AMA Analytical Services, Inc.
Focused On Results.

CERTIFICATE OF ANALYSIS

Chain of Custody: 625947
Client: US Food & Drug Administration
Address: Office of Cosmetics & Colors
4300 River Road
College Park, MD 20740
Attention: John Gasper

Job Name: Assignment DFPG #21-18
Job Location: Batch No. 02232021B (Batch #2B)
Job Number: CLIN 0001
PO Number: 75F40119P10689

Date Submitted: 3/29/2021
Date Analyzed: 4/29/2021-4/30/2021
Report Date: 5/20/2021
Date Sampled: Not Provided
Person Submitting: Martha Schwartz
Revised: 5/21/2021 (Revision #1)

SUMMARY OF ANALYSIS

AMA Sample ID	Client Sample ID	TEM LOD Using ASTM D5756 Mass Calculation	TEM LOQ Using ASTM D5756 Mass Calculation	% Chrysotile by TEM Using ASTM D5756 Mass Calculation	% Tremolite by TEM Using ASTM D5756 Mass Calculation	% Total Chrysotile & Tremolite by TEM Using ASTM D5756 Mass Calculation	% Asbestos by PLM	% Organics	% Acid Soluable	% Other	Comments
625947-1A	02232021-12	0.00000179%	0.00000717%	ND	ND	< 0.00001%	ND	27.39%	5.98%	66.63%	
625947-1B	02232021-12	0.00000253%	0.00001013%	ND	ND	< 0.00001%	ND	27.68%	10.78%	61.55%	
625947-1C	02232021-12	0.00000165%	0.00000661%	ND	ND	< 0.00001%	ND	27.35%	5.50%	67.15%	
625947-2A	02232021-13	0.00000246%	0.00000983%	ND	ND	< 0.00001%	ND	27.08%	8.76%	64.16%	
625947-2B	02232021-13	0.00000251%	0.00001006%	ND	ND	< 0.00001%	ND	27.17%	10.15%	62.68%	
625947-2C	02232021-13	0.00000357%	0.00001427%	ND	ND	< 0.00001%	ND	27.30%	9.17%	63.53%	

LOD = Limit of Detection **LOQ** = Limit of Quantification **ND** = Not Detected **PLM** = Polarized Light Microscopy **TEM** = Transmission Electron Microscopy

Analytical Method(s): PLM by Modified NY ELAP 198.6
TEM by Modified NY ELAP 198.4/ASTM D5756

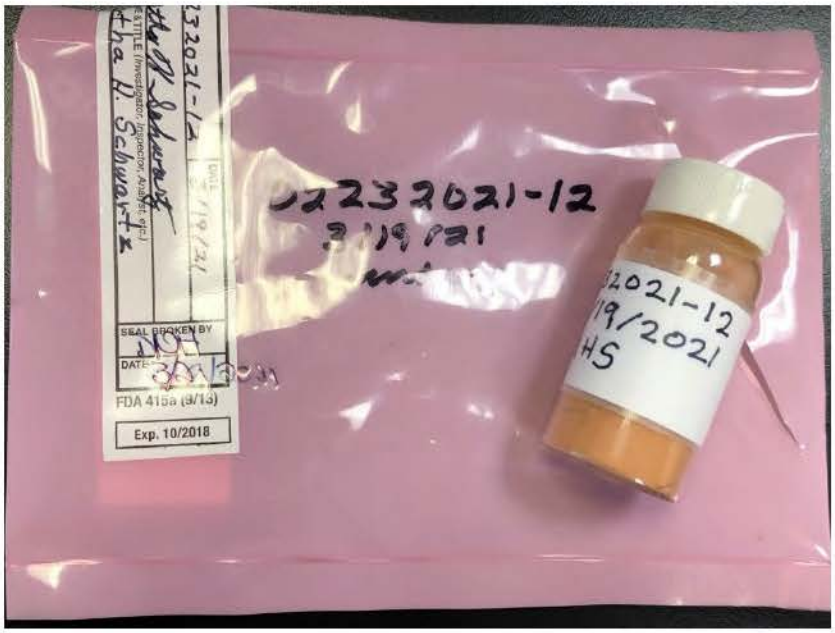
Analyst(s): **PLM** (b)(6)
TEM (b)(6) Andreas Saldivar

(b)(6)

Technical Director: (b)(6)

All results are to be considered preliminary and subject to change unless signed by the Technical Director or Deputy

625947-1A, 1B, 1C/02232021-12



625947-2A, 2B, 2C/02232021-13

Sample Preparation

Samples were gravimetrically reduced and filtered by (b)(6) on: April 23, 2021 through April 28, 2021. PLM slide preparations were made by (b)(6) on April 28, 2021. TEM grid preparations were made by (b)(6) on April 28, 2021. Sample preparation consisted of the following steps:

1) Label and weigh two 8mL glass vials for each sample in the set – one vial for the PLM preparation and one vial for the TEM preparation.
2) Weigh out 0.1 to 0.8-grams of material and place in the corresponding 8mL glass vial. Record weight.
3) Burn samples at 480° C for at least 12-hours.
4) Record Post-Ash weight.
5) Treat ashed sample with reagent grade hydrochloric acid.
6) Filter acid reduced material with a pre-weighed disposable filtration apparatus onto a 47mm 0.4µm PolyCarbonate filter.
7) Place disposable filtration apparatus with filter into drying oven for 3 hours and then record Post-Acid Reduced weight.
8) Make four PLM slide preparations from the PLM residue for each sample in 1.550 dispersion oil. Make additional preparations in 1.605, 1.625, 1.680 and 1.700 dispersion oil(s) as necessary for particle identification.
9) Weigh a portion of the material from the TEM residue and place it into the corresponding pre-weighed 100mL jar.
10) Fill the 100mL jar with deionized water
11) Sonicate the jar for ~5-minutes.
12) Filter 0.1mL to 2mL of the solution onto a 47mm 0.22µm MCE filter.
13) Dry the filter for ~10-minutes then collapse, carbon coat, and place on a 3 TEM grids.

TEM grid preparations were examined prior to analysis and were rejected if they meet the following criteria:

1) Less than 50% of the carbon coating was intact
2) The grid was too dark due to incomplete dissolution of the filter
3) Heavy particulate loading in excess of 25%
4) Light particulate loading below 10%
5) Uneven distribution of particulate

Problems Encountered During Preparation & Resolutions:

No problems were encountered during sample preparation. All gravimetric data was consistent among each group of aliquots and all TEM grid preparations were deemed acceptable for analysis.

PLM Analysis

Analysis was performed in accordance with NY ELAP 198.6 protocols. The analysis was conducted using an Olympus BH-2 polarized light microscope (PLM) equipped with a dispersion staining objective. All four slide preparations for each aliquot were examined; each slide preparation consisted of two (2) coverslips for a total of eight (8) coverslips. 400-point count was performed for those samples on which asbestos was observed. If no asbestos was detected on any of the slides, the percentage of fibrous components was determined by visual estimation. The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

Point Counting

If asbestos was observed on the slide preparations, the amount of asbestos was quantified using point count techniques. Point counting is form of quantifying PLM samples. One of the oculars of each PLM microscope is etched with a crosshair. When point counting, whatever is under the crosshair is counted as one point of whatever the material is. Four (4) slide preparations with a total of eight (8) coverslips are prepared for each sample. The microscope mechanical stage is used to randomly move the slide. After each movement, whatever is under the crosshair, provided the point is not empty, is counted. Fifty (50) non-empty points are counted on each of the eight (8) coverslips for a total of four hundred (400) points. The total asbestos points counted are divided by the total points counted to calculate the percentage.

Example:

11 points of asbestos were counted out of the 400 total points

Slide percentage = (11pts/400pts) * 100%
Slide percentage = 2.75%

This number is not the final asbestos percentage. To calculate the final percentage, this number must be corrected to account for the material lost during gravimetric reduction preparation. See the *Calculations* section below for additional details.

TEM Analysis

Analysis was performed in accordance with modified NY ELAP Method 198.4 protocols. The analysis was performed using JEOL JEM-100CX II and JEOL JEM-100CX transmission electron microscopes (TEM) equipped with Thermo Fisher NSS System 7 Energy Dispersive X-Ray Analyzers (EDXA), at magnifications of 19,000x – 20,000x. All TEM scopes are equipped with a Selective Area Electron Diffraction (SAED) setting that allows the operator to view the diffraction pattern of any mineral substance. Twenty (20) grid openings over two (2) grids were examined for each aliquot.

Modifications to the NY ELAP 198.4 Method were:

1) The residue was not placed in alcohol and prepared using the quick drop method. To obtain a more uniform preparation, the residue was placed in a jar and filled with 100mL of deionized water. The jar was sonicated, and a portion of the solution was filtered onto a 47mm 0.22µm MCE filter.
2) Any amphibole or chrysotile particle(s) observed were not quantified by visual estimation. The length and width of the observed particle(s) were measured, and the mass of each amphibole and chrysotile particle was calculated using the ASTM D5756 method.
3) All particles identified as amphibole were included with the counts/concentrations, regardless of size and aspect ratio.

The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

Calculations

ASTM D5756 Mass:

$M = \pi/4\ L * W^2 * D * 10^{-12}$

Where: M: Mass
L: Length
W: Width
D: Density

Gravimetric Reduction Loss Percentages:

Organic: ((W1 – W2) * 100)/W1
Acid Soluble: ((W2 – W3) * 100)/W1
Other*: ((W3/W1) * 100) – Calculated Asbestos %

*Other is defined as the non-asbestos, inorganic, acid insoluble portion of the sample

Where: W1: Weight of sample prior to ashing/acid wash
W2: Weight of sample after ashing
W3: Weight of sample after acid treatment

Asbestos Percent Calculations:

TEM

$$\frac{EFA(mm^2) * 100ml * MA(g) * RW(g)}{VF(ml) * IW(g) * AA(mm^2) * RJ(g)}$$

(The calculated value is then multiplied by 100 to convert it to percent)

Where: EFA: Effective filter area
MA: Mass of asbestos
RW: Weight of residue
VF: Volume filtered
IW: Initial weight of the sample
AA: Area analyzed
RJ: Weight of residue placed into the jar

PLM

(ASB * W3)/W1

Where: W1: Weight of sample prior to ashing/acid wash
W3: Weight of sample after acid treatment
ASB: Calculated Point Count Result

Note: All reported concentrations were calculated using the gravimetric data from the TEM preparations.

Limit of Detection and Quantification

We used the mass of a 0.5 x 0.04-micron tremolite fiber as the basis for our calculations. Limit of detection (LOD) was defined as 1 fiber and limit of quantification (LOQ) was defined as 4 fibers.

Discussion and Interpretation of Analytical Findings:

625947-1A, 1B, 1C/Client Sample: 02232021-12

PLM

All three aliquots of sample 02232021-12 were analyzed by (b)(6) on April 30, 2021. No asbestos or non-asbestos amphibole variants were observed during analysis. The results were calculated using the equations detailed in the *Calculations* section above.

