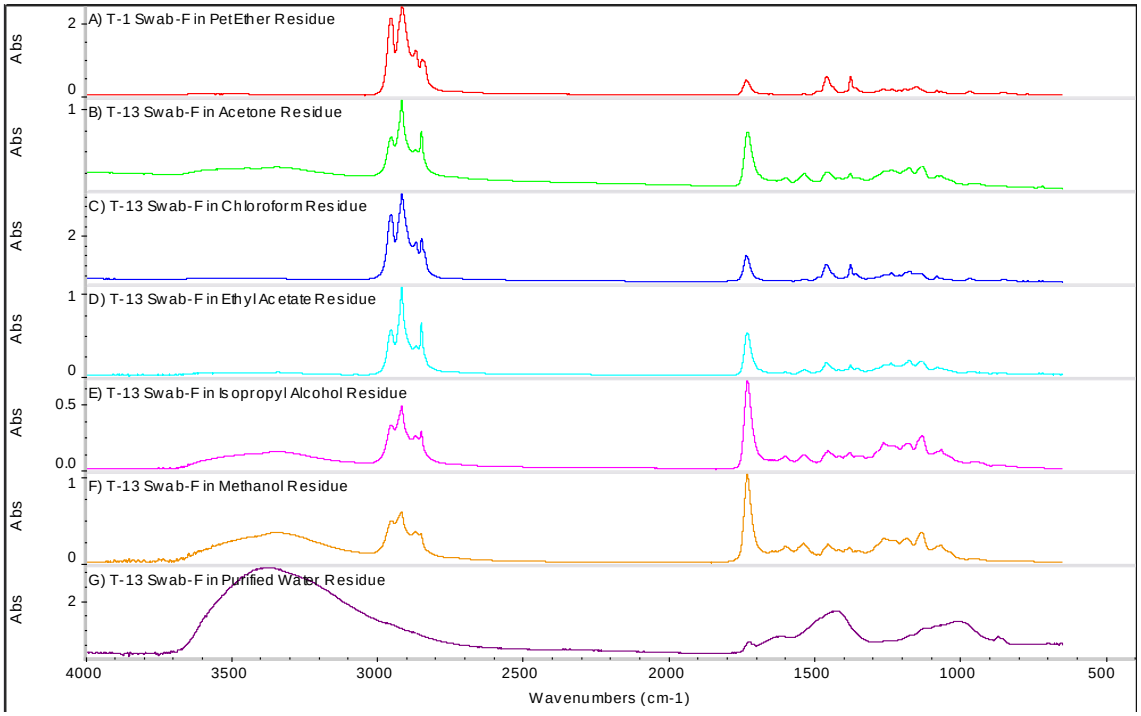

Figure 27 - Comparison of (A) T-1 Swab-F in petroleum ether residue spectrum and (B-G) T-13 residue spectra.

in all ranges (Figure 27: C). The features were, however, qualitatively consistent with the other solvent extractions of Swab-F. Due to its high reactivity with Swab-F, chloroform was deemed to be inappropriate for use as a sample or extraction solvent when using Swab-F.

Summary

Solvents were analyzed and several solvents were found to contain significant concentration of spectroscopically visible contaminants. The results were as follows:

- T-0 determined that the vials used for sample storage and laboratory extractions did not introduce analytically significant contaminants into petroleum ether.

- T-1 determined that both swab types introduce a significant concentration of contamination into petroleum ether extractions.
- T-2 and T-3 determined that the introduction of sampling solvents into the research methodology did not introduce spectroscopically visible contaminants relative to the swab contaminants.
- T-5 and T-6 determined that the introduction of aluminum foil as wrapping for sample swabs did not introduce spectroscopically visible contaminants relative to swab contaminants.
- T-8 and T-9 determined that plastic bags did not introduce spectroscopically visible contaminants relative to swab contaminants when used to store foil wrapped sample swabs prior to analysis.
- T-11 determined that the vials used for sample storage and laboratory extractions did not introduce spectroscopically visible contaminants intro acetone, chloroform, ethyl acetate, isopropyl alcohol, methanol, or purified water solvent extractions.
- T-12 and T-13 determined that, although the various solvents listed in T-11 did recover slightly different components from the sample swabs than petroleum ether, no analytically significant changes appeared that would complicate interpretation of earlier (T-0 _T-9) experiments.

This experimental research has tested the methodology used in the Bathtub Research Project. It can be used now both to assist in evaluating initial results of the BRP and to revise the methodology. These will be discussed in Chapter 5.

