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The following lessons were created by **Anna Alinda**, a teacher participating in the National Endowment for the Humanities Summer Institute for Teachers entitled Touch the Past: Archaeology of the Upper Mississippi River Region.

Any views, findings, conclusions or recommendations expressed in this publication do not necessarily represent those of the National Endowment for the Humanities.

### **Organic Residue Analysis in Archaeology**

**Subject and Grade Level:** Advanced Chemistry, Organic Chemistry

#### **Objectives**

Students will be able to:

- explain application of organic residue analysis to archaeology
- define vocabulary listed below
- distinguish between mono- and disaccharides, starches and lipids from their structural formula
- recognize patterns in the names of associated organic compounds
- interpret results of Benedict's test, iodine test, and Sudan IV test
- summarize lab results
- connect lab demonstration to current archaeological research

**Standards (following National Science Standards found here:**

[http://www.nap.edu/openbook.php?record\\_id=4962](http://www.nap.edu/openbook.php?record_id=4962))

U.2 Evidence, models, and explanation

A.1 Abilities necessary to do scientific inquiry

B.3 Chemical reactions

E.2 Understandings about science and technology

F.5 Science and technology in society

#### **Duration**

45 minute lesson and 75 minute lab

#### **Materials/Supplies**

- *Organic Residue Analysis in Archaeology Lesson Resource Document* (referred to as Lesson Document)

- Resource for reading an academic paper:  
Mayyas, Abdulraouf, et al. "Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan." *Mediterranean Archaeology and Archeometry* Vol. 13, No. 2 (2013): 189-206 (Available online.)
- Additional resources for background information listed in Lesson Document
- Naming, Structure and Literature Worksheet
- Non-Quantitative Analysis of Organic Residues Lab Document (including data sheet)

For lab:

|                          |                          |
|--------------------------|--------------------------|
| Corn starch              |                          |
| Skim milk                | Disposable pipets        |
| Olive oil                | Hot plate/hot water bath |
| Deionized water          | Test tube holders        |
| Acetone                  | Unknown solutions        |
| Benedict's Solution      | Treated pottery sample   |
| Iodine                   | Mortar and pestle        |
| Sudan IV Solution        | Metal scoop              |
| 18 test tubes            | DI water wash bottle     |
| Test tube holder (metal) | 6 test tube stoppers     |
| Wax pencil               | Acetone wash bottle      |
| Stirring rod             |                          |

### **Vocabulary**

Organic compound  
Organic residue  
Lipid  
Fatty acid  
Starch  
Sugar  
Amino acid  
Hydrocarbon  
Qualitative  
Quantitative  
Chromatography  
Mass spectrometry  
Single bonds  
Double bonds  
Extraction  
Sherds  
Resin  
Porosity  
Monosaccharides  
Disaccharides

## Background

This lesson is an application where organic chemistry works in conjunction with archaeology. For students to be prepared for content, they should be comfortable with organic compounds. In particular, they should be familiar with the basics of naming, drawing line structures, and identifying functional groups. Students should understand how to interpret graphical data. Finally, they should have a strong understanding of lab procedures, including lab safety.

## Setting the Stage

To set the stage, introduce how chemistry can enrich archaeology. This involves a brief discussion of the field of archaeology, the inferences that can be made, and the information missing from artifacts (without analysis). Short student activity involving what information is missing about school's culture from the garbage bin can clarify gaps in prehistoric data. Follow up by discussing general methods where chemistry applications can inform archaeology. See *Lesson* document for more detailed plan.

## Procedure

After an initial introduction to how chemistry can enrich archaeology, a specific focus is taken on organic residues analysis. The lesson reminds students of previous information known about organic compounds and functional groups, and then focuses on organic compounds present in common food residues. A general explanation of the analytical process of residue analysis is provided in as much depth as appropriate for students' comprehension level. Eventually, applications to inferences in archaeology can be made. Once students understand the process and significance of organic residue analysis, academic research on the topic can be used to introduce students to the skills necessary to tackle high level published research. Beginning as a class, together students and instructor can dissect the more complicated elements, pulling out vocabulary and strategizing on the function of each section. Finally, the instructor sets up the lab by introducing the three tests and what they can determine. See *Lesson* document for more detailed plan of Lesson 1.

