William E. Longo, Ph.D.

"The Heavy Liquid Separation Method for the Analysis of Cosmetic Talc to Detect Amphibole and Chrysotile Asbestos"

February 4, 2020

wlongo@mastest.com

Dr. Longo is President of MAS LLC. located in Suwanee, Georgia. Dr. Longo has served as an expert witness in litigation on behalf of individuals who have claimed that either their ovarian cancer or mesothelioma was caused by their use of cosmetic talc. Dr. Longo has also served as an expert witness at the request of companies who have been sued for their asbestos-containing products. Dr. Longo has not been compensated for this FDA presentation and no lawyers have had any input or review of this presentation.

Overview

- Why use heavy liquid separation/concentration methods for cosmetic talc analysis
- History of HLS/concentration methods for talc analysis
- Analytical sensitivity or "how high can you go"
- Amphibole asbestos: tremolite series & anthophyllite series
- Development of chrysotile asbestos HLS methodology
- Reporting of results: fibers per gram

Why?

It's all about the sensitivity

- Standard TEM bulk analysis (ASTM, EPA & ISO) approximate detection limit: 2,000,000 to 14,000,000 fibers/gram or approx. 0.001 to 0.01 weight percent

- Standard PLM analysis (EPA R-93): DL 0.1-0.5 wt.%

- XRD: Useless for the detection of asbestos in cosmetic talc analysis

Standard TEM Bulk Detection Limits

Based on the Detection of 1 Fiber

Company	Method	Detection Limit Fibers/gram
Johnson & Johnson	7024 Current Method	7,000,000 -14,000,000
R.J. Lee Group	ASTM 5756 Current Method	11,000,000
AMA Analytical Services Inc. FDA 2010 contract	NY ELAP Method 198.6	10,000,000
AMA Analytical Services, Inc. FDA 2019 Contract Off-the-Shelf JBP Container	NY ELAP Method 198.6	2,000,000 - 6,000,000
McCrone 1970's to 1980's	In-house Method	7,000,000

Quantifiable Level

For both the McCrone and J&J's 7024 TEM methods, must detect 5 asbestos fibers of each type (tremolite, actinolite, anthophyllite & chrysotile) for it to be quantifiable

Total number of Asbestos fibers found in 20 Grid Openings	Total Asbestos Fiber Concentration Per gram of cosmetic Talc (0.009mg)
4 tremolite fibers	28,000,000 asbestos fibers
4 tremolite fibers 4 anthophyllite fibers	56,000,000 asbestos fibers
4 tremolite fibers 4 anthophyllite fibers 4 actinolite fibers	84,000,000 asbestos fibers
4 tremolite fibers 4 anthophyllite fibers 4 actinolite fibers 4 chrysotile fibers	112,000,000 asbestos fibers

How to Increase TEM Sensitivity

1. Concentrate the material examined in talc sample.

Gravimetric reduction: acid dissolution to remove calcite & pigments

Muffle Furnace: remove organics @ 400 C° for 1 hr.

2. Increase the number of TEM grid openings examined.

Standard TEM analysis typical analyzes 10 to 20 grid openings

Example: 20 grid openings = 7,000,000 - 14,000,000 f/g of talc

500 grid openings = 280,000 – 560,000 f/g

1000 grid openings = 140,000 - 280,000 f/g

Time of analysis: 500 to 1000 grids openings 3 to 5 days 8 hr. days

How to Increase TEM sensitivity

9/22/1972 Dr. Hutchinson (University of Minnesota), J&J consultant analyzed Dr. Lewin's (FDA) J&J's Shower to Shower sample to determine is XRD results showing chrysotile was accurate. Analyzed two sample splits by TEM

200 mesh grid size & number of grid openings examined

1st sample: 900 grid openings & 2nd sample 2100 grid openings

Results: 6 chrysotile fibers total, estimated at 0.1% by weight

How to Increase TEM Sensitivity

9/22/1972 Dr. Hutchinson (University of Minnesota), J&J consultant analyzed Dr. Lewin's (FDA) J&J's Shower to Shower sample to determine if Dr. Lewin's XRD results showing chrysotile was accurate or not.

Hutchinson analyzed two sample splits by TEM

200 mesh grid & number of grid openings examined

1st sample (Lewin's) 900 grid openings

2nd sample (STS) 2100 grid openings

Results: Both samples positive

6 chrysotile fibers total, estimated at 0.1% by weight

Brief Early History of HLS Method for Talc: Developed for J&J

1. 10/5/1971: Colorado School of Mines (CSM) memo to file, meeting W.H. Ashton of J&J, Item 3; will write report describing our efforts in removing tremolite by flotation and elutriation from 1'-5' talc ore composites

2. 4/2/1973: CSM memo to W.H. Ashton of J&J, using HLS <2.65 g/cc to separate out light fraction (chrysotile) and using >2.90 g/cc to separate out heavy fraction (tremolite), used 1% iodine in glycerine to stain the chrysotile four samples positive for chrysotile - 0.0001% to 0.0007% by PLM.