625947-1A	No Asbestos Detected
625947-1B	No Asbestos Detected
625947-1C	No Asbestos Detected

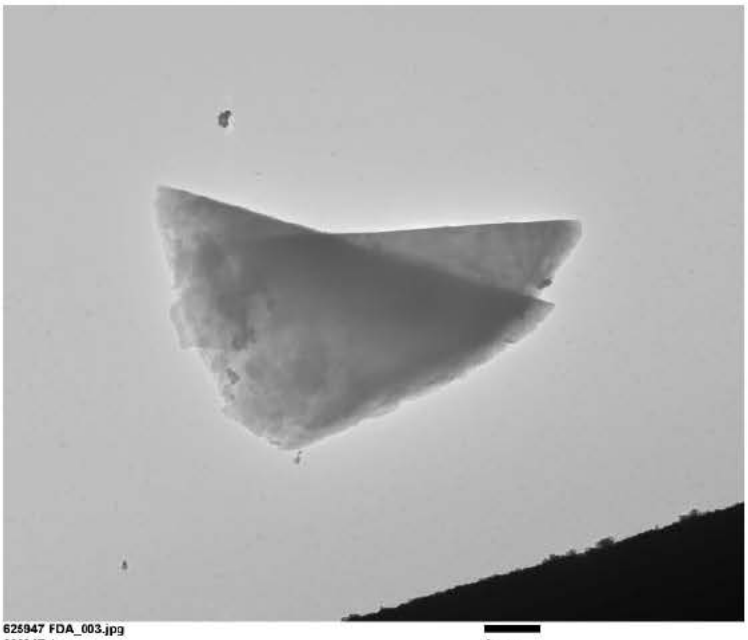
TEM

(b)(6) analyzed aliquot 1A April 29, 2021. Andreas Saldivar analyzed aliquots 1B and 1C on April 29, 2021. The primary particle observed was talc; barium sulfate particles were also observed along with some silica spheres and a few iron particles and talc fibers. No asbestos or non-asbestos amphibole variants were observed during analysis. The results were calculated using the equations detailed in the *Calculations* section above.

625947-1A	No Asbestos Detected
625947-1B	No Asbestos Detected
625947-1C	No Asbestos Detected

Below are pictures, diffraction patterns, and chemistry from some of the observed particles. The copper and carbon peaks in the chemistry spectra are from the TEM grid. The zinc peak in the chemistry spectra is from the TEM specimen holder.

Sample 625947-1A, Talc Particle

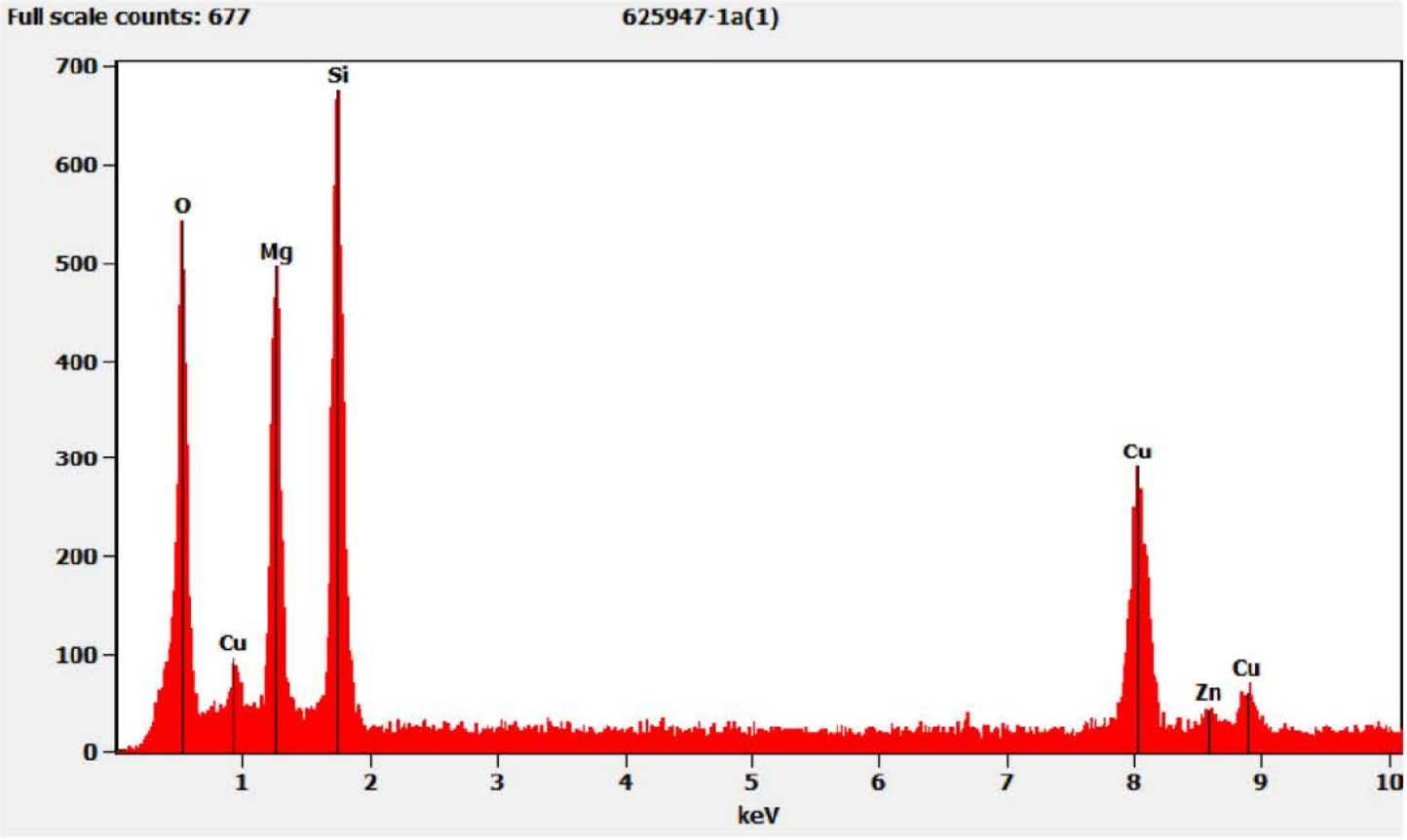


Hexagonal Diffraction Pattern from the Talc Particle pictured above

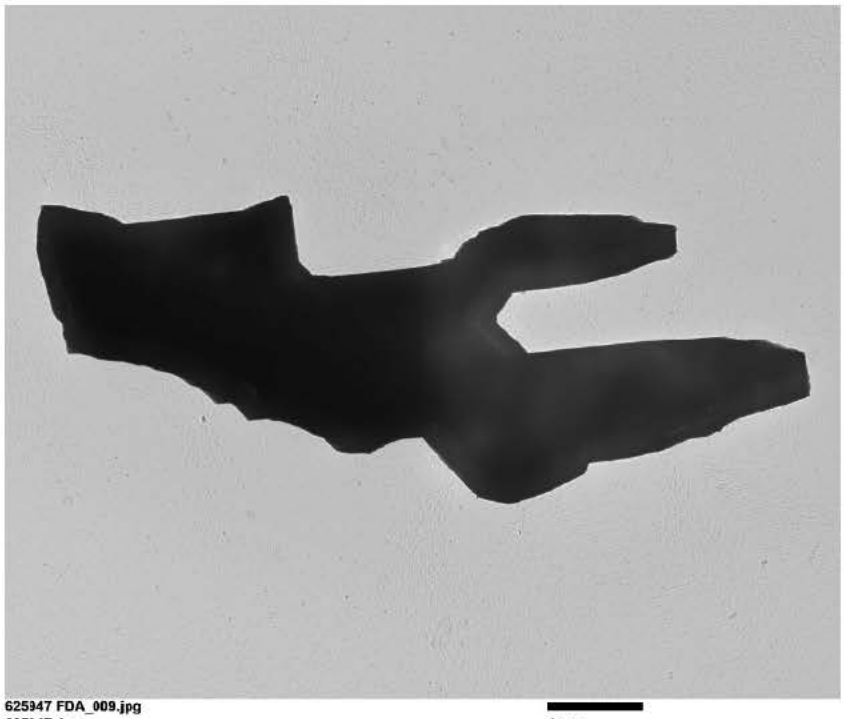
625947 FDA_002.jpg
625947-1a
Talc Particle
18:11 4/29/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from the Talc Particle pictured above



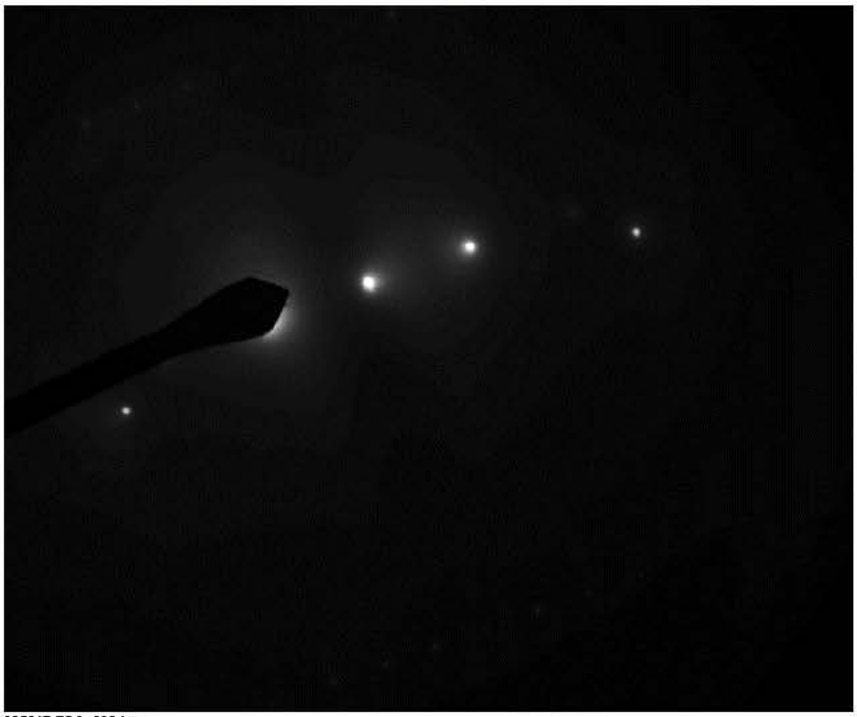
Sample 625947-1A, Barium Sulfate Particle



625947 FDA_009.jpg
625947-1a
Barium Sulfate Particle
Cal: 0.003548 µm/pix
18:38 4/29/2021
TEM Mode: Imaging
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