The lab focuses on showing students the qualitative indicator of the presence of certain nutrients that might be present in archaeological samples. By first establishing standard positive indicators, students will then determine the compounds present in an unknown mixture and test to see if those compounds can also be extracted from a sample of modern treated pottery. The lab that serves as Lesson 2 is detailed in *Non-Quantitative Analysis of Organic Residues Lab* Document.

## Closure

Students will process results through the lab write up outlined at the end of the *Lab* document. Students can be given the following quick write prompt the day they hand in the lab write up.

*Based on the information you discovered about your piece of pottery, describe the life of your pot. Pick a general time period (that makes sense – no one in the mid-1900s would be using pottery for storing corn, for example), and talk about its function. You might want to include something about its formation, uses, and/or eventual end.*

## **Evaluation**

This lesson includes *Naming, Structure and Literature Worksheet* with an answer key. Students will complete worksheet for a grade. Additionally, students will complete a simple lab write up following the completion of the residue lab.

## **Links/Extension**

Extensions could include:

- an investigation of prehistoric ceramic-making techniques
- more in-depth study of nutrients and biochemistry
- case study of subsistence patterns and their change over time
- lesson on development of agriculture and its effects on formation of complex societies

## **References**

Additional resources for background information listed in *Lesson Document*.

## **Attachments**

- *Organic Residue Analysis in Archaeology Lesson Resource Document*
- *Naming, Structure and Literature Worksheet*
- *Non-Quantitative Analysis of Organic Residues Lab Document (including data sheet)*

## Organic Residue Analysis in Archaeology Lesson Resource Document

### Necessary Skills:

This lesson is an application of organic chemistry in archaeology. Students should have an introductory understanding of nomenclature and functional groups, as well as skills in drawing and interpreting structural formulas.

### Lesson 1 – Introduction, Literature, and Lab Basics

1. How can chemistry enrich archaeology?
  - a. What is archaeology?
  - b. Observations and inferences in archaeology
    - i. e.g. observation: stone materials are found far from sources, inference: Archaic people are trading with one another
  - c. Data that cannot necessarily be observed
    - i. have students generate list of what information cannot be learned about the school from its garbage
    - ii. bring up difference between cultures with written record and permanent dwellings vs. prehistoric cultures with structures that would not survive
    - iii. discuss materials that most often would not survive being buried (e.g. wood, paper, fabric, plant remains)
  - d. Chemical processes that can provide more data
    - i. radiocarbon dating
    - ii. isotopic analysis
    - iii. organic residue analysis
    - iv. material analyses (e.g. pottery, glazes, pigments)

Resources for background information:

Introduction to Archaeological Chemistry

<http://pubs.acs.org/doi/pdf/10.1021/ba-1978-0171.ch001> (older source, but good introduction)

Short Royal Society of Chemistry Article

<http://www.rsc.org/chemistryworld/Issues/2008/October/TheInsiderArchaeologicalInvestigation.asp>

Lambert, Joseph B. *Traces of the Past*. Cambridge: Perseus Publishing, 1997.

Pollard, A. M., et al. *Analytical Chemistry in Archaeology*. Cambridge: Cambridge UP, 2007.