MAS LLC HLS Analysis for Amphibole Asbestos by PLM

- PLM Analysis using the Blount method published in 1991

  2.81g/cc HLS, 100 mg of talc

  Centrifuge at 7,000 rpm for 10 minutes

  Remove heavy minerals from bottom of centrifuge tube

  Analyze by PLM using ISO 22262-1 protocol

  Validated detection limit of approximately 0.001% by weight

  Detected both tremolite/actinolite & anthophyllite asbestos

  57 historical J&J JBP & STS containers (1960 to 1996) and 15 Imerys RC samples (1989 to 2003) provided by J&J and Imerys

- Results: 41 positive out of 72 samples or 57%

MAS LLC HLS Analysis for Amphibole Asbestos by TEM

- TEM Analysis using the ISO-22262-1 &2

  2.85g/cc HLS, 20 to 30 mg of talc

  Centrifuge at 9,000 rpm for 90 minutes

  Remove heavy minerals from centrifuged tube, filter/wash residue & dry

  Prepare and analyze TEM grids by standard TEM methods

  Detection limit of approximately 4,000 to 9,000 f/gram of cosmetic talc

  (increase in detection limit by 800 to1,750 times)

  Detected both tremolite/actinolite & anthophyllite asbestos

  55 historical J&J JBP & STS containers (1960 to 1996) and 15 Imerys RC samples (1989 to 2003) provided by J&J and Imerys

- Results: 42 positive out of 70 samples or 60%

MAS LLC HLS Analysis for Chrysotile Asbestos by PLM

- **PLM Analysis using the 1974 Colorado School of Mines/J&J Method**

  Stain 200 mg of cosmetic talc with Betadine (2% Iodine) solution
  filter stained talc material and wash in alcohol/Di-water

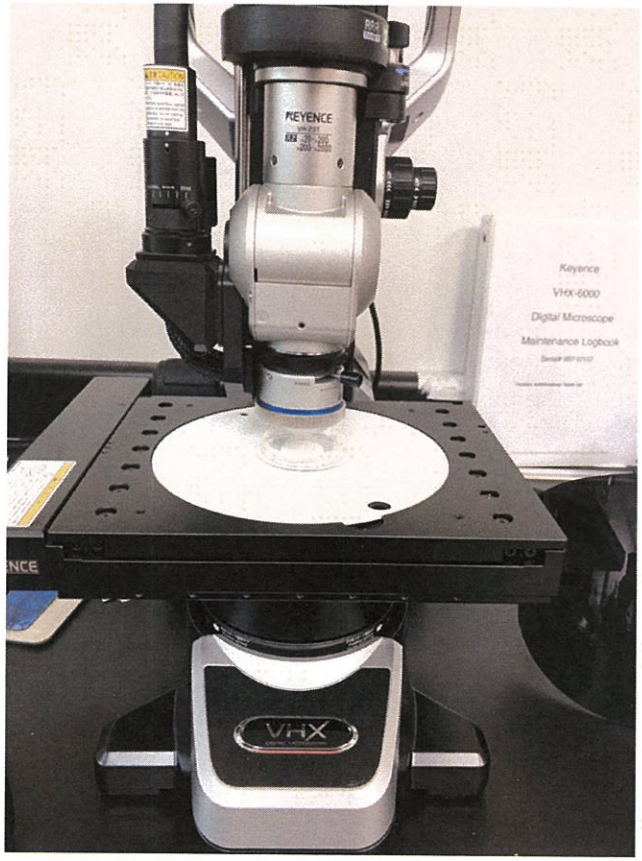
  2.72g/cc HLS, 200 mg of talc, centrifuge at 500 rpm for 5 minutes then 1800 rpm for 5 minutes