1 µm
HV=100kV
Direct Mag: 2900 x
AMA Analytical Services, Inc

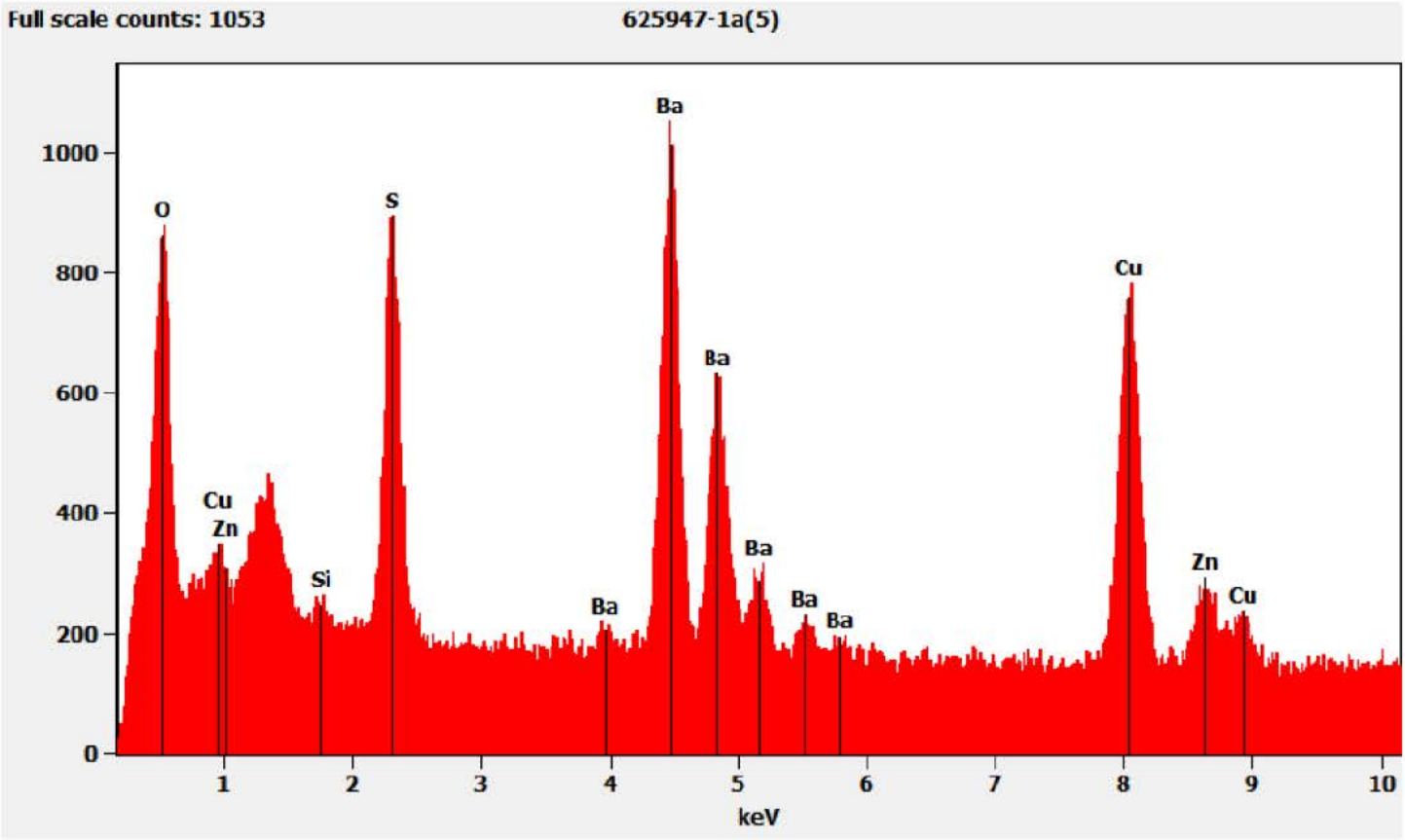
Diffraction Pattern from the Barium Sulfate Particle pictured above



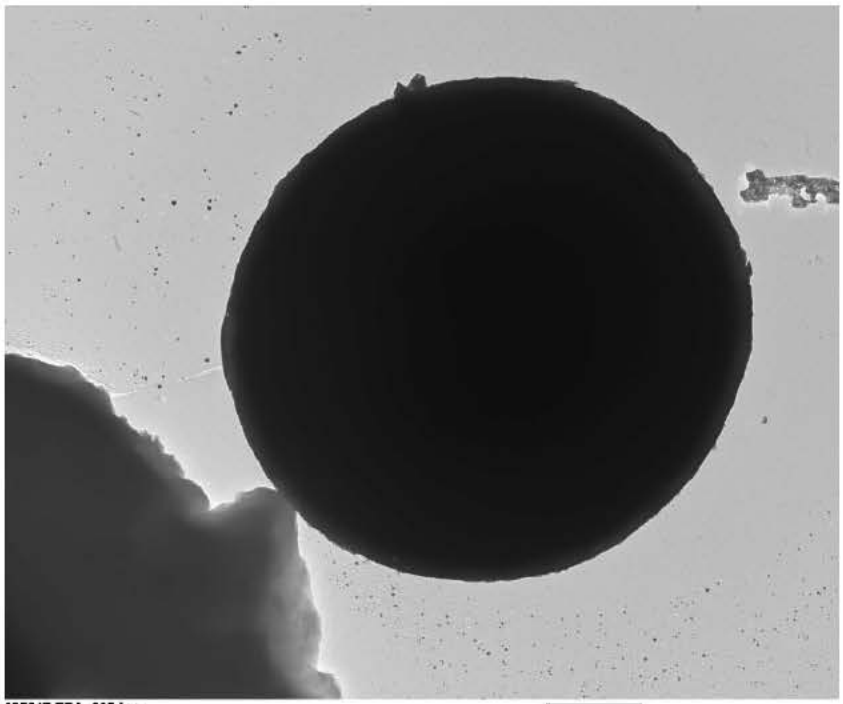
625947 FDA_008.jpg
625947-1a
Barium Sulfate Particle
18:37 4/29/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/A)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

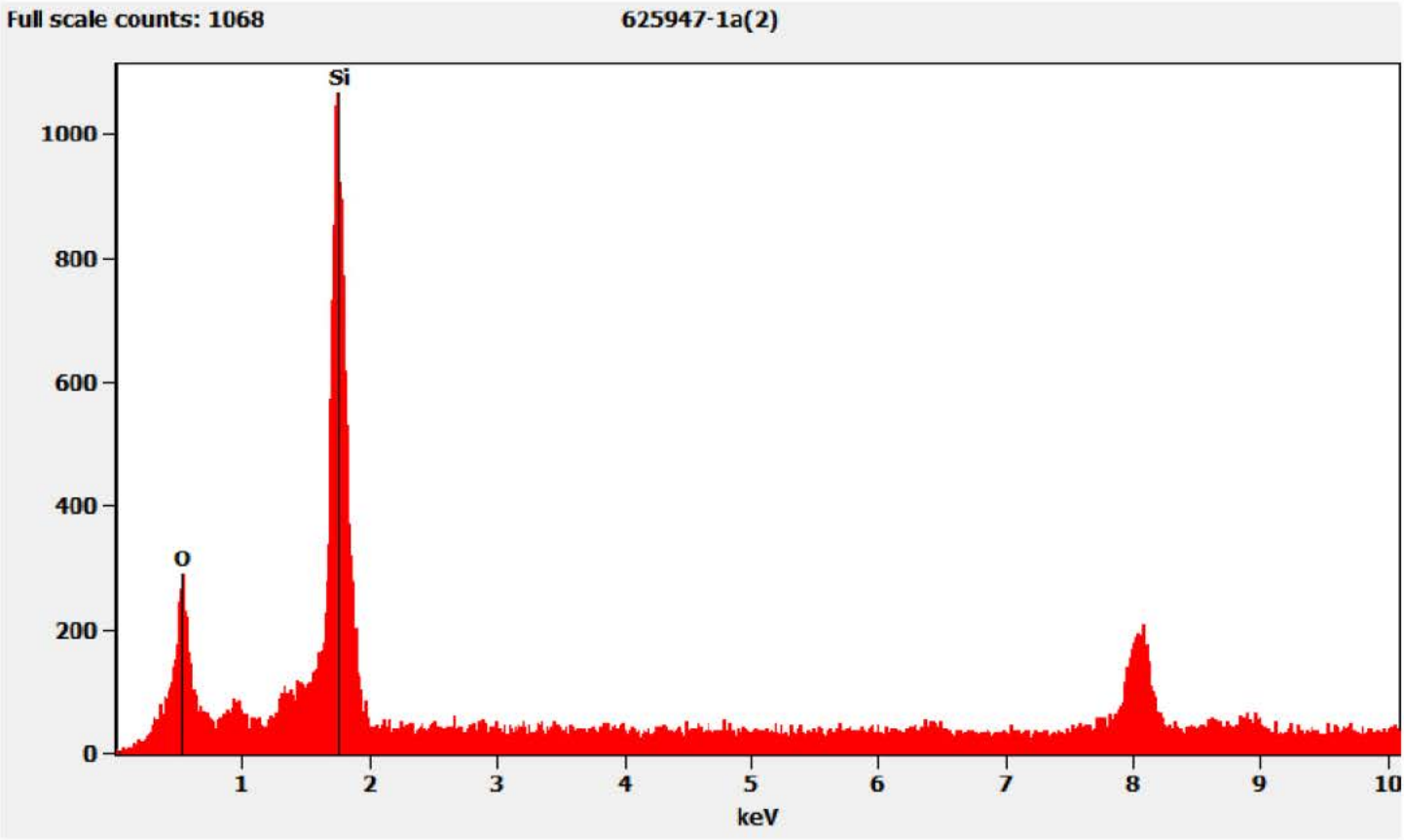
Chemistry from Barium Sulfate Particle pictured above