2. Organic residue analysis: how it works, what it tells us
  - a. Reminder: What are organic compounds? What are functional groups and which have been discussed?
  - b. Organic compounds that interest archaeologists
    - i. Food residues
      1. sugars
      2. starches
      3. lipids
    - ii. Others
      1. amino acids (soils, tools)
      2. hydrocarbons (tars, waxes)

- c. The science of organic residue analysis
  - i. Specific and quantitative (different than our lab will be)
  - ii. Chromatography
  - iii. Mass spectrometry
  - iv. This YouTube video gives a decent visual for the process of GC-MS (begins around 1:00) but isn't the most engaging style  
<https://www.youtube.com/watch?v=ozJ2oZboIFc>
- d. Information from results
  - i. Diet
  - ii. Activity areas
  - iii. Identity of materials
  - iv. Origin of materials

Resources for background information:

Price, T. Douglas, and James H. Burton. *An Introduction to Archaeological Chemistry*.

New York: Springer, 2012. 102-114. (This is an EXCELLENT resource.)

Introduction to Lipid Analysis chapter from larger analytical text

Barnard, H., A.N. Dooley and K.F. Faull. "An Introduction to Archaeological Lipid Analysis by Combined Gas Chromatography Mass Spectrometry (GC/MS)." *Theory and Practice of Archeological Residue Analysis*. British Archaeological Reports: 2007.

General Introduction on blog of two ECU staff members

<http://organicresidueanalysis.wordpress.com/>

Hoekman-Sites, Hanneke A., and Julia I. Giblin. "Prehistoric animal use on the Great Hungarian Plain: A synthesis of isotope and residue analyses from the Neolithic and Copper Age." *Journal of Anthropological Archaeology* Vol. 34, Issue 4 (2012): 515-527 (Available through Science Direct)

Evershed, R. P. "Organic residue analysis in archaeology: the archaeological biomarker revolution." *Archeometry* Vol. 50, 6 (2008): 895 – 924

Article on lipids and culinary practices

<http://www.pnas.org/content/108/44/17910.full>

- 3. What this looks like in current research – reading an academic paper
  - Resource:** Mayyas, Abdulraouf, et al. "Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan." *Mediterranean Archaeology and Archeometry* Vol. 13, No. 2 (2013): 189-206 (Available online.)
  - a. Discuss the importance of structure and the role of each section
    - i. Abstract – summary of paper and its goals
    - ii. Introduction – describes the concepts in some details, good overall with a little more specificity than abstract
    - iii. Method – gives details to reproduce experiment (not necessarily key in first approaching the paper, if approaching for conclusions instead of process)
    - iv. Data – not always as straightforward as data students might be used to, pull apart a chart or two to explain how footnotes can help in interpreting tables
      - 1. For Table 2, can discuss fatty acid notation (# carbons:# double bonds) and what that translates to in a structure

- v. Results – discusses results of experiments and what inferences can then be made in response to the research question
  - b. As a class, read through Abstract, Introduction and Conclusion
  - c. Pull out vocabulary from reading through Abstract, Introduction, and Conclusion, and have students look up definitions (e.g. GC-MS, extraction, lipids, fatty acids, alkaline hydrolysis, sherds, resin, porosity)
4. Lab introduction (Can be moved to Lesson 2 if time runs out)
- a. What do tests tell us
    - i. Benedict’s test: presence of monosaccharides and disaccharides (this includes sucrose, fructose and lactose)
    - ii. Iodine test: presence of starches (flour, corn starch)
    - iii. Sudan IV test: presence of lipids (fatty acids like olive oil, waxes)
  - b. What results to look for (great visual on this website:  
[http://emp.byui.edu/wellerg/Molecules\\_of\\_the\\_Cell\\_Lab/instruction/Molecules\\_of\\_the\\_Cell\\_Instructions.html](http://emp.byui.edu/wellerg/Molecules_of_the_Cell_Lab/instruction/Molecules_of_the_Cell_Instructions.html))

Resources for background information:

Website BYU from General Botany describing tests and showing positive results

[http://emp.byui.edu/wellerg/Molecules\\_of\\_the\\_Cell\\_Lab/instruction/Molecules\\_of\\_the\\_Cell\\_Instructions.html](http://emp.byui.edu/wellerg/Molecules_of_the_Cell_Lab/instruction/Molecules_of_the_Cell_Instructions.html)