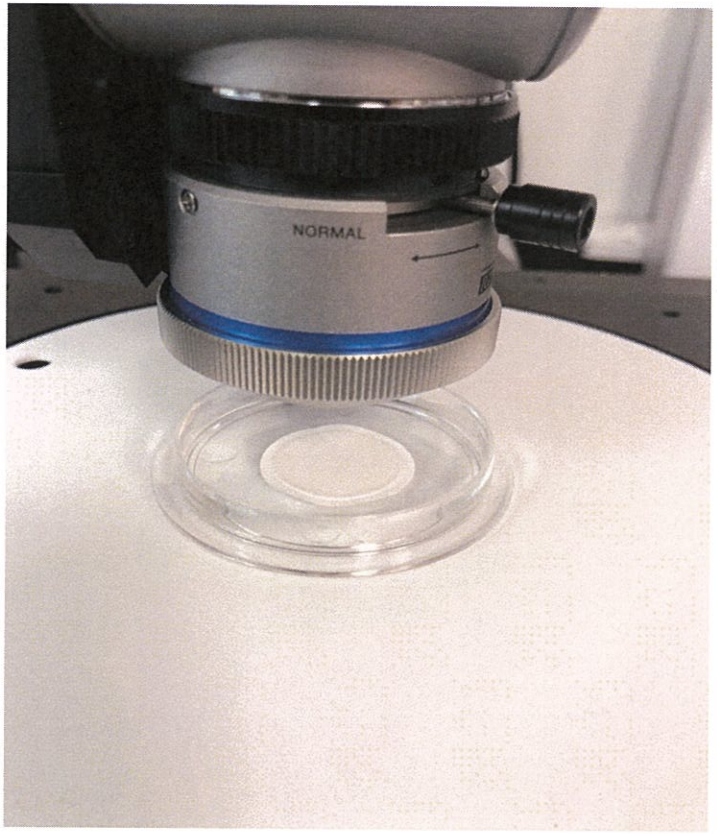
  Remove supernatant (light minerals) from centrifuged tube
  Filter/wash residue & dry

  Examine talc/filter under optical microscope at 1000x, and with fine tweezer remove stained chrysotile bundles from filter & place on glass slide

  Examine and verify chrysotile by PLM

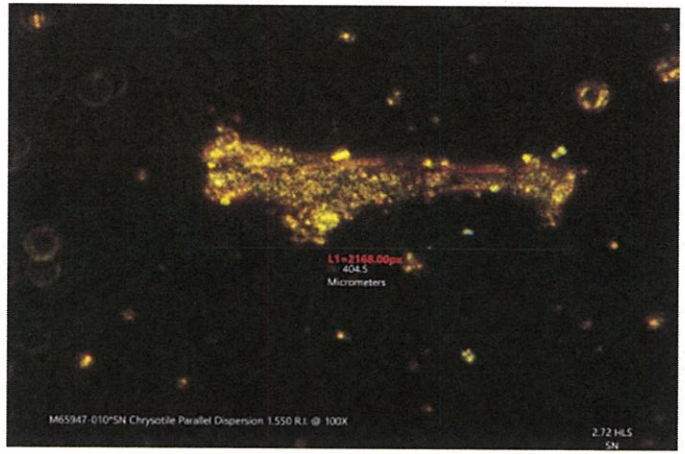
  Have validated detection limit of approximately 0.0001 % by wt. f/gram of talc