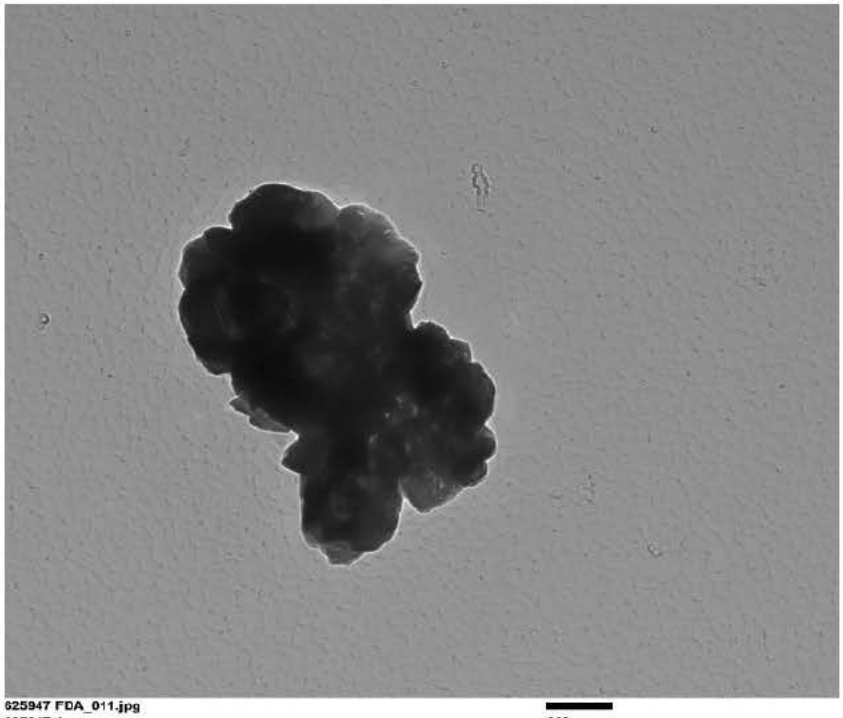
625947-1A, Silica Sphere



Chemistry from the Silica Sphere pictured above

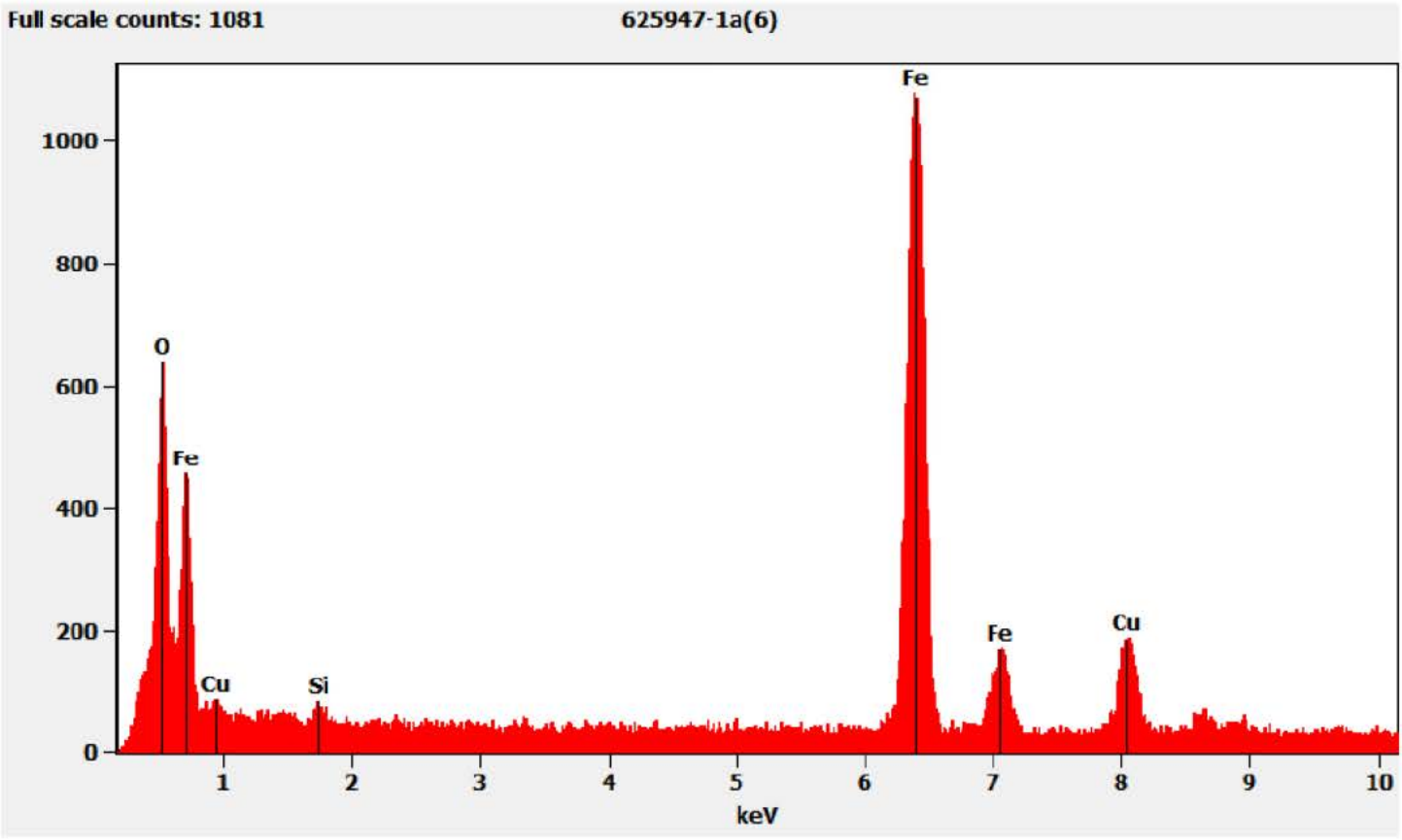


625947-1A, Iron Particles

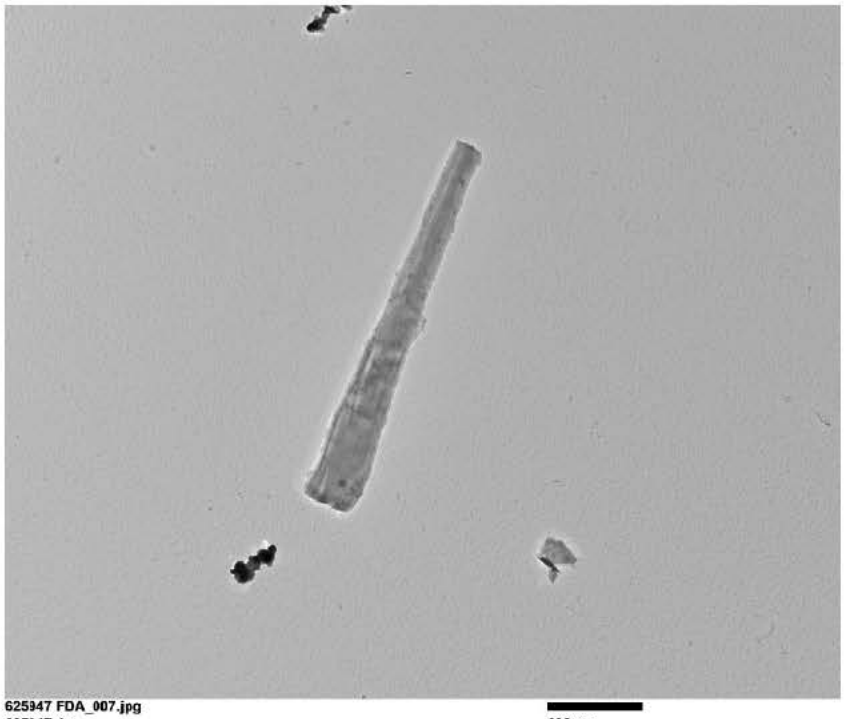


Diffraction Pattern from the Iron Particles pictured above

Chemistry from the Iron Particles pictured above



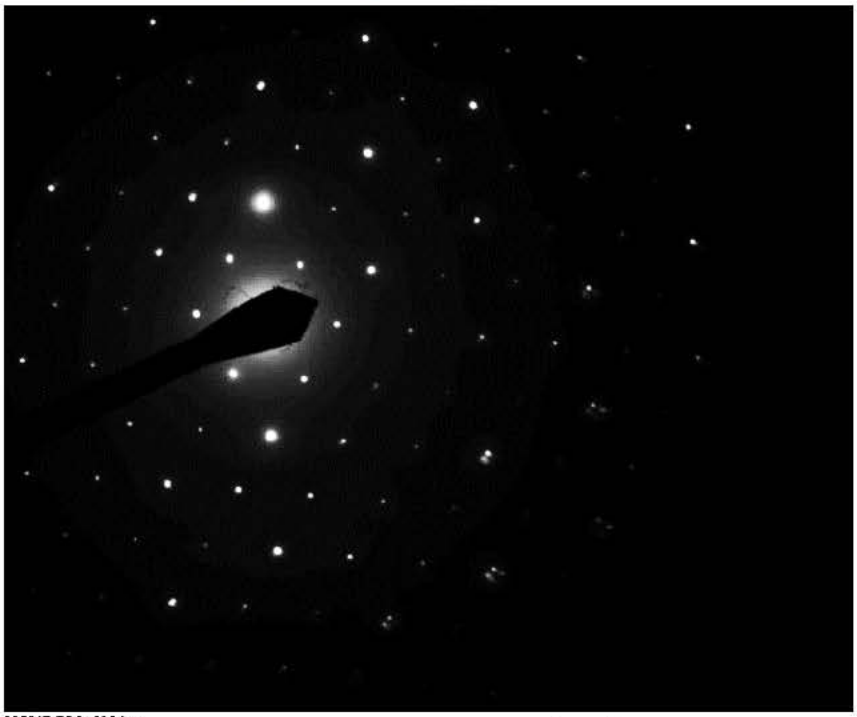
625947-1A, Talc Fiber



625947 FDA_007.jpg
625947-1a
Talc Fiber
Cal: 0.002858 µm/pix
18:31 4/29/2021
TEM Mode: Imaging
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

800 nm
HV=100kV
Direct Mag: 3600 x
AMA Analytical Services, Inc

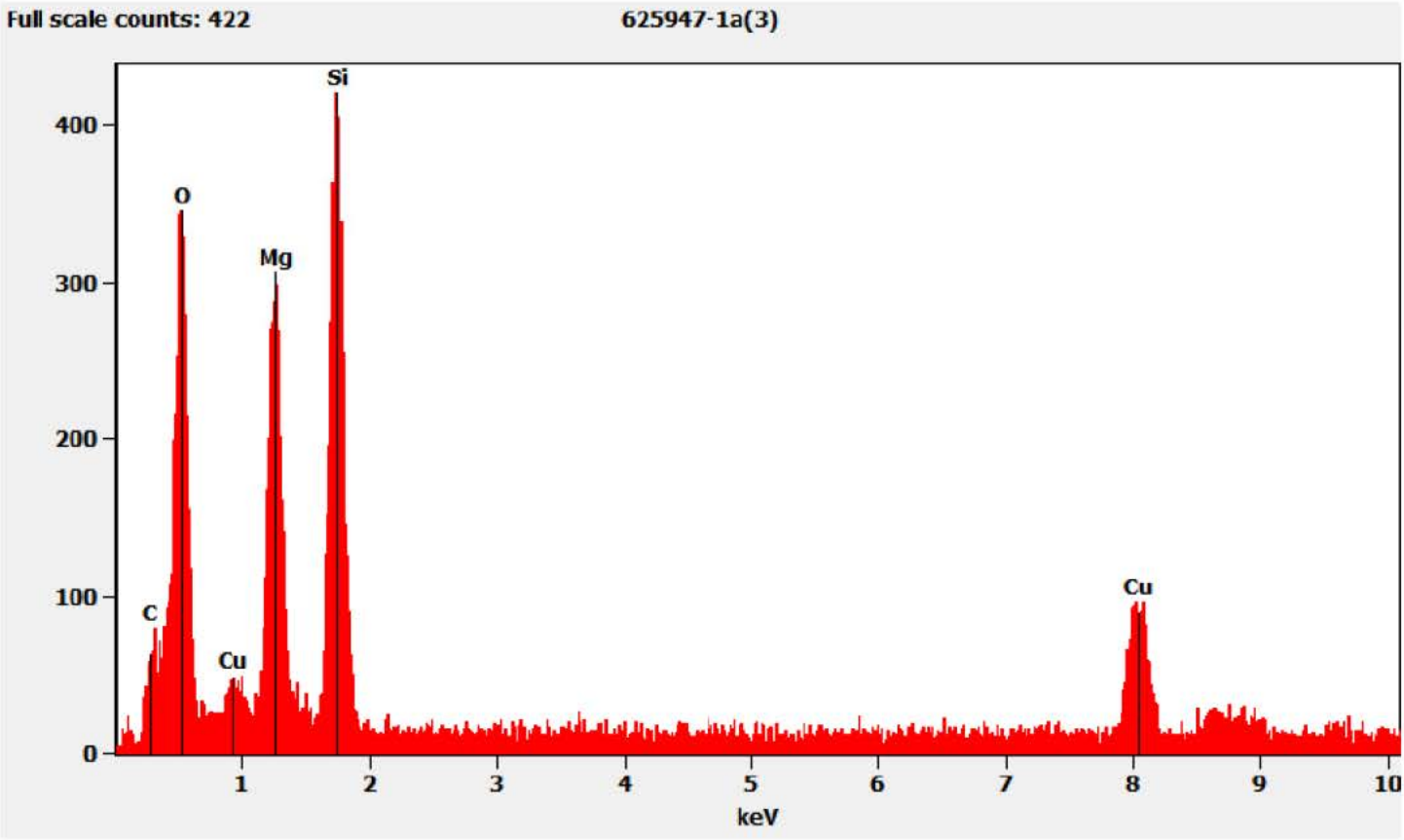
Hexagonal Diffraction Pattern from the Talc Fiber pictured above