UNM Biology Lab site again describing tests and showing positive results

[http://biology.unm.edu/ccouncil/Biology\\_124/Summaries/Macromol.html](http://biology.unm.edu/ccouncil/Biology_124/Summaries/Macromol.html)

Harper College site showing chemical process for Benedict’s Test

<http://www.harpercollege.edu/tm-ps/chm/100/dgodambe/thedisk/carbo/bened/benedict.htm>

Wikipedia page for Sudan IV compound

[http://en.wikipedia.org/wiki/Sudan\\_IV](http://en.wikipedia.org/wiki/Sudan_IV)

Food nutrient lab with similar procedures to following lab

<http://www.scienceteacherprogram.org/biology/Lillieno2.html>

More in-depth nutrients lab with additional tests

[http://www.hccfl.edu/media/571427/3-indicator\\_tests\\_for\\_important\\_nutrients.pdf](http://www.hccfl.edu/media/571427/3-indicator_tests_for_important_nutrients.pdf)

Assignment: *Structure, Naming and Literature Worksheet*

### **Lesson 2 – Non-Quantitative Analysis of Organic Residues Lab**

See *Non-Quantitative Analysis of Organic Residues Lab* document for detailed instructions. In general, the lab lets students see a positive result for each of the tests, then use that information to determine the components in an unknown mixture. Additionally, they will investigate the possible application of qualitative analysis to residues on modern pottery pieces.

Assignment: Lab write-up (instructions included in *Non-Quantitative Analysis of Organic Residues Lab* document)

### **Closure Activity**

Students can be given the following quick write prompt the day they hand in the lab write up to strengthen the connection between data and cultural understanding.

*Based on the information you discovered about your piece of pottery, describe the life of your pot. Pick a general place and time period (that makes sense – very few Americans in the late-1900s would be using pottery for storing corn, for example), and talk about its function. You might want to include something about its formation, uses, and/or eventual end.*



Name: \_\_\_\_\_ Date: \_\_\_\_\_

Naming, Structure and Literature Worksheet

Using class notes and “Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan.”<sup>1</sup>

1. Looking at Table 2 (p. 195), find the fatty acids retrieved for Sherd 1 (D624/2).

a. Name the first three listed (remember, they are fatty acids).

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

b. Draw the line structure for the last three listed. For C<sub>18:1</sub> there is more than one correct answer, but you only need to provide one.

Structure 1

Structure 2

Structure 3

c. Choose a dioic acid, oxo-acid, hydroxyl acid and sterol from the table. Look up the structure online, and draw the line drawing. After drawing the line drawing, write the name and chemical formula on the line.

Dioic acid \_\_\_\_\_

Oxo-acid \_\_\_\_\_

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<sup>1</sup> Mayyas, Abdulraouf, et al. “Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan.” *Mediterranean Archaeology and Archeometry* Vol. 13, No. 2 (2013): 189-206

Hydroxyl acid \_\_\_\_\_

Sterol \_\_\_\_\_

2. Looking at Figure 3 (p. 197), answer the following questions.
  - a. Which compound was found in the highest concentration on the interior of sherd 1?
  - b. Which compound was not found in the soil on the interior surface of sherd 1?
  - c. Which compound was found to have a concentration of 6  $\mu\text{g-g}$  on the interior of sherd 1?
3. Looking at Figure 4 (p. 198), answer the following questions.
  - a. Which compounds were found in the soil on the internal surface of sherd 2?
  - b. Which compound had the highest concentration on the exterior of sherd 2?
  - c. Roughly, what is the highest concentration of a residue found on sherd 2?
4. Rereading the Conclusions of the paper (p. 202) and doing research online as necessary, answer the following questions.
  - a. What substance would you expect to find in the interior of the sherds?
  - b. What are the saturated fats present in that substance?
  - c. The compound with the highest concentration present in the interior of sherd 1 and 2 is  $\text{C}_{16:0}$ . Find out the name of that compound.
  - d. Based on your answers to 4a. and 4b., do the findings in 4c. make sense?