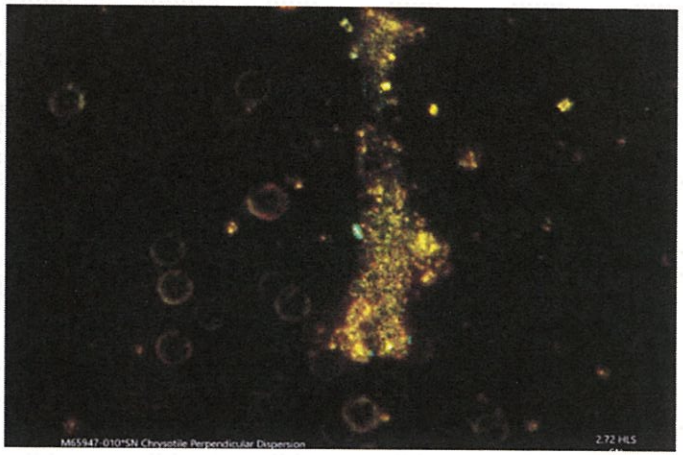

KEYENCE
Keyence
VHX-6000
Digital Microscope
Maintenance Logbook
VHX

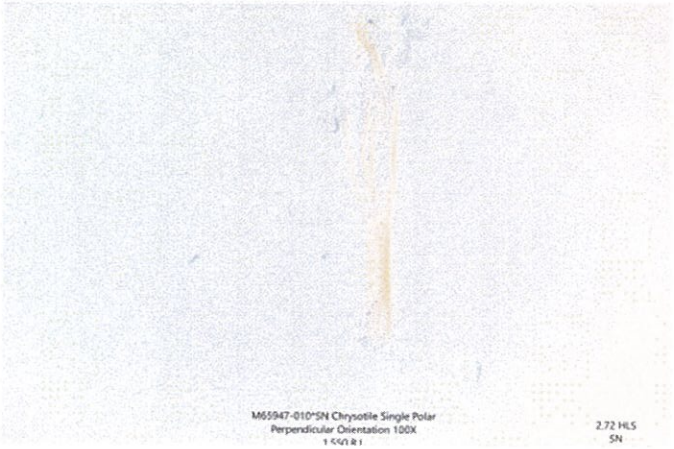

NORMAL

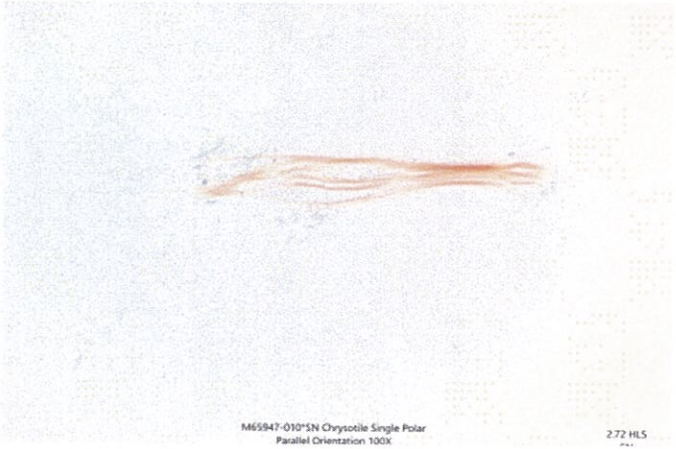
0.0001% Chrys & 2.72 HLS post stain 1/24/2020
Magnification: X200.0
500.00µm

0.0001% Chrysotile in Filtered Supernatant from Heavy Liquid Separation 2.72 gm/cc
Iodine Stained Chrysotile bundle in 1.550 R.I. Dispersion Staining / Single Polar

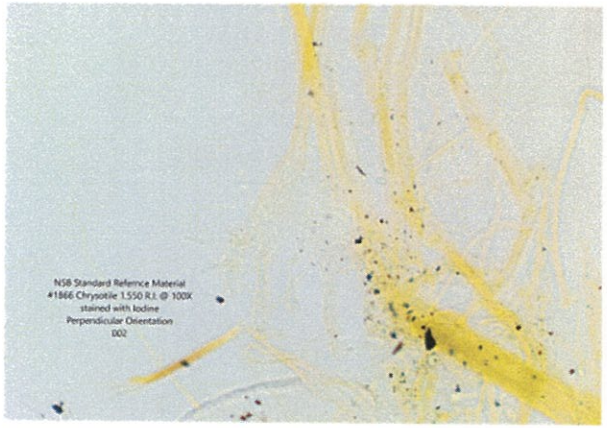


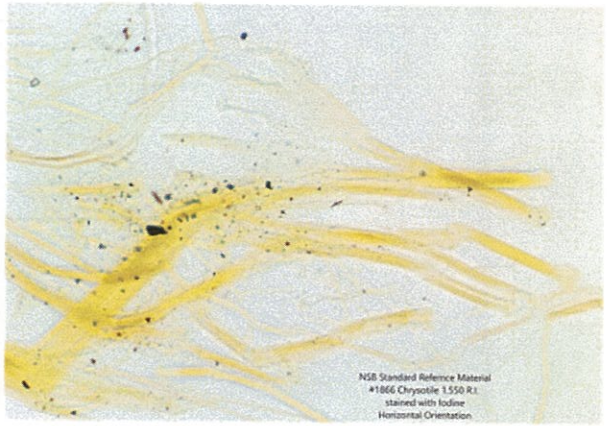


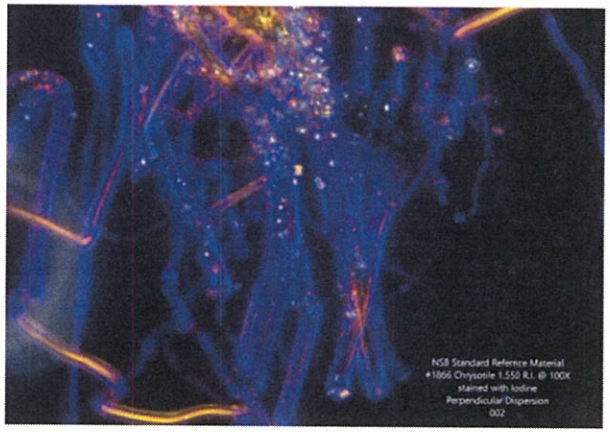


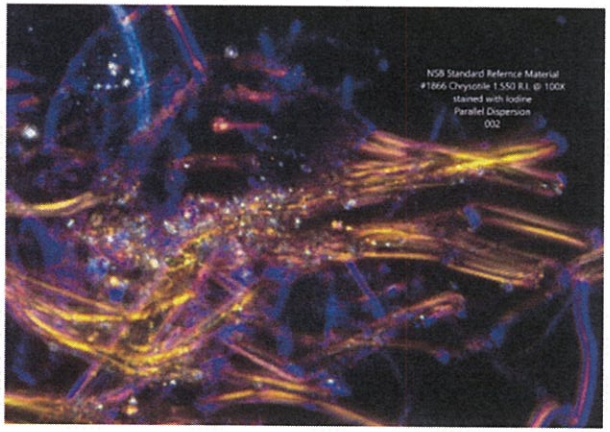


Iodine Stained NSB #1866 Chrysotile Standard Reference Material Mounted in 1.550 R.I. Liquid
Stained fibers in Single Polar Light / Dispersion Staining