625947 FDA_006.jpg
625947-1a
Talc Fiber
18:30 4/29/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 1/Å
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from the Talc Fiber pictured above



625947-2A, 2B, 2C/Client Sample: 02232021-13

PLM

All three aliquots of sample 02232021-13 were analyzed by (b)(6) on April 30, 2021. No asbestos or non-asbestos amphibole variants were observed during analysis. The results were calculated using the equations detailed in the *Calculations* section above.

625947-2A	No Asbestos Detected
625947-2B	No Asbestos Detected
625947-2C	No Asbestos Detected

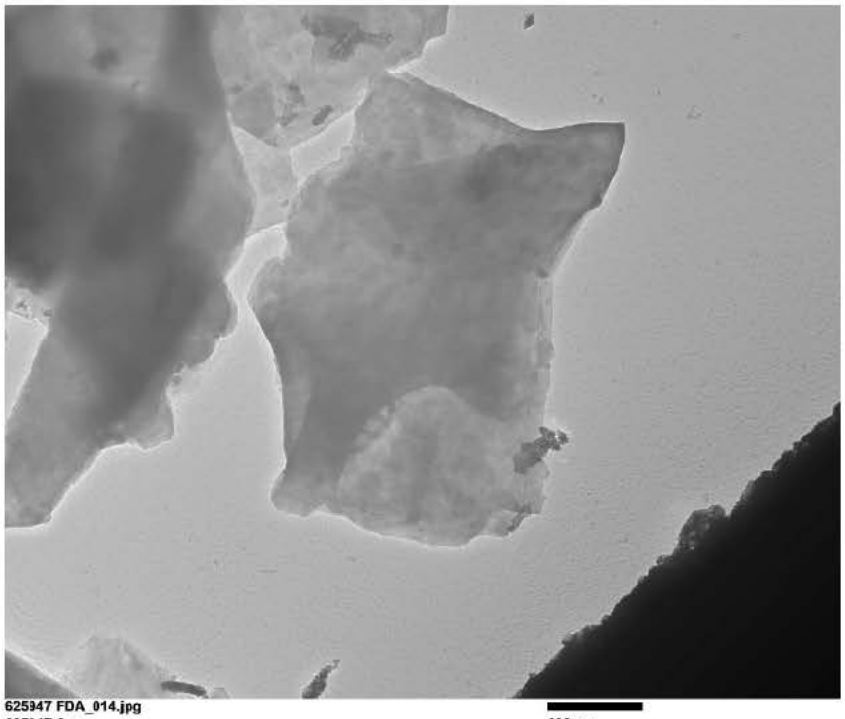
TEM

(b)(6) analyzed aliquot 2A on April 30, 2021. Andreas Saldivar analyzed aliquots 2B and 2C on April 30, 2021. The primary particle observed was talc; barium sulfate particles were also observed along with some silica spheres and a few iron particles, iron fibers and talc fibers. No asbestos or non-asbestos amphibole variants were observed during analysis. The results were calculated using the equations detailed in the *Calculations* section above.

625947-2A	No Asbestos Detected
625947-2B	No Asbestos Detected
625947-2C	No Asbestos Detected

Below are pictures, diffraction patterns, and chemistry from some of the observed particles. The copper and carbon peaks in the chemistry spectra are from the TEM grid. The zinc peak in the chemistry spectra is from the TEM specimen holder.

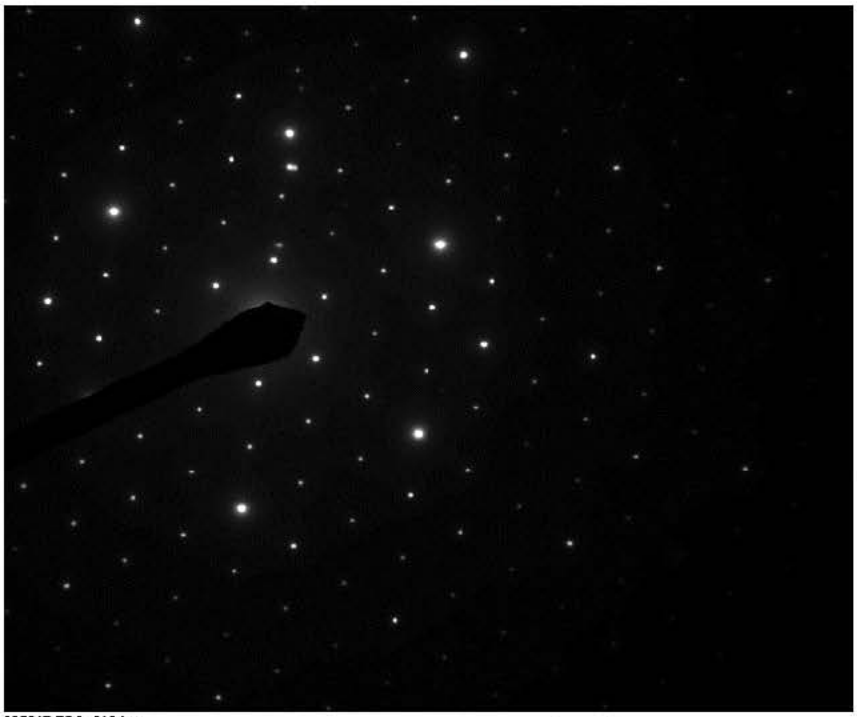
625947-2A, Talc Particle with Iron



625947 FDA_014.jpg
625947-2a
Talc Particle with Fe
Cal: 0.002858 µm/pix
10:44 4/30/2021
TEM Mode: Imaging
Microscopist:(b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

800 nm
HV=100kV
Direct Mag: 3600 x
AMA Analytical Services, Inc

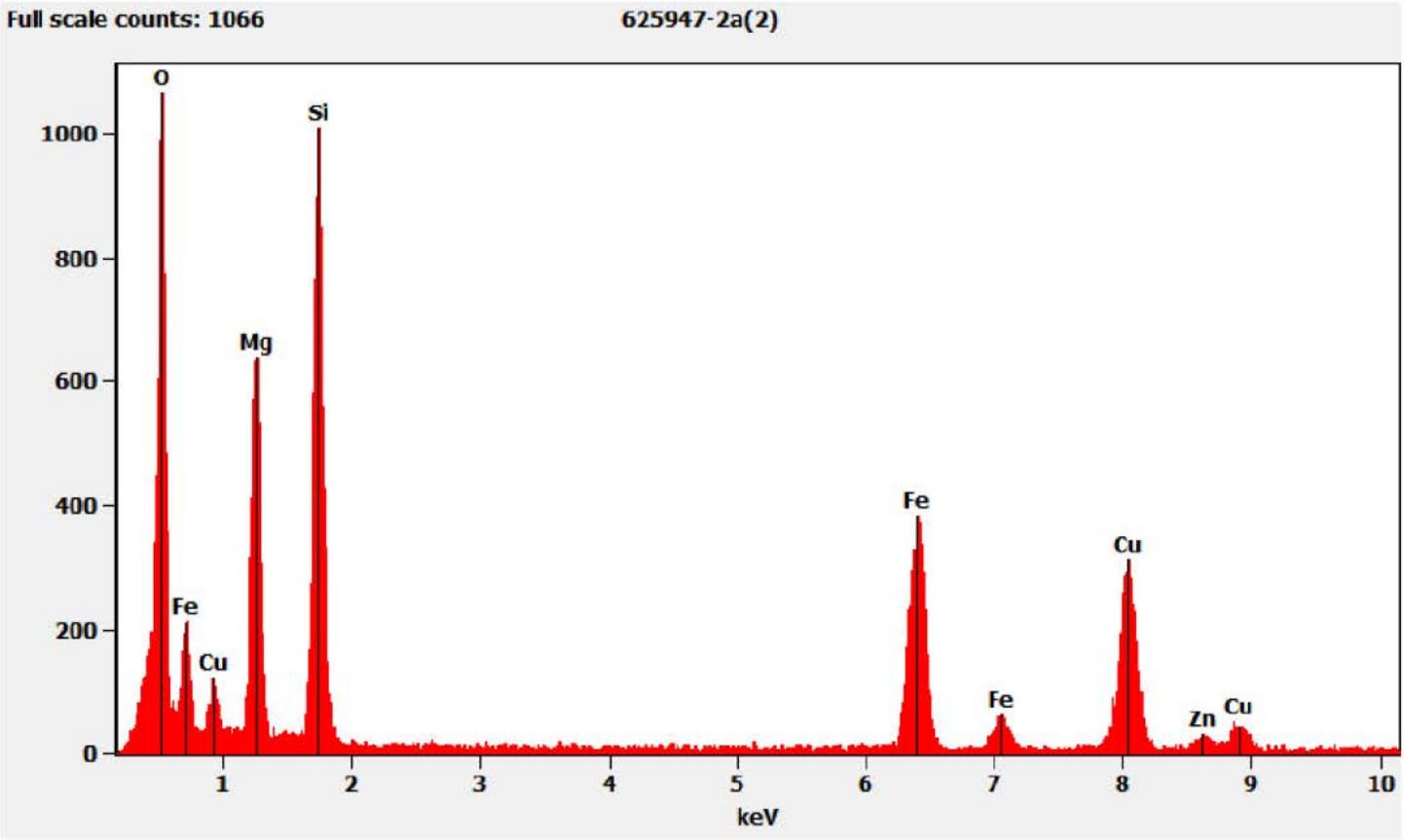
Hexagonal Diffraction Pattern from the Talc Particle pictured above