## TEACHER'S KEY

### Naming, Structure and Literature Worksheet

Using class notes and "Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan."<sup>2</sup>

1. Looking at Table 2 (p. 195), find the fatty acids retrieved for Sherd 1 (D624/2).
- a. Name the first three listed (remember, they are fatty acids).

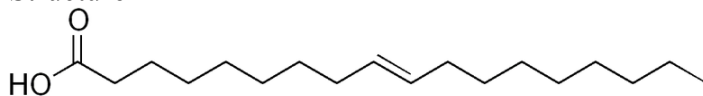
octanoic acid

nonaioic acid

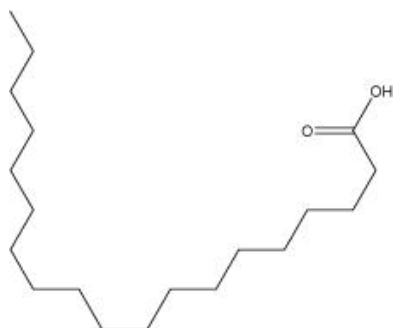
decanoic acid

- b. Draw the line structure for the last three listed. For C<sub>18:1</sub> there is more than one correct answer, but you only need to provide one.

Structure 1



Structure 2



Structure 3



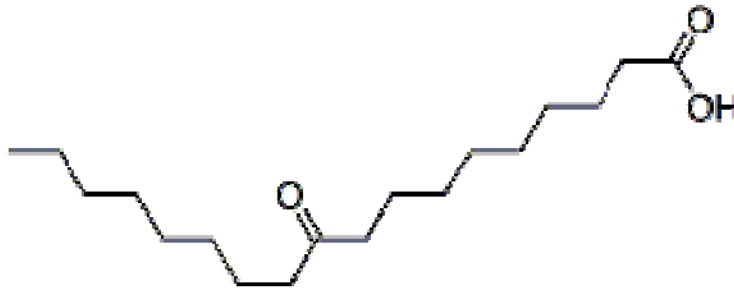
- c. Choose a dioic acid, oxo-acid, hydroxyl acid and sterol from the table. Look up the structure, and draw the line drawing. After drawing the line drawing, write the name and chemical formula on the line.

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<sup>2</sup> Mayyas, Abdulraouf, et al. "Organic Residues Preserved in Archaeological Ceramics from the Early Bronze Age Site of Khirbet Al-Batrawy in North-Central Jordan." *Mediterranean Archaeology and Archeometry* Vol. 13, No. 2 (2013): 189-206

Answers will vary. An example is provided for oxo-acid.

Oxo-acid     10-oxo-octadecanoic acid     C<sub>18</sub>H<sub>34</sub>O<sub>3</sub>



Hydroxyl acid \_\_\_\_\_

Sterol \_\_\_\_\_

2. Looking at Figure 3 (p. 197), answer the following questions.
  - a. Which compound was found in the highest concentration on the interior of sherd 1?  
**C<sub>16:0</sub> (or hexadecanoic acid)**
  - b. Which compound was not found in the soil on the interior surface of sherd 1?  
**10-oxo- (or 10-oxo-octadecanoic acid)**
  - c. Which compound was found to have a concentration of 6 µg-g on the interior of sherd 1? **C<sub>9:0</sub> (or nonanoic acid)**
  
3. Looking at Figure 4 (p. 198), answer the following questions.
  - a. Which compounds were found in the soil on the internal surface of sherd 2?  
**C<sub>9:0</sub>, C<sub>16:0</sub>, C<sub>18:1</sub>, C<sub>18:0</sub>**
  - b. Which compound had the highest concentration on the exterior of sherd 2?  
**C<sub>18:0</sub>**
  - c. Roughly, what is the highest concentration of a compound found on sherd 2?  
**34 µg-g**