Chapter 5
Case Study Results

A set of goals was proposed in this case study. The first goal was to isolate contaminants in field and laboratory methodology for the Bathtub Research Project. My research has identified numerous sources of contaminants in both field and lab methodologies. There are three key observations concerning contamination in organic residue analysis that are demonstrated by the identification of contaminants in this study. First, methodological contaminants can be introduced into samples by solvents commonly employed in organic residue analysis during field and laboratory methodologies. This research has shown that over-the-counter solvents that are stored and shipped in plastic containers introduce plastics during sample collection and laboratory analysis. More importantly, high-grade solvents procured from reputable chemical manufacturers that are specifically manipulated to reduce contaminants can, in fact, contain contaminant materials at a concentration that can interfere with analysis by IR spectroscopy. High-grade solvents cannot be assumed to contain no significant contaminants. Second, along with solvent contaminants, sample collection utensils such as swabs are also a source of methodological contaminants in residue analysis. This is particularly true in cases where solvent extraction is used to recover sample materials from swabs. The use of plastic swabs in residue analysis can introduce significant concentrations of plastics into residue samples. Cotton and wood swabs are also just as likely a source for significant contaminants as their plastic peers. Third, plastics are a critically important material to avoid during organic residue analysis projects. Plastics contain many of the same chemical components as organic compounds, and they can obscure the identification of organics in residues by masking critical

molecular components, or, in the worst of cases, lead to misidentification of samples. This research has demonstrated that the environment and plastic bags are not the only significant sources for hydrocarbon and carbonyl contaminants in residue analysis projects as others have suggested. Solvents of both low and laboratory grade, and sampling swabs, have been shown to introduce plastic contaminants into residue analysis that can interfere with data collection, analysis, and interpretation.

The second major goal of the case study was to build a spectral database of contaminants for comparative analysis in the Bathtub Research Project. My research has produced a full database of spectral characterizations of all field and laboratory material contaminant residues produced by sample collection and analysis methodologies. All future samples can be compared with the control spectra generated during this research project to aid in analysis and interpretation.

The third goal of the case study was to remove methodological materials that introduce analytically significant contaminants into recovered residues. My research has allowed the Bathtub Research Project to successfully remove contaminating materials from its sample methodology by identification of contamination sources. Isopropyl alcohol and acetone shipped in plastic containers have been removed from field methodologies due to significant plastic contaminants. Bottled purified water has also been removed from the methodology due to its significant amount of mineral contaminants and the threat it poses in harboring microbial growth. Plastic swabs are no longer being employed as sample collection utensils in the bathtub project. This research has also demonstrated that many of the sample materials used such as aluminum foil, scintillation vials, and several solvents will not introduce significant

contaminants within the existing project methodologies. They will remain in use now that they have been experimentally verified.

Chapter 6
Conclusions

The research question asked was; “Are common methodological materials used in organic residue analysis projects introducing analytically significant contaminants into samples.” The answer is “Yes.” My research has demonstrated that there are many sources of methodological contamination in common materials used in residue analysis projects. It has been demonstrated that high-grade solvents can introduce analytically significant contaminants into sample extractions despite the claims of manufacturers to the contrary. It has also been shown that while plastics are a major and significant contaminant in residue analysis, appearing in many of the materials tested, there are other potentially significant contaminants in residue analysis, such as the as yet unidentified contaminants from cotton-tipped and foam-tipped swabs that can be introduced into samples during research.

As discussed in Chapter 1 many research publications on residue analysis do not publish contamination methodologies. In research publications, contamination issues are given short shrift or overlooked completely. Solvents and other methodological materials are assumed to be contaminant free and not independently verified within research methodologies. In light of the findings presented in this research, two major conclusions can be drawn concerning the lack of contamination methodologies in publications. First, no material used in organic residue analysis sample collection, preparation, or analysis can be assumed to be contaminant-free. Some of the “high-grade” solvents tested in this research contained spectroscopically visible concentrations of contaminant residues (e.g., Petroleum Ether, Methanol). One of the major factors in organic residue analysis in archaeology is the relatively small sample size available for

analysis. Due to this restriction, residues are significant at the smallest visible concentrations, and equally minute concentrations of contaminants can mask and obscure residues during analysis. Any contaminant that is spectroscopically visible during FTIR analysis of organic residues is therefore analytically significant. It follows that identifying and isolating all sources of contamination becomes critical in preventing sample corruption and validating spectral interpretation. Organic Residue Analysis projects using FTIR as the sole means of chemical analysis are cautioned to acknowledge all potential sources of methodological contamination, particularly methodological materials.

Second, the positive identification and isolation of methodological contaminants requires rigorous control methodologies in organic residue analysis projects. The research methods presented in this thesis should serve as a common example for contaminant control protocols when using FTIR. Many publications take the first step in addressing contamination by acknowledging that such issues exist. However, very few residue analysis publications include primary control data. My research has identified not only a need for such control methodologies, but a need for publication of both the methods and primary control data. This research has demonstrated that the purity of methodological materials cannot be safely assumed without adequate controls (e.g., high grade solvents can introduce analytically significant contaminants). In addition, the source of identified contaminants cannot be inferred from the sample methodologies, but must be independently verified. For example, in archaeological publications, plastic bags and buckets are pointed to without independent assessment as sources for plasticizer and antioxidants in residue samples (Garner 2008). Other materials, however, may be introducing contaminants that are not being identified. In this

research, plastic bags and unwashed aluminum foil, which were used to wrap sample materials, did not introduce analytically significant contaminants, while other materials normally assumed to be contaminant-free did introduce contaminants (e.g., high-grade solvents). To assume the source of contaminants in analytical research without verification is a critical flaw in many studies. When reading organic residue analysis literature, which does not contain explicit control methods and primary control data, one cannot effectively evaluate the data and interpretations being presented.