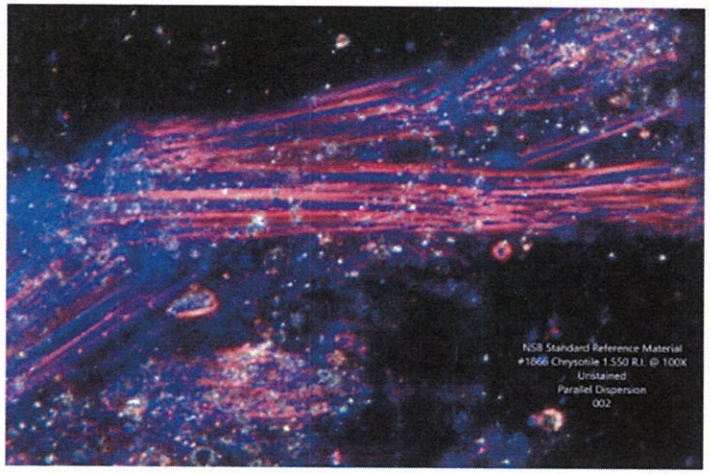


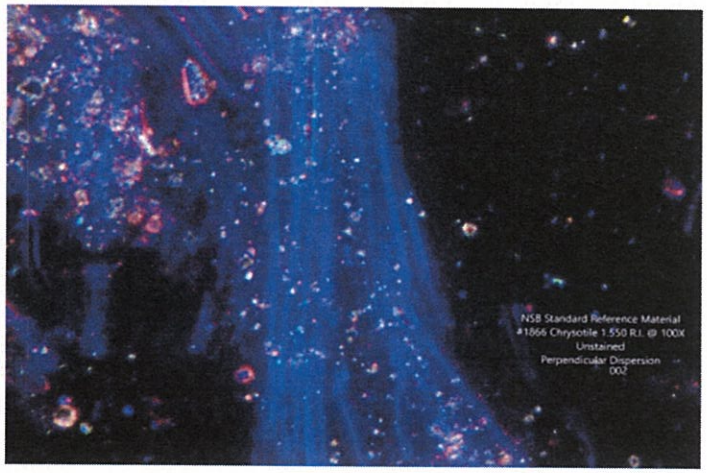


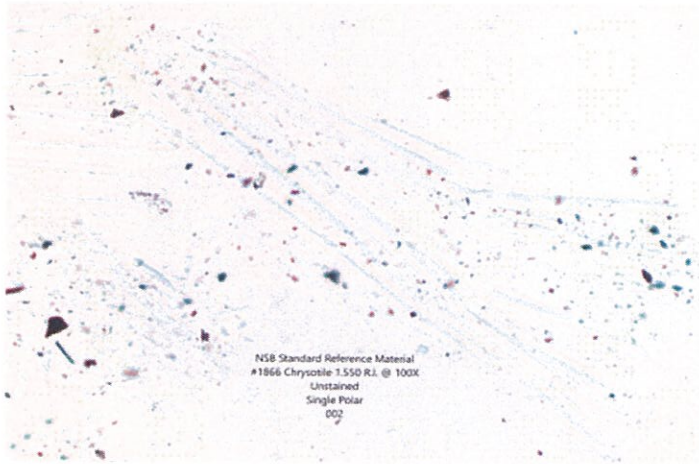




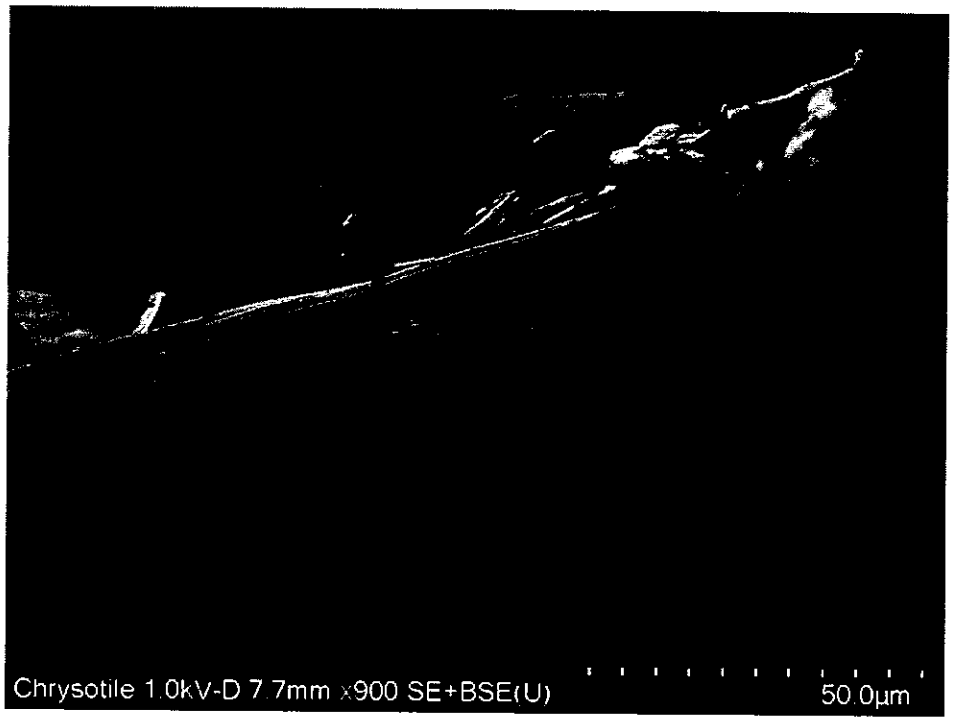
Unstained NSB #1866 Chrysotile Standard Reference Material Mounted in 1.550 R.I. Liquid Dispersion Staining / Fibers in Single Polar light