4. Rereading the Conclusions of the paper (p. 202) and doing research online as necessary, answer the following questions.
- What substance would you expect to find in the interior of the sherds (based on the authors' conclusion)?  
olive oil
  - What are the two saturated fats present in that substance?  
palmitic acid, stearic acid
  - The compound with the highest concentration present in the interior of sherd 1 and 2 is  $C_{16:0}$ . Find out the IUPAC and common name of that compound.  
hexadecanoic acid, palmitic acid
  - Based on your answers to 4a. and 4b., do the findings in 4c. make sense?  
Yes

## Non-Quantitative Analysis of Organic Residues Lab

### Advanced Chemistry, Organic Chemistry

**NOTE: This lab was constructed for first use in the classroom during the 2014-2015 school year. As written, it has not yet been tested with students.**

#### Introduction

Archaeologists are constantly working to gain as much information about past societies as possible. Information can be gathered from artifacts in multiple ways. Archaeologists can study the context of the materials as they discover them. For example, they can look at what artifacts are found together, what geographic features are nearby, what materials are the artifacts made of, and what type of workmanship or use is present on the artifacts.

Chemists can enrich archaeology by analyzing artifacts, soils, and much more for data that cannot be seen on the surface. A common example of this is the use of radiocarbon dating to date charred artifacts (or any artifacts containing carbon). A growing use of chemistry in archaeology is organic residue analysis. In organic residue analysis, scientists take samples of pottery (or other materials), and process them to determine what compounds are present in the remaining residue. At times, residue is visibly caked on the surface, while at other times, residue is captured within the pores of the item. Typically, chemists want to know the concentrations of organic compounds present in residues and use a gas-chromatography-mass spectrometry system to analyze the samples in great detail. For the purposes of this lab, the process of analysis will be simplified to simply test for presence, not concentrations.

#### Pre-Lab Questions

1. What functional group is present in a fatty acid?
2. What functional groups are present in a starch?
3. What functional groups are present in lactose?
4. What is the chemical formula of lactose?
5. Once a scientist can determine the compounds present in the residue of a large piece of pottery, what conclusions do you think they can make?

#### Materials

|                          |                          |
|--------------------------|--------------------------|
| Corn starch              | Hot plate/hot water bath |
| Skim milk                | Test tube holders        |
| Olive oil                | Unknown solutions        |
| Deionized water          | Treated pottery sample   |
| Acetone                  | Mortar and pestle        |
| Benedict's Solution      | Metal scoop              |
| Iodine                   | DI water wash bottle     |
| Sudan IV Solution        | 6 test tube stoppers     |
| 18 test tubes            | Acetone wash bottle      |
| Test tube holder (metal) |                          |
| Wax pencil               |                          |
| Stirring rod             |                          |
| Disposable pipets        |                          |

## Procedure

### Determining Standard Results

1. Mark 6 medium test tubes as shown below.

| Test Tube 1 | Test Tube 2 | Test Tube 3 | Test Tube 4 | Test Tube 5 | Test Tube 6 |
|-------------|-------------|-------------|-------------|-------------|-------------|
| CS – W 1    | CS – W 2    | CS – W 3    | CS – A 4    | CS – A 5    | CS – A 6    |

*The following notations will be used for this lab:*

CS = corn starch

W = water

M = milk

A = acetone

O = olive oil

1-6 = test tube number

2. Add 1 scoop of cornstarch to the test tubes.
3. In the 3 test tubes marked “W,” students should add water until the test tube is about  $\frac{3}{4}$  full. In the 3 test tubes marked “A,” students should add acetone until the test tube is about  $\frac{3}{4}$  full.
4. One member of the lab group can use a stirring rod to dissolve the solutes in solvent while the remaining members move on in the procedure. Stirring rod should be rinsed between test tubes with the appropriate solvent (meaning: rinse with water between “W” tubes, rinse with acetone before putting stirring rod into “A” tubes).
5. Mark 6 more medium test tubes as shown below.