FTIR spectroscopy is being promoted by the archaeological community, as well as the product manufacturers, as an effective method for residue analysis (e.g., Weiner 2010, Shearer 1988). However, these publications and marketers do not communicate to consumers the limitations and dangers of the technique as a sole means of analysis. Data are easily generated using FTIR, but not as easily or clearly interpreted by untrained individuals. This is not to say that researchers in organic residue analysis are untrained, but merely that many consumers of organic residue analysis literature are not. They, therefore, must be presented with contamination and control methods in publications when asked to accept research methods, analysis, and conclusions.

Assessment of archaeological materials by independent, for-profit laboratories is also problematic. For-profit laboratories are often hesitant to provide complete methodological information to clients due to proprietary concerns. In the context of academic research, however, the nondisclosure of analytical methodology amounts to a “black-box” scenario in which academic researchers are asked to accept third party interpretations without the primary data and methodology necessary for critical assessment. Archaeological samples are exposed

to many potential sources of methodological contamination between the point of excavation and the final laboratory location. For proper analytical assessment of the methodological materials, independent labs would need access to the source materials used by the archaeological project for the laboratory to produce adequate method blanks for comparative analysis. Without blank materials, with which proper controls and method blanks can be executed, the data from these kinds of analyses cannot be validated. Independent, for-profit laboratories do not seem to be requesting these blank materials from academic research projects that are requesting their services (e.g., Paleoresearch Institute 2012). When data generated by these labs are being incorporated into primary research literature, the archaeological community should be asking independent laboratories to publish their contamination and control methodologies for critical assessment in peer-reviewed journals.

This research has provided a real world assessment of the potential for contamination in a working organic residue analysis project using FTIR. The data and analysis presented serves as evidence to the importance of controls and the discussion of contamination in residue analysis research using infrared spectroscopy. As the cost and ease-of-use of Infrared spectroscopy instruments decrease, their prevalence in archaeological research will no doubt increase. The increased application of FTIR in antiquities research has the potential to advance the field of organic residue analysis and the body of knowledge in archaeology and conservation dramatically. However, these fields can only be harmed by the improper application of the technique and the misinterpretation of data by untrained, intrepid researchers. I suggest that all social scientists venturing into organic residue analysis resist the temptation of immediate gratification, and engage in heavy research on the techniques of residue analysis and FTIR, and

conduct preliminary control experimentation of proposed field methods prior to field sample collection and analysis. A humble attitude towards a powerful and complex method of analysis can no doubt prevent a great many flaws in organic residue analysis research.

The research presented in this thesis applies to research in organic residue analysis using FTIR spectroscopy. The results and conclusions of this research may or may not be directly applicable to other analytical techniques or projects. Each analytical technique is impacted by contamination in its own way. Further research using various chemical and analytical techniques will be needed to determine the relevance of methodological contamination issues for each case respectively. It is my wish that such research begins in earnest, with open and critical debate in primary literature, conference proceedings, and academic departments.

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Appendix A:
Research Materials

Solvents

***Petroleum Ether**:* 40-60 degrees, for Analysis, 5L
FisherScientific#18021-0050

***Chloroform**:* 99+%, Spectrophotometric Grade, 1L
FisherScientific#AC16773-0010

***Methanol**:* 99.9%, Spectrophotometric Grade, 500mL
FisherScientific#AC16783-5000

***Ethyl Acetate**:* 99.5+%, 500mL
FisherScientific#16781-5000

***Purifed Water**:* **"**Evian"*,* Reverse Osmosis, 12.0 oz. Plastic bottle
UPC#07929878

***Isopropyl Alcohol**:* 91%, 16.0 oz. Plastic Bottle, Rite Aid Pharmacy
UPC#1182239358

Acetone: Studio 35 100% Acetone, 16FL OZ.
UPC#4902239416

Containers and Implements

***Scintillating Vials* w/plastic caps**: 28x61mm, 20mL, 500 count case,
FisherScientific#0334025, WSP#986580

***Aluminum Foil**:* standard gauge, 12"x25' roll
FisherScientific#01-213-100

***Erlenmeyer Flask**:* 250mL, narrow-mouth, 12pk
FisherScientific#FB-500-250

***Beaker**:* glass Berzelius, 100mL, Case of 4 (4x12 packs)
FisherScientific#FB-102-100

***Swab, Flat Foam-tipped**:* Polypropylene handle, 5 inches, 50pk
FisherScientific#14-960-3L

***Swab, Cotton-tipped**:* Wooden Handle, 6 inches, 1000pk
FisherScientific#23400106

***Plastic Bags**:* Hefty OneZip Click Freezer Bags, 1 quart, 18 count.
UPC#2570002150

Miscellaneous Laboratory Materials

***Kimwipes**:* Kimberly Clark, 1 ply, 4.4”X8.4”
FisherScientific#34155

Gloves: Nitrile Gloves, Large
FisherScientific#57373-17

***Pipets**:* Corning, Glass Pasteur Pipets, 5 ¾ inch
FisherScientific#7095B-5X