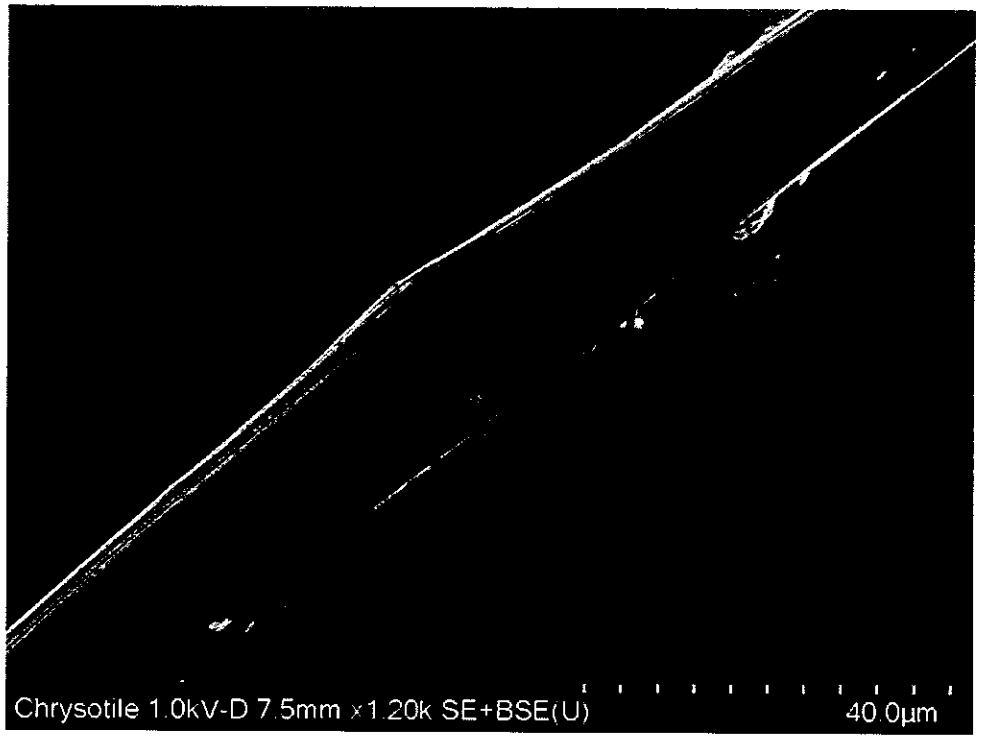




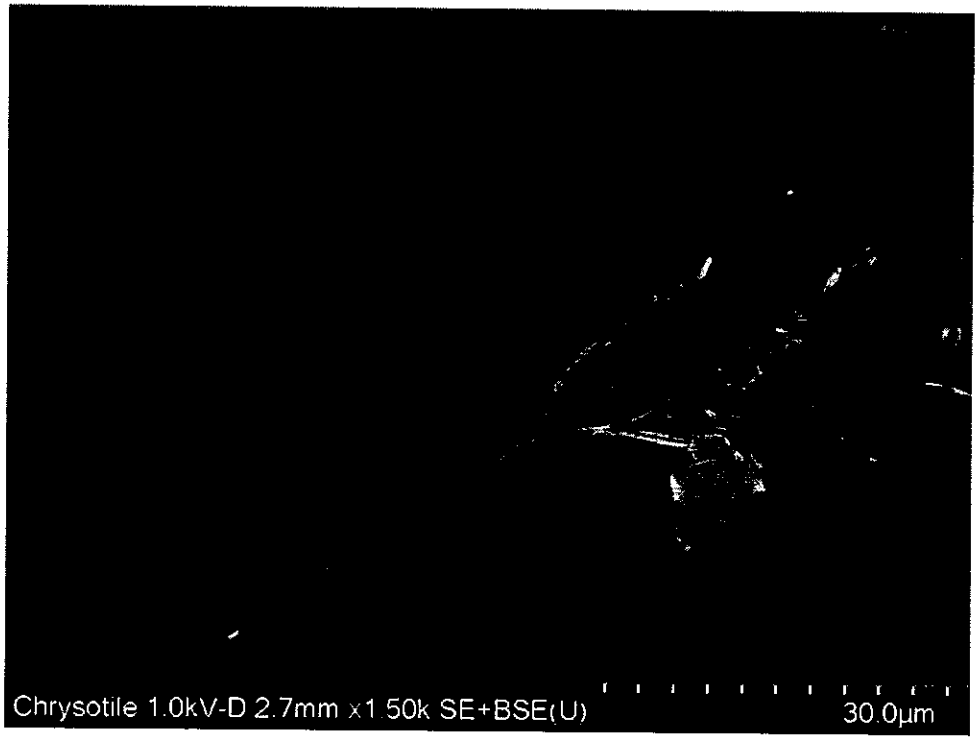
FE-SEM 0.0001% Chrysotile – Iodine Stained