| Test Tube 1 | Test Tube 2 | Test Tube 3 | Test Tube 4 | Test Tube 5 | Test Tube 6 |
|-------------|-------------|-------------|-------------|-------------|-------------|
| M – W 1     | M – W 2     | M – W 3     | M – A 4     | M – A 5     | M – A 6     |

6. Add roughly 3-5 mL of milk to the test tubes (using a disposable pipet).
7. In the 3 test tubes marked “W,” students should add water until the test tube is about  $\frac{3}{4}$  full. In the 3 test tubes marked “A,” students should add acetone until the test tube is about  $\frac{3}{4}$  full.
8. One member of the lab group can use a stirring rod to dissolve the solutes in solvent while the remaining members move on in the procedure. Stirring rod should be rinsed between test tubes with the appropriate solvent (meaning: rinse with water between “W” tubes, rinse with acetone before putting stirring rod into “A” tubes).
9. Mark 6 more medium test tubes as shown below.

| Test Tube 1 | Test Tube 2 | Test Tube 3 | Test Tube 4 | Test Tube 5 | Test Tube 6 |
|-------------|-------------|-------------|-------------|-------------|-------------|
| O – W 1     | O – W 2     | O – W 3     | O – A 4     | O – A 5     | O – A 6     |

10. Add roughly 3-5 mL of olive oil to the test tubes (using a disposable pipet).
11. In the 3 test tubes marked “W,” students should add water until the test tube is about  $\frac{3}{4}$  full. In the 3 test tubes marked “A,” students should add acetone until the test tube is about  $\frac{3}{4}$  full.
12. One member of the lab group can use a stirring rod to dissolve the solutes in solvent while the remaining members move on in the procedure. Stirring rod should be rinsed between test tubes with the appropriate solvent (meaning: rinse with water between “W” tubes, rinse with acetone before putting stirring rod into “A” tubes).

**Benedict's Test**

13. All test tubes labeled with the number 1 and 4 (CS – W 1, CS – W 4, M – W1, M – W4, O – W1, O – W4) will undergo Benedicts Test.
14. Set up a hot water bath using a hot plate. Have a member of the lab group monitor the hot water bath. Water should be lightly boiling and water level should NOT be higher than the height of the test tubes.
15. Add roughly 5mL of Benedict's solution to all test tubes labeled with the number 1 and 4. Solution should be added to 6 test tubes total.
16. Once the water is lightly boiling, use a test tube holder to move the six test tubes to the water bath. Leave the test tubes in the water bath for 4-5 minutes. Observe any color changes. Turn off hot plate after 5 minutes.
17. Remove test tubes using test tube holder. Write observations on data sheet. DO NOT place test tubes in plastic test tube holder. Use metal or wooden holder, or place back in water bath after observation.

**Starch Test**

18. All test tubes labeled with the number 2 and 5 (CS – W 2, CS – W 5, M – W2, M – W5, O – W2, O – W5) will undergo Starch Test.
19. Add roughly 5 mL of iodine to all test tubes labeled with the number 2 and 5. Iodine should be added to 6 test tubes.
20. Observe any color changes and record on data sheet.

**Sudan IV Test**

21. All test tubes labeled with the number 3 and 6 (CS – W 3, CS – W 6, M – W3, M – W6, O – W3, O – W6) will undergo Sudan IV Test.
22. Add 5-10 drops of Sudan IV solution to all test tubes labeled with the number 3 and 6. Sudan IV should be added to 6 test tubes.
23. Observe any color changes and record on data sheet.