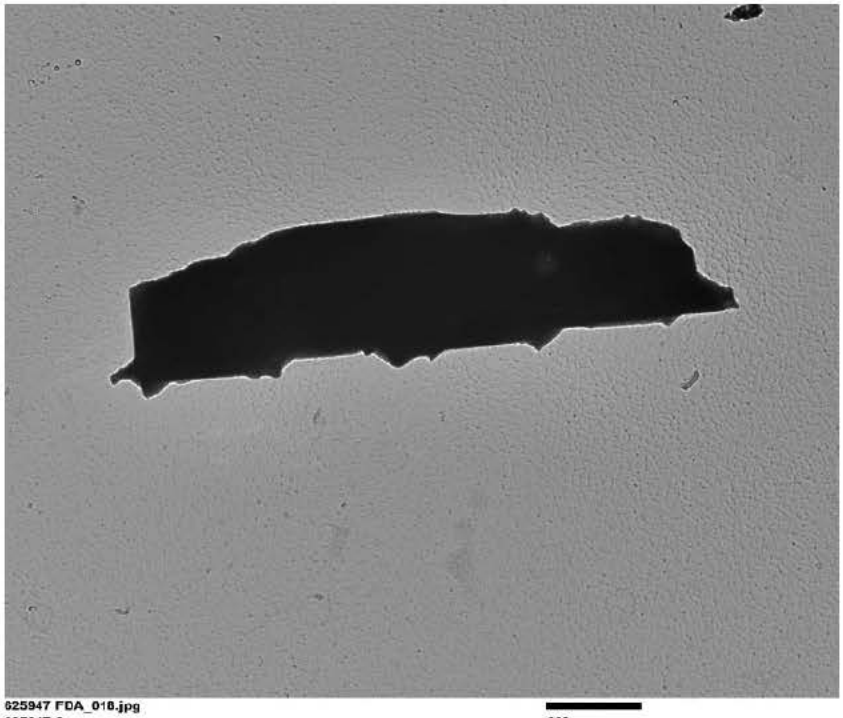
625947 FDA_013.jpg
625947-2a
Talc Particle with Fe
10:43 4/30/2021
TEM Mode: Diffraction
Microscopist:(b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/A)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from the Talc Particle pictured above



625947-2A, Barium Sulfate Particle

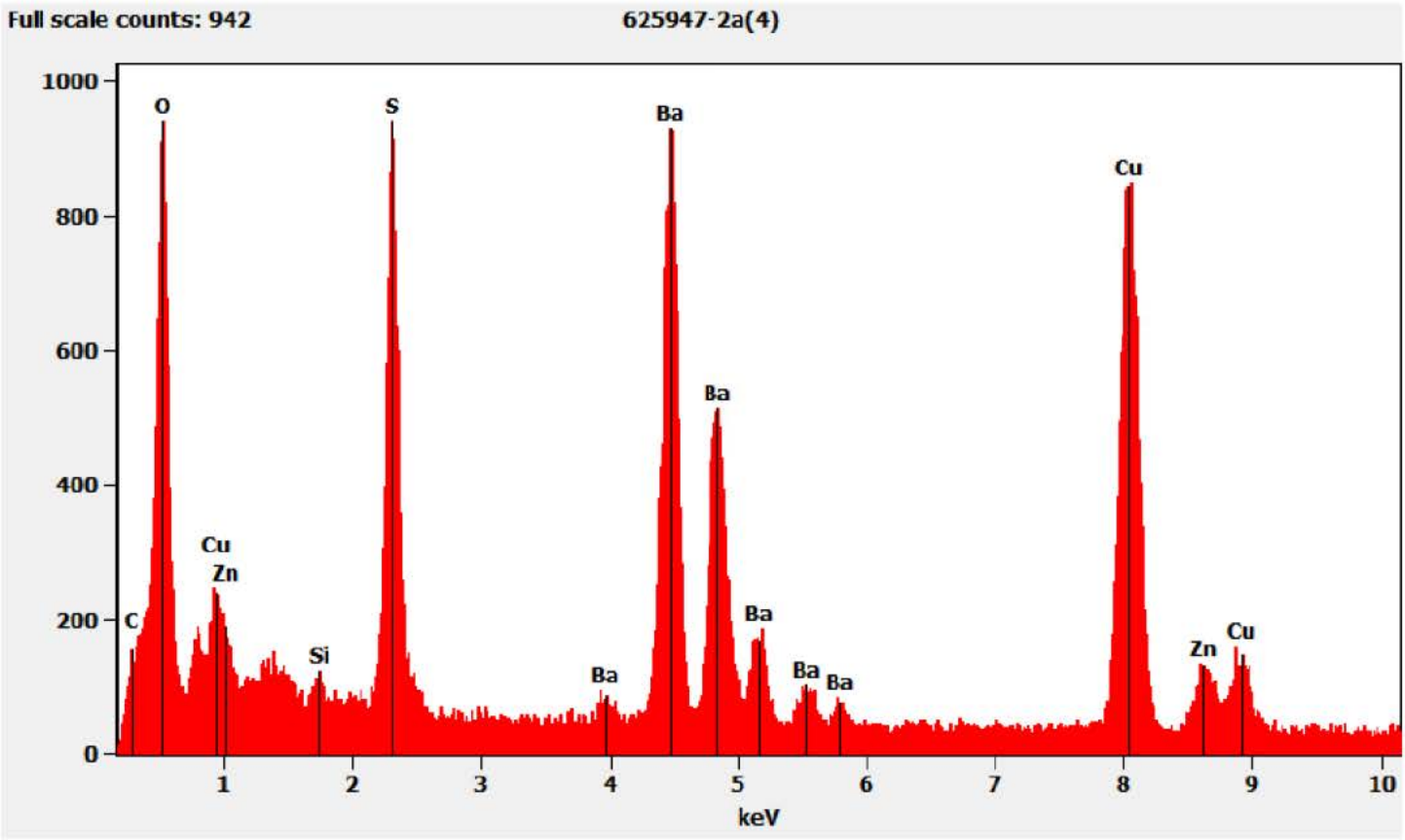


Hexagonal Diffraction Pattern from the Barium Sulfate Particle pictured above

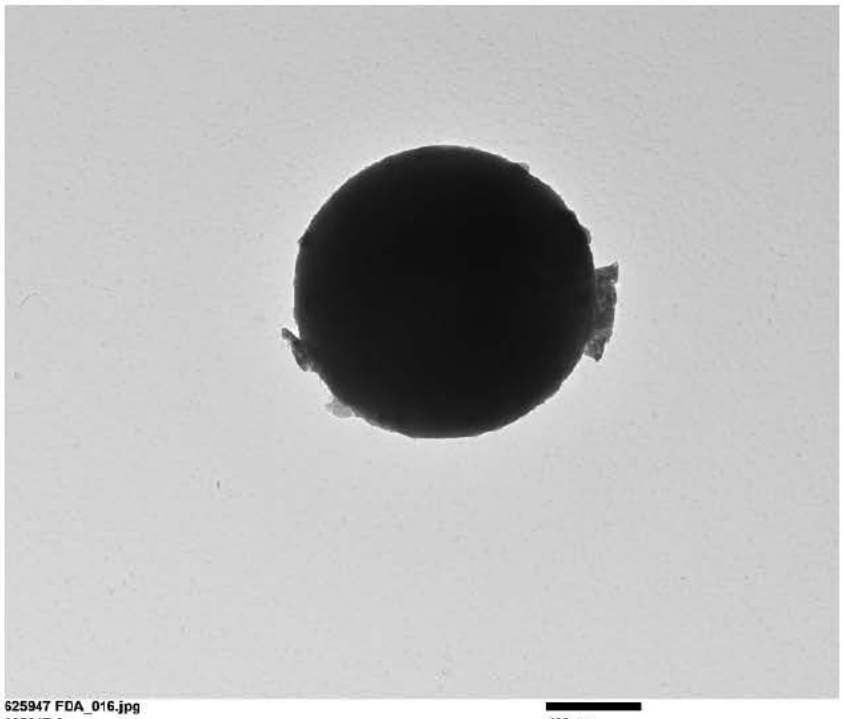
625947 FDA_017.jpg
625947-2a
Barium Sulfate
10:51 4/30/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from the Barium Sulfate pictured above