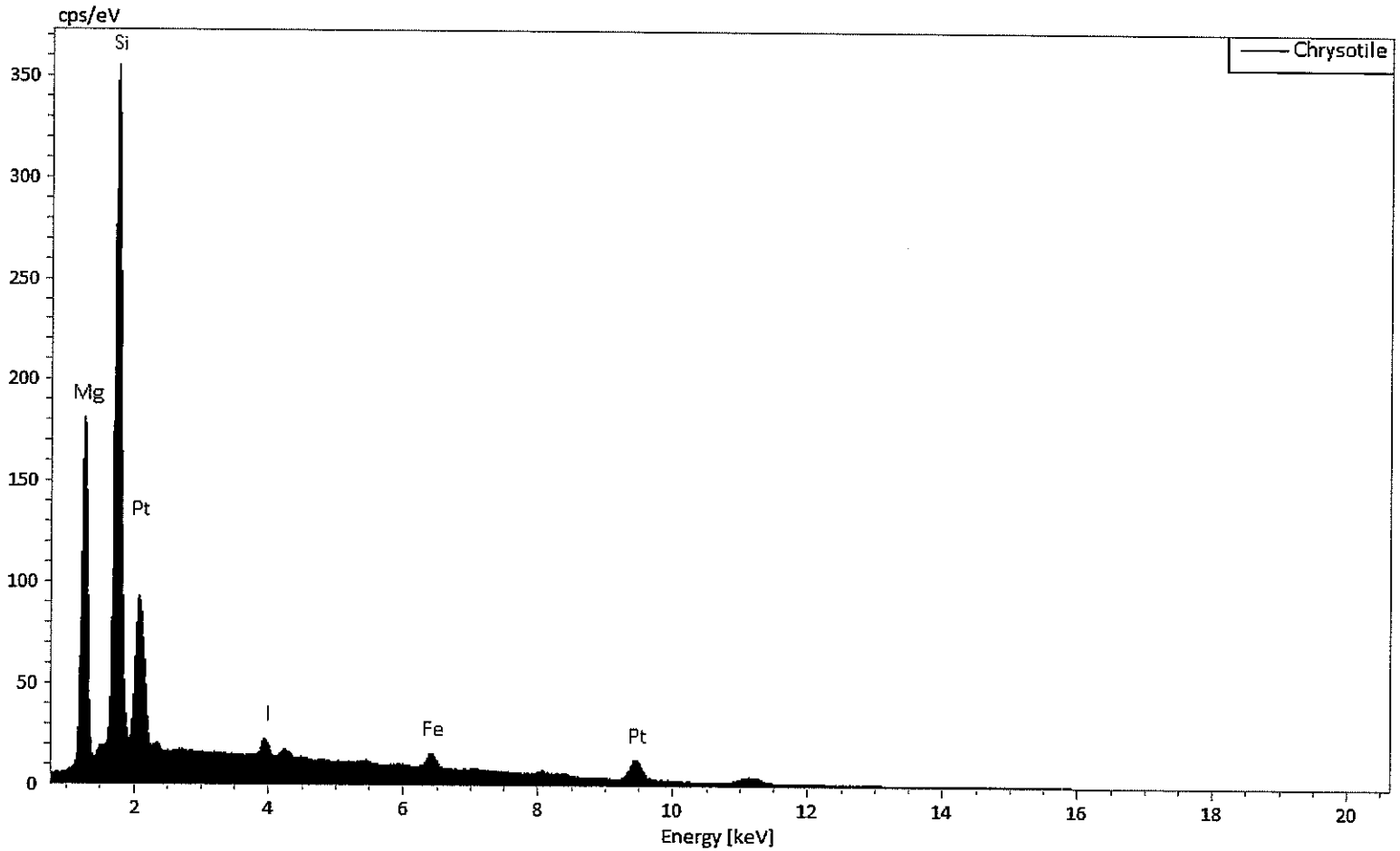


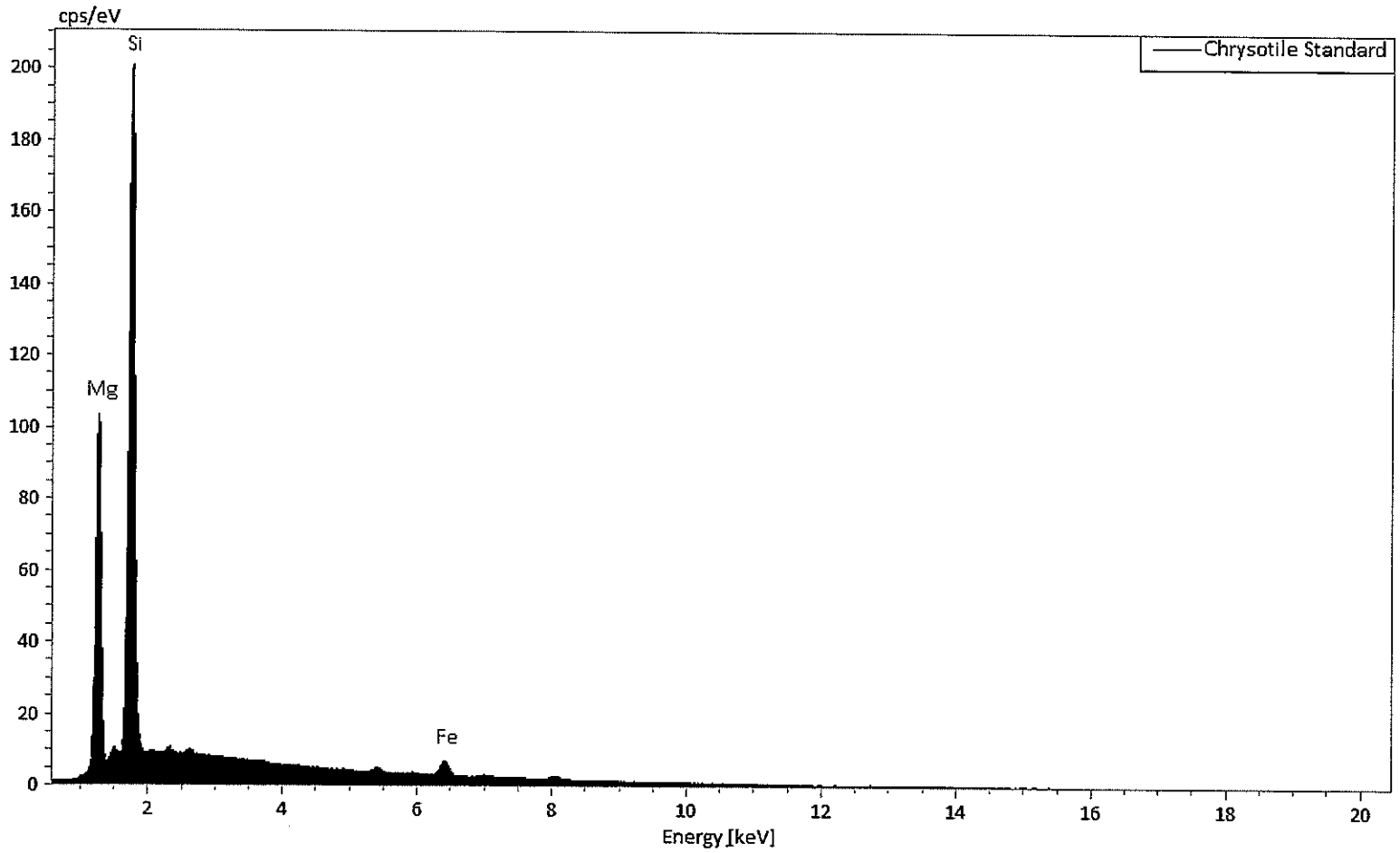
FE-SEM 0.0001% Chrysotile – Iodine Stained



FE-SEM 0.0001% Chrysotile – Iodine Stained




cps/eV
Chrysotile
Si
Mg
Pt
I
Fe
Pt
350
300
250
200
150
100
50
0
2
4
6
8
10
12
14
16
18
20
Energy [keV]


cps/eV
Si
Mg
Fe
Chrysotile Standard
200
180
160
140
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Energy [keV]