Jordi Labs

MATERIAL SOLUTIONS. UNCOMPROMISING INTEGRITY.

Company Name

Client Name

Released by:
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President

Job Number: J10635

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Introduction

Particulates and residues are major types of contamination found in the pharmaceutical industry. These may be generated from a wide variety of sources including packaging, undissolved residuals in buffer and media solutions, and various system components such as gaskets and seals. Contamination can also result from side reactions related to the manufacture of the product, including charred products and detergent residues or from degradation or maintenance of the processing equipment such as metal corrosion, lubricant oils, or scoring of Teflon gaskets. Particulates are found to be composed of a wide range of materials including glass, rubber, aluminum, plastics, and wood-based products.

Particulates can spread to the surrounding air volume of a clean room through airborne contamination or by transport attached to people or containers. This can then result in contamination of both products and the manufacturing area. These particles, if carried over to the final drug product, can have unfavorable effects such as impairment of microcirculation, blockages of blood vessels, damage to various organs, phlebitis etc. The FDA requires documentation and investigation of unexpected particles or adulterated drugs[1], and has taken action against companies that fail to perform adequate investigations for violations involving particle contamination[2].

Identifying and understanding the source of particulates is critical to controlling their spread. Once the source is known, then elimination of the particulate contamination becomes substantially simpler. In this regard, FTIR-microscopy and SEM-EDX are powerful tools for identifying particles, as well as providing information about shape, size and surface topography. The following analyses were conducted using a FTIR-Microscope (Thermo Nicolet iN10 MX FTIR microscope) and a SEM-EDX (Tescan Vega S 3 LMU with EDAX Octane Plus EDX detector) to investigate the chemistry of a brown colored particle found in a steel bioreactor in addition to a brown residue on the surface of the same steel reactor.

FTIR microscopy and SEM-EDX were able to identify the ***brown residue*** as iron oxide/iron hydroxide (rust). It was also able to determine that the ***brown particle*** was composed of a mixture of poly(tetrafluoroethylene) (PTFE, Teflon) and rust (iron oxide/hydroxide). By comparing the results of the brown residue and the brown particle, SEM-EDX and FTIR microscopy were able to show that particle source was consistent with degradation of the metal container. These techniques were also able to show that the particle was primarily composed of Teflon, which was correlated with the material specifications to a larger stirring apparatus made from Teflon.

[1] FDA Regulation 21 CFR 211 Subpart E

[2] FDA fines drug manufacturer $500 million for violations including insufficient investigation of rejected lots http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/EnforcementActivitiesbyFDA/ucm118411.htm

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February 8, 2016

Client Name
Company name
Address line 1
Address line 2

P: Phone Number
E: email

Dear Client,

Please find enclosed the test results for your samples described as:

1. *Brown Residue*
2. *Brown Particle*

The following tests were performed:

1. Fourier Transform Infrared Spectroscopy Microscopy (FTIR-Micro)
2. Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (SEM/EDX)

Objective

The purpose of this analysis was to identify a brown particle which was found in a bioreactor. A brown residue was also noted in the reactor and it was desired to determine if the particle was chemically related to the residue.

Summary of Results

FTIR-Micro and SEM-EDX results for *Brown Residue*, and *Brown Particle* are summarized in **Table 1.** It was found that *Brown Residue* (shown in **Figure 1**) was consistent with iron oxide/hydroxide (rust). *Brown Particle* (shown in **Figure 1**) was found to have an FTIR spectrum most consistent with a chemical composition of poly(tetrafluoroethylene) (PTFE) and iron oxide. FTIR and SEM-EDX maps acquired of the *Brown Particle* are consistent with particles of iron oxide embedded in a PTFE fragment. The chemical composition of the *brown particle* was found to correlate with the material specifications of a larger stirring apparatus made from PTFE.

Table 1: Particle Identifications

Sample Name	FTIR Best Match	SEM major Elements
Brown Residue	Iron oxide/hydroxide	Fe, O, Ni
Brown Particle	Iron oxide,/hydroxide PTFE	F, Fe, C, O

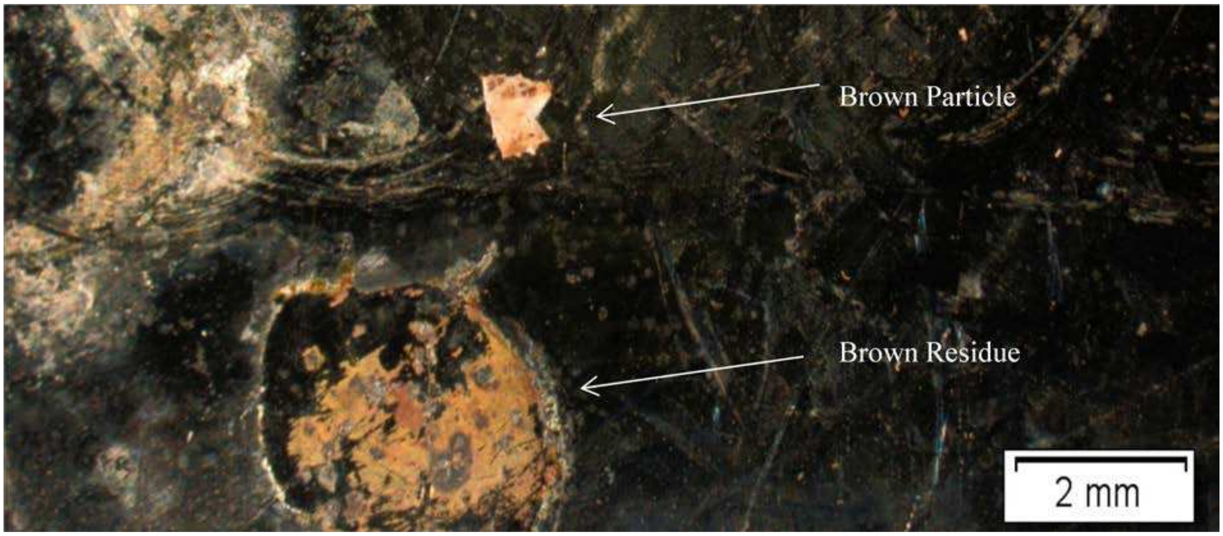

Figure 1: Optical Micrograph of *Brown Particle* and *Brown Residue*

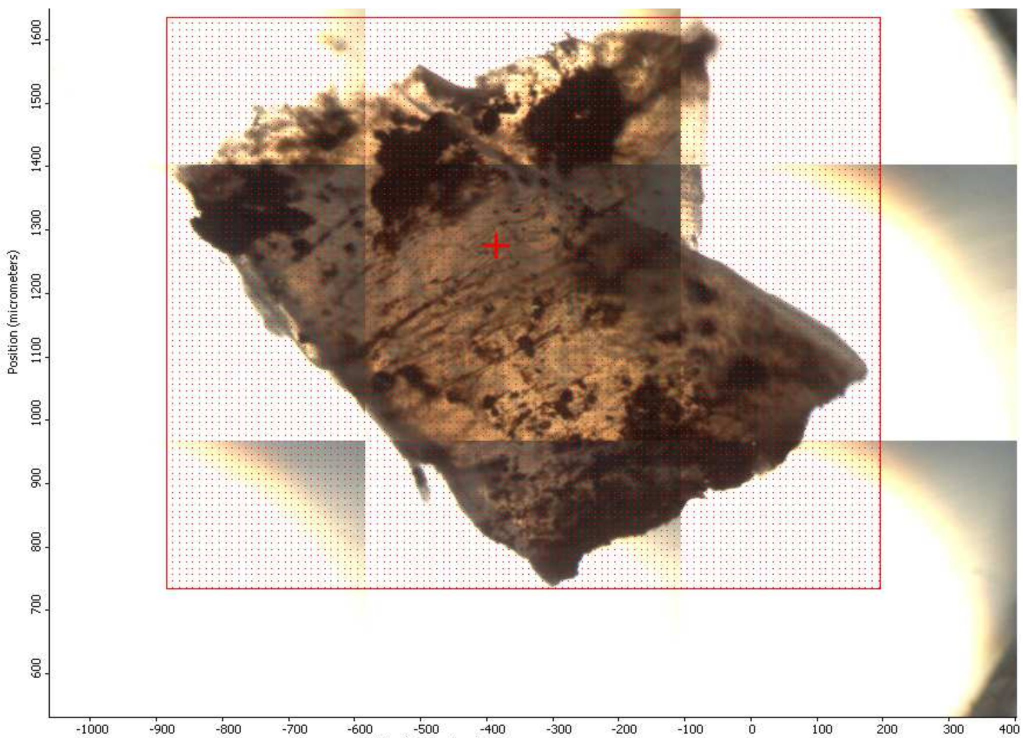

Figure 2: FTIR micrograph of mapped area

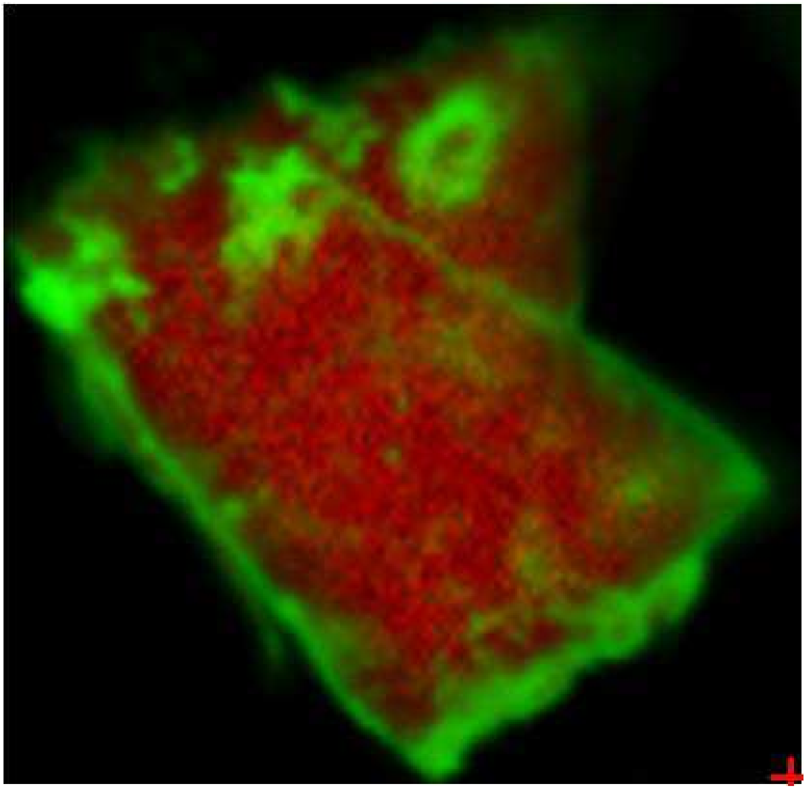

Figure 3: Overlay of iron oxide absorbances (green) and PTFE absorbances (red)

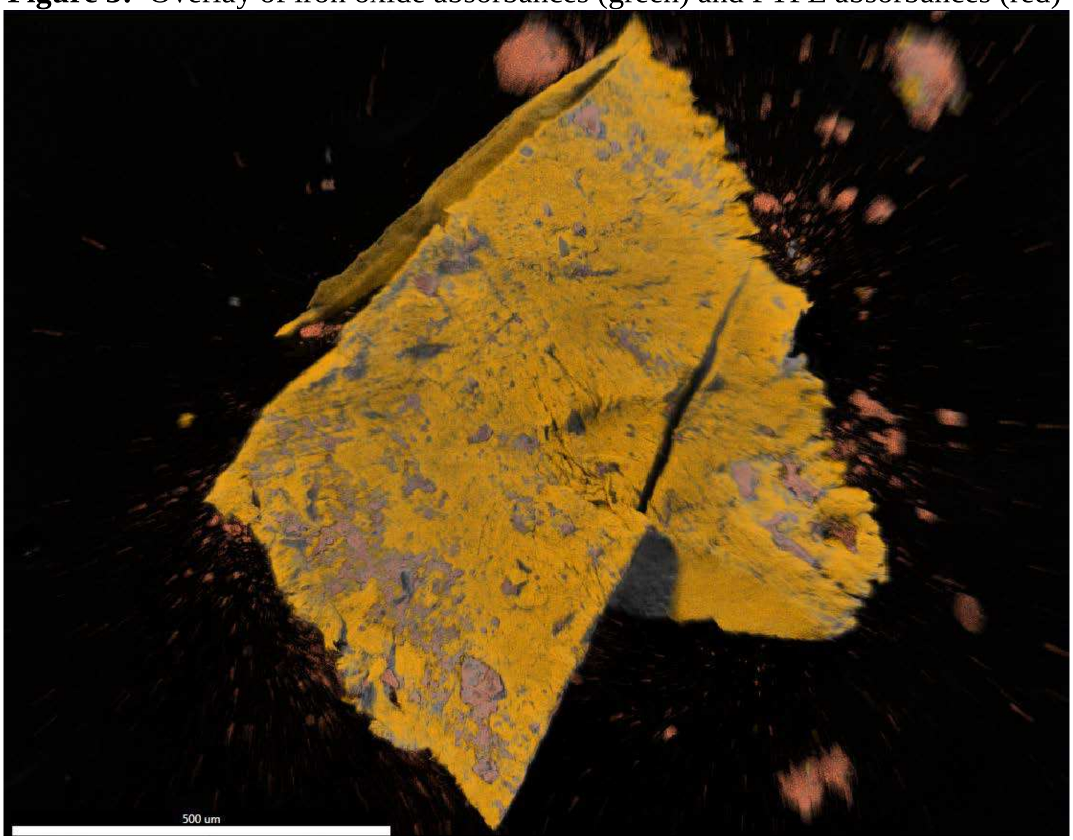

Figure 4: EDX map overlay of Fluorine (yellow, correlates with PTFE) and Iron (reddish-orange, correlates with iron oxide)

Individual Test Results

A summary of the individual test results is provided below. All accompanying data, including spectra, has been included in the data section of this report.

Brown Residue

FTIR-Microscopy

Brown Residue was received as a brown stain on a metal surface as seen in **Figure 1**. A portion was removed and placed on a sampling cell as seen in **Figure 5**.

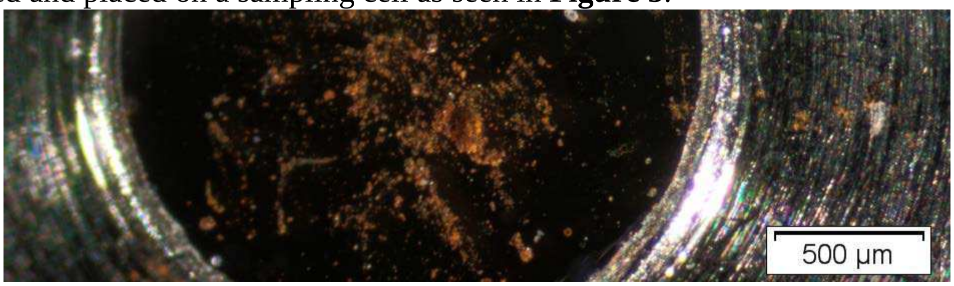

Figure 5: Optical Micrograph of *Brown Residue* on a sampling cell

Results

It was found that *Brown Residue* was consistent with iron oxide/hydroxide. Specific absorbance assignments for *Brown Residue* are provided in **Table 2**. An overlay of *Brown Residue* and iron oxide/hydroxide can be seen in **Figure 6.** This identification is further confirmed by the SEM-EDX.

Table 2
FTIR Peaks and Identifications Of Brown Residue

IR Frequency (cm^{-1})	Possible Functional Group	Possible Source
3480	O-H stretch	Iron Oxide/Hydroxide
1541, 1427, 1345	Fe-O comb/overtone	Iron Oxide/Hydroxide
804	Fe-OH stretch	Iron Oxide/Hydroxide
~675	Fe-O stretch	Iron Oxide/Hydroxide

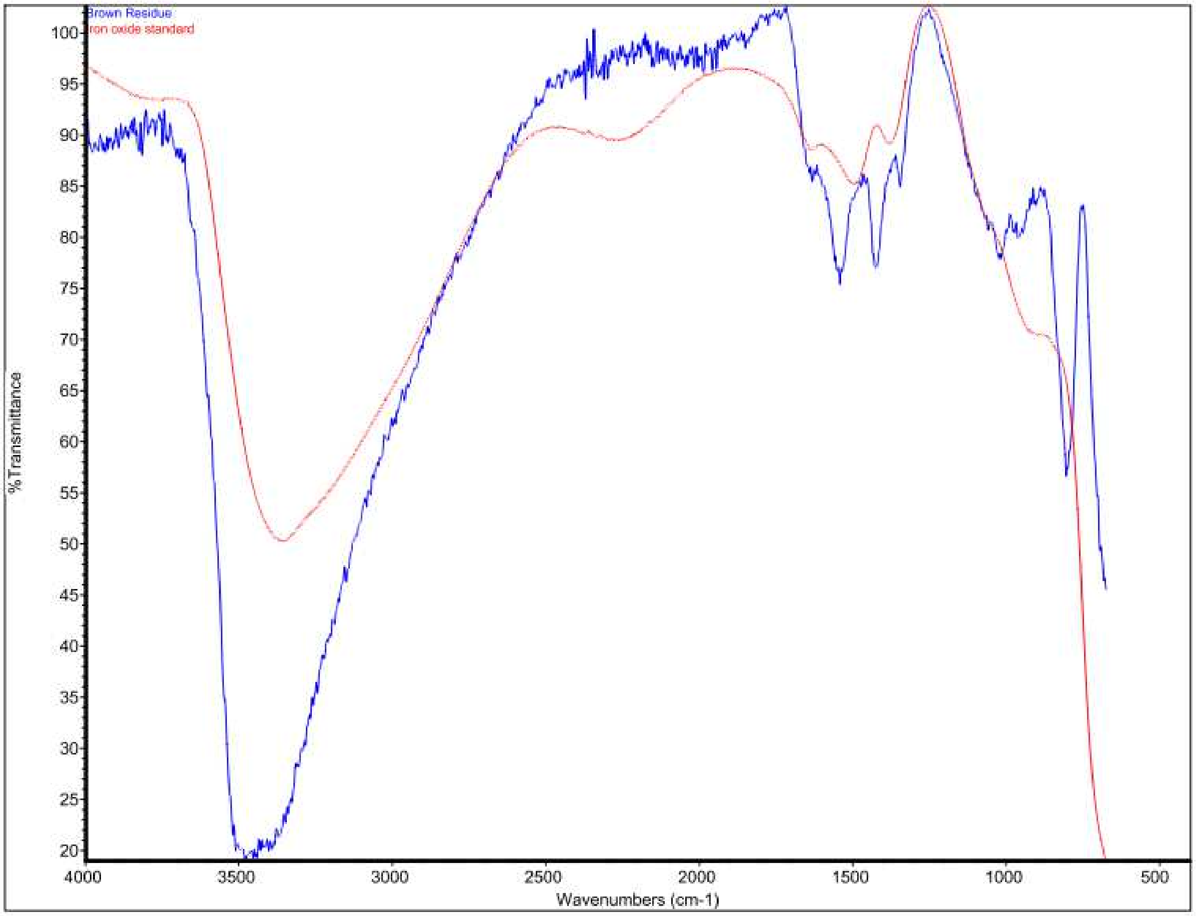

Figure 6: Overlay of *Brown Residue* and an iron oxide/hydroxide standard

SEM-EDX

Brown Residue was analyzed by SEM-EDX at one sampling location and a control location. **Figures 7-8** show the SEM-EDX images and the elemental composition of each sampling location is summarized in **Tables 3-4**. **Figure 9** shows the secondary electron (SE) and backscattered electron (BSE) images of the sample.

Brown Residue showed the presence of Fe, O and Ni. This is consistent with the FTIR identification of iron oxide/hydroxide. The presence of Ni suggests that the reactor composition is consistent with steel.

Table 3: Elemental concentration at area 1 (control) on *Brown Residue*