**Determining Substances in Unknown Samples**

24. Clean out all test tubes with negative results. Keep samples of positive results for each test.
25. Ask your instructor for your group's unknown and pottery sample.
26. Label 12 cleaned test tubes as indicated below, filling in “#” with your group's sample numbers.

|             |             |             |             |             |             |
|-------------|-------------|-------------|-------------|-------------|-------------|
| Test Tube 1 | Test Tube 2 | Test Tube 3 | Test Tube 4 | Test Tube 5 | Test Tube 6 |
| U# - W1     | U# - W2     | U# - W3     | U# - A4     | U# - A5     | U# - A6     |

|             |             |             |              |              |              |
|-------------|-------------|-------------|--------------|--------------|--------------|
| Test Tube 7 | Test Tube 8 | Test Tube 9 | Test Tube 10 | Test Tube 11 | Test Tube 12 |
| P# - W1     | P# - W2     | P# - W3     | P# - A4      | P# - A5      | P# - A6      |

The following notations will be used for this lab:

W = water

A = acetone

U# = unknown \_\_\_\_\_ (each group will be given a different numbered unknown)

P# = pottery \_\_\_\_\_ (each group will be given a different numbered pottery sample)



27. Add roughly 3-5 mL of the unknown solutions to the test tubes marked with "U."
28. Using a mortar and pestle, grind up the pottery sample.
29. Add 1 scoop of the finely ground pottery to each of the test tubes marked with "P."
30. Following the earlier procedures from determining standards, test all the test tubes marked 1 and 4 with Benedict's Test. **\*\* Note: After adding solvent to pottery test tubes, cap them with a stopper and shake vigorously for 1 minute. Then continue by adding the Benedict's solution.**
31. Record results in data table.
32. Following the earlier procedures from determining standards, test all the test tubes marked 2 and 5 with Starch Test. **\*\* Note: After adding solvent to pottery test tubes, cap them with a stopper and shake vigorously for 1 minute. Then continue by adding the iodine.**
33. Record results in data table.
34. Following the earlier procedures from determining standards, test all the test tubes marked 3 and 6 with Sudan IV Test. **\*\* Note: After adding solvent to pottery test tubes, cap them with a stopper and shake vigorously for 1 minute. Then continue by adding the Sudan IV solution.**
35. Record results in data table.
36. Dispose of materials as directed and clean up glassware. DO NOT touch hot plate until it has cooled.

## Conclusions

Write up a brief lab report (three paragraphs) in the following format. Turn in typed lab report with data sheet attached. Assignment will be graded.

### Introduction

- What did this lab seek to accomplish?
- What should each test tell you?
- What does a positive result for each test look like?

### Data

#### Standards

- For each test, which substances gave positive results?
- Describe appearance of positive results and any difference from expected appearance.
- Which solvent worked best?

#### Unknowns

- Which of the following was present in your unknown solution: lactose, starch, and/or fatty acids?
- Give data to support your conclusions.
- Which of the following was present in your pottery sample: lactose, starch, fatty acids, or none?
- Give data to support your conclusions.

## Conclusion

- Summarize your findings for the unknown and pottery sample.
- Can the composition of pottery residues be conclusively determined by the basic analysis conducted in this lab?
- What problems might a scientist anticipate with archaeological samples (unlike the modern samples present in this lab)?

Name: \_\_\_\_\_  
Organic Residues Data Sheet

Date: \_\_\_\_\_

### Determining Standards

Fill in Test Tube IDs as indicated in Procedure. Record observations in the appropriate box (including any color change) and indicate any positive test by checking the box.

| Test Tube ID | Benedict's Test                   | Iodine Test                       | Sudan IV Test                     |
|--------------|-----------------------------------|-----------------------------------|-----------------------------------|
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |

### Determining Substances in Unknown Samples

Fill in Test Tube IDs as indicated in Procedure. Record observations in the appropriate box (including any color change) and indicate any positive test by checking the box.

| Test Tube ID | Benedict's Test                   | Iodine Test                       | Sudan IV Test                     |
|--------------|-----------------------------------|-----------------------------------|-----------------------------------|
|              | <input type="checkbox"/> positive | <input type="checkbox"/> positive | <input type="checkbox"/> positive |