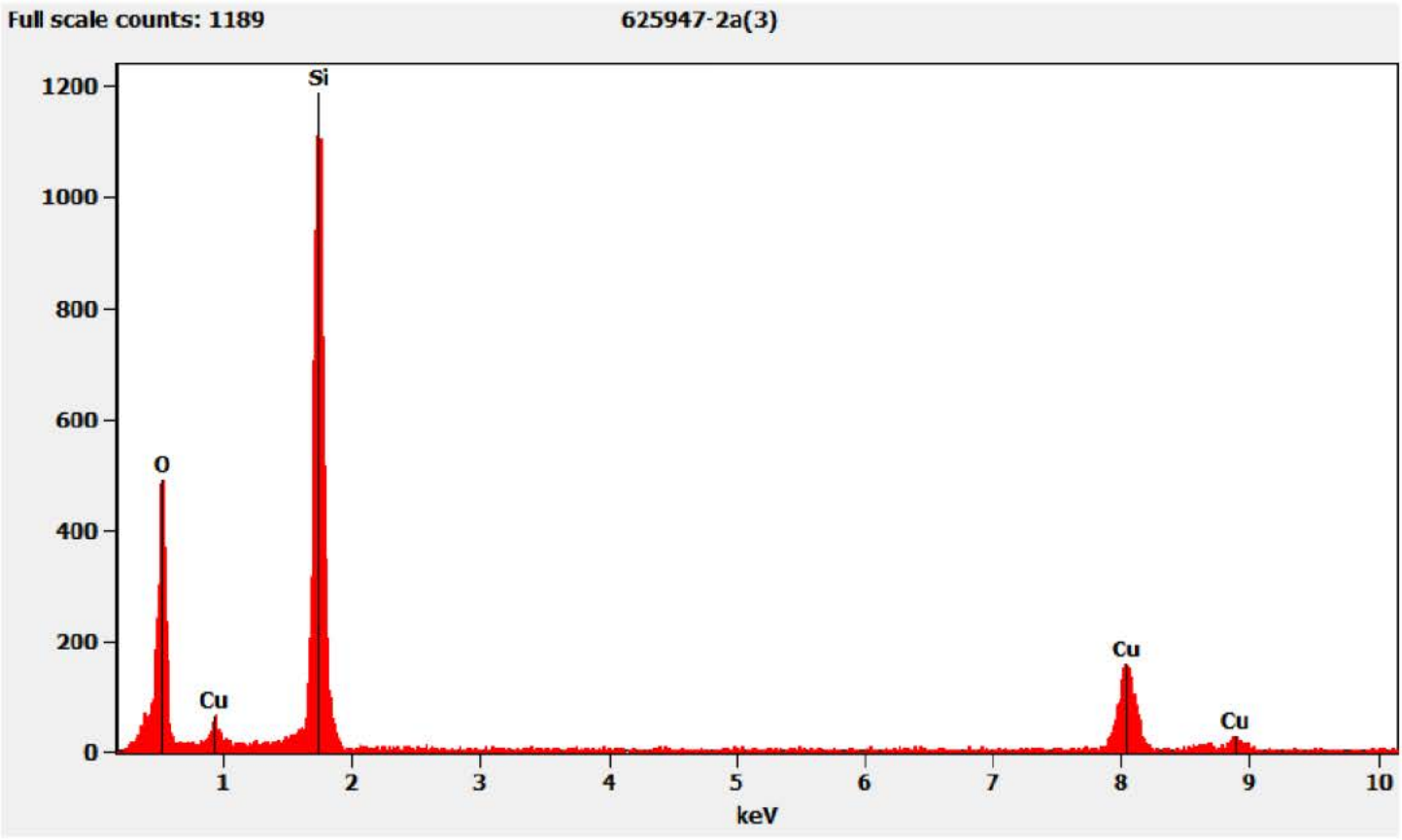
625947-2A, Silica Sphere



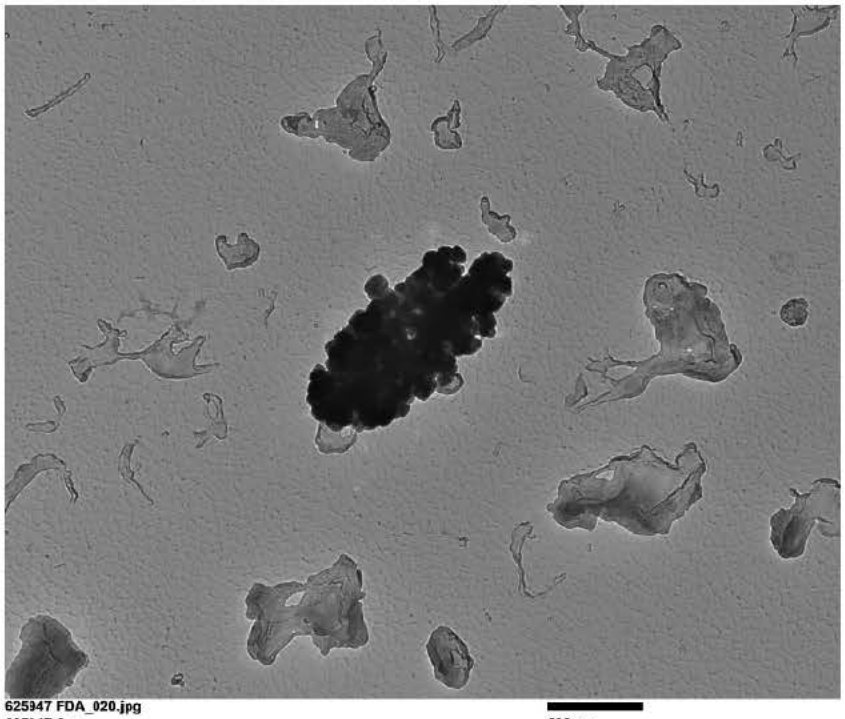
625947 FDA_016.jpg
625947-2a
Silica Sphere
Cal: 0.001429 µm/pix
10:47 4/30/2021
TEM Mode: Imaging
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

400 nm
HV=100kV
Direct Mag: 7200 x
AMA Analytical Services, Inc

Chemistry from the Silica Sphere pictured above



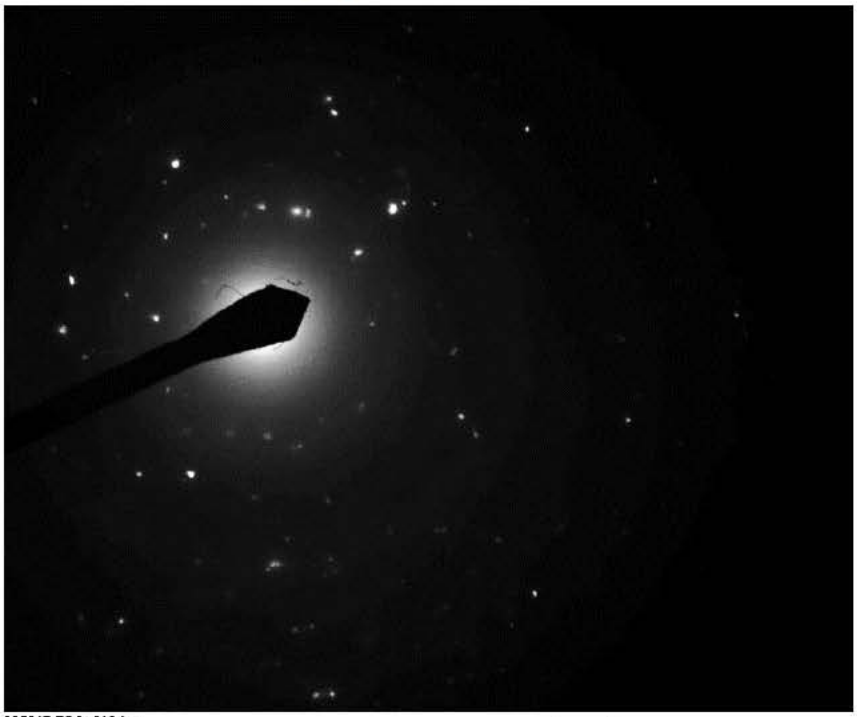
625947-2A, Iron Particles



625947 FDA_020.jpg
625947-2a
Fe Particles
Cal: 0.001774 µm/pix
10:59 4/30/2021
TEM Mode: Imaging
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

500 nm
HV=100kV
Direct Mag: 5800 x
AMA Analytical Services, Inc

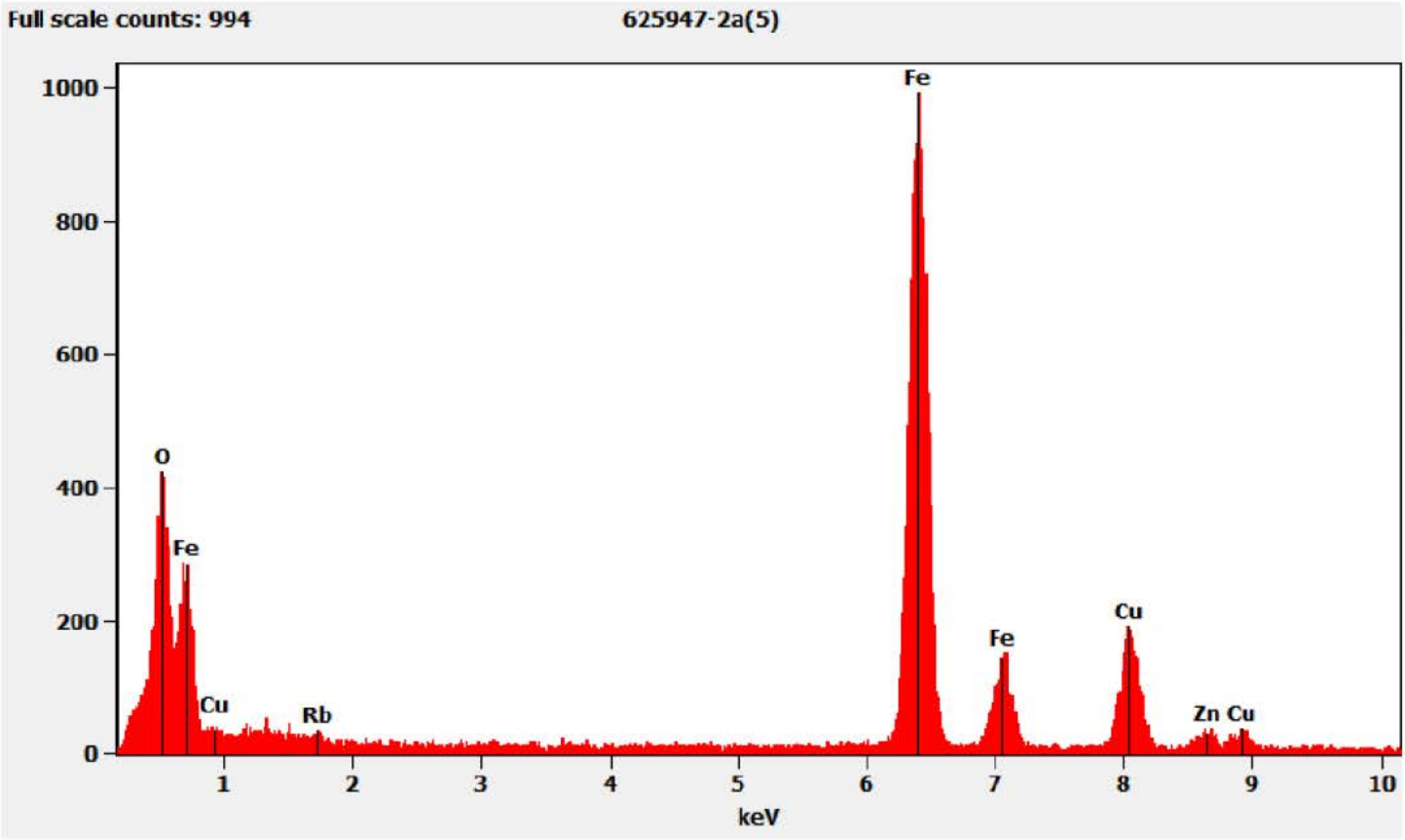
Diffraction Pattern from the Iron Particles pictured above



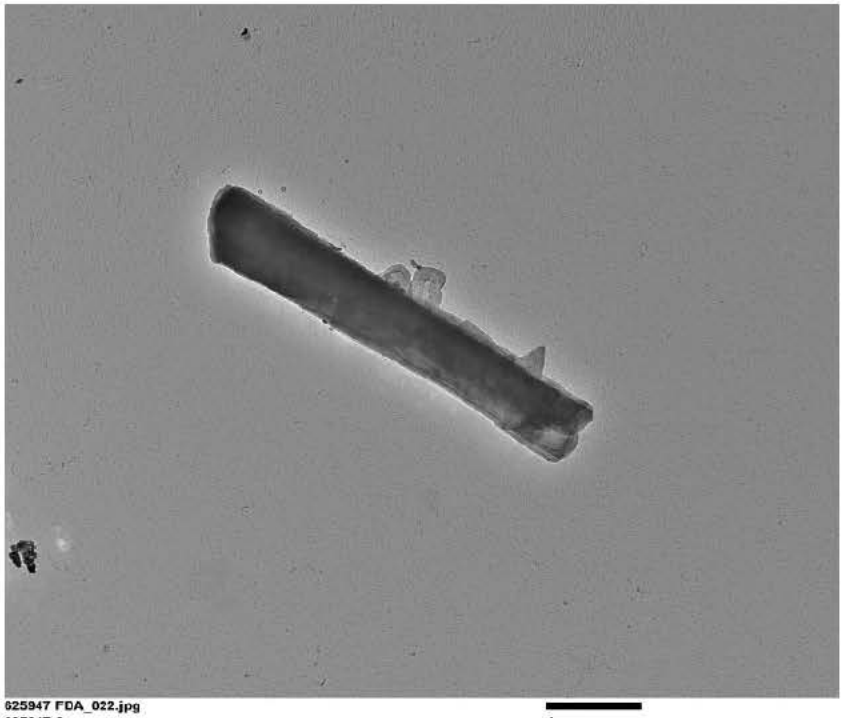
625947 FDA_019.jpg
625947-2a
Fe Particles
10:57 4/30/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

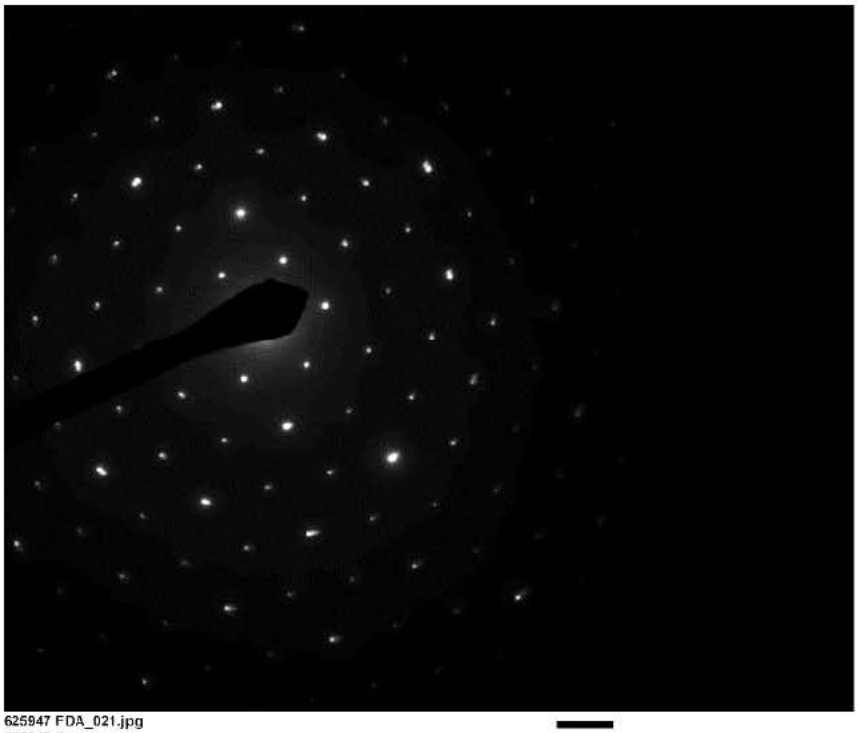
Chemistry from the Iron Particles pictured above



625947-2A, Talc Fiber



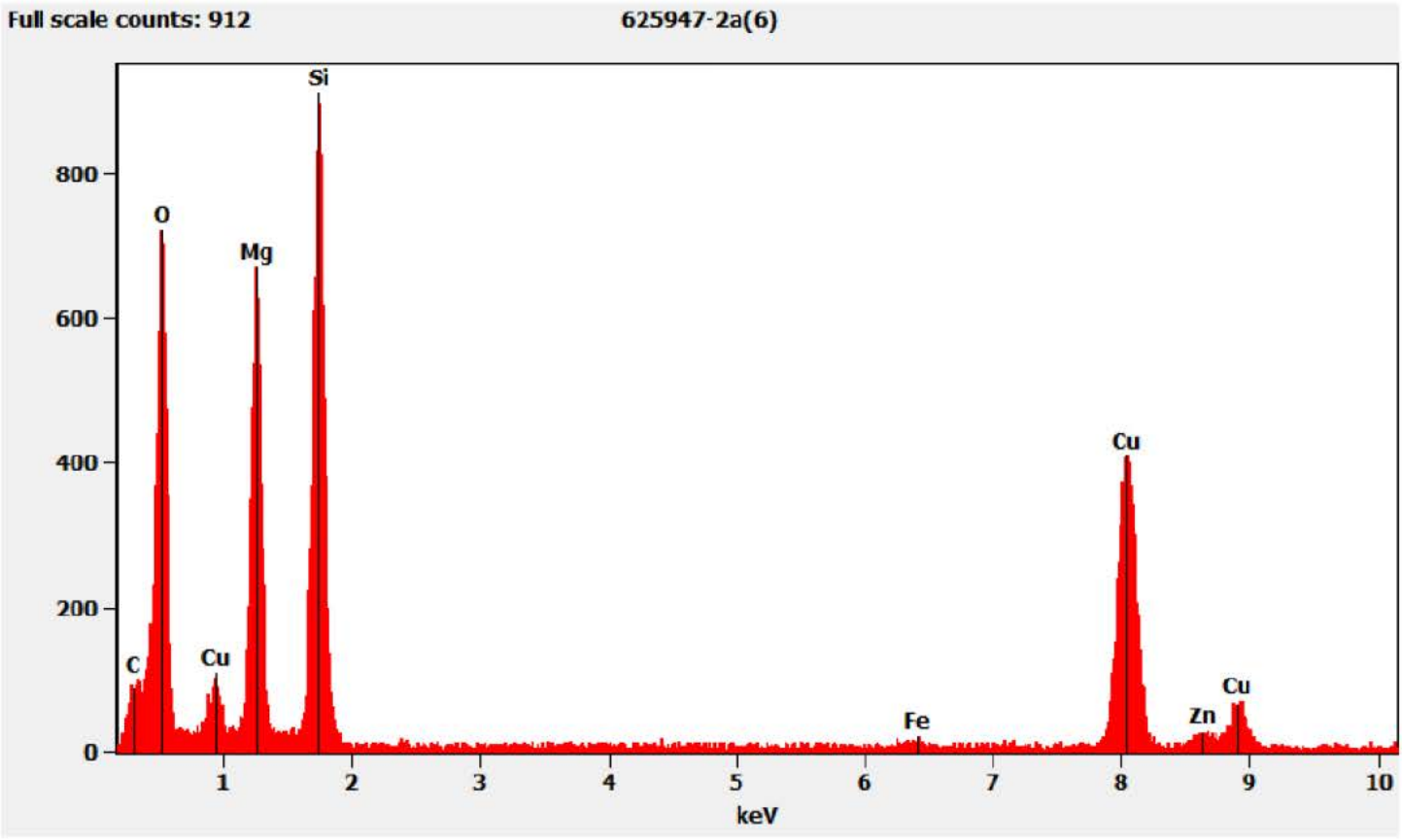
Hexagonal Diffraction Pattern from the Talc Fiber pictured above



625947 FDA_021.jpg
625947-2a
Talc Fiber
11:02 4/30/2021
TEM Mode: Diffraction
Microscopist: (b)(6)
Camera: NANOSPR15, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from the Talc Fiber pictured above



QC Discussion:

Microscope alignment and calibration for both the PLM and TEM scopes, and EDXA unit calibration were performed on each day of analysis as specified by method requirements and standard laboratory operating procedures. The analytical balance used for gravimetric reduction is verified weekly at three (3) tare levels using three NIST-traceable weights – 10.0-g, 0.1-g, 0.5-g – and on each day of operation using the 0.1-g and 0.5-g weights tared with an 8-mL glass vial. The muffle furnace is verified monthly at a temperature of 480°C. All equipment was functioning within normal operating parameters

Matrix blank samples were prepared at rate of 10% or greater alongside the client samples with each series of samples that were put into the muffle furnace together. The matrix blank samples were prepared using Sigma-Aldrich Talc Powder 18654 (Cas No. 14807-96-6; EC No. 238-877-9, Lot 82330). Analysis of the matrix blank samples was only required if asbestos, or the non-asbestos versions of the regulated minerals, was found on the associated client samples unless otherwise noted. Matrix blank sample number NB21-288 was not analyzed since no asbestos was observed on the associated client samples.

A talc reference control sample was randomly selected from our library of TEM grid preparations made from Sigma-Aldrich Talc Powder, <10 micron (Product No. 643604-500G; Batch No. 10830AJ) spiked with various levels of Chrysotile ranging from 0.4%-10%. One (1) reference control sample, sample number 625547/625947-RB1, was analyzed with this set. It was analyzed by (b)(6) on May 7, 2021 and found to be within acceptable limits.

Filtration blank samples were prepared alongside the client samples with each use of the filtration apparatus. Analysis of these samples was only required on those blanks associated with a client sample on which asbestos, or the non-asbestos versions of the regulated minerals, was found unless otherwise noted. Filtration blank sample numbers DI-Blank-01 and DI-Blank-02 were not analyzed since no asbestos was observed on the associated client samples.

TEM grid preparation (EB) blank samples were prepared with each batch of carbon coated filters. AMA policy is to analyze these blank samples whenever asbestos, or the non-asbestos versions of the regulated minerals, is detected on an associated client sample or when the laboratory blank identification number ends in a "0" or "5." Since no asbestos was observed on any of the client samples, only EB Blank ID 56645 was analyzed. (b)(6) analyzed this sample on April 30, 2021. No asbestos was detected on the sample.

Our laboratory information management system (LIMS) randomly selected samples 625947-2/02232021-13 for additional duplicate QC analysis. Separate preparations were made for the PLM and TEM portions of analysis. The duplicate QC analysis was performed by (b)(6) on April 30, 2021 for PLM and by (b)(6) on May 20, 2021 for TEM. The QC results were consistent with the original findings.

No samples in this set were randomly selected for additional replicate QC analysis.

Attachments:

The following items are attached to this case narrative for your reference:

1) Sample Log-In Sheet
2) Analytical Balance Verification Log
3) Daily PLM Scope Verification Log
4) Refractive Index Oil Verification Log
5) Daily TEM Scope Verification Log(s)
6) QC Results Summary for 625947
7) NB (Matrix) Blank Preparation Log
8) RB (Reference) Sample Bench Sheet(s)
9) EB (TEM Grid) Blank Preparation Log
10) EB (TEM Grid) Blank Bench Sheet(s)
11) Duplicate & Replicate QC Charts for (b)(6) or samples analyzed between 1/1/2021 & 4/30/2021
12) Duplicate & Replicate QC Charts for (b)(6) for samples analyzed between 1/1/2021 & 4/30/2021

13) Duplicate & Replicate QC Charts for Andreas Saldivar for samples analyzed between 1/1/2019 & 4/30/2021
14) Raw Data Sheets
    a. PLM Gravimetric Reduction Bench Sheet
    b. TEM Gravimetric/Filtration Bench Sheet
    c. PLM Analysis
    d. TEM Analysis
    e. Duplicate QC Analysis

I certify that all information contained in this report pertaining to laboratory events, procedures, and protocols is true to the best of my knowledge and accurately describes the handling of this project by AMA Analytical Services, Inc., and its personnel.

(b)(6) 5/20/2021
(b)(6) Date
Laboratory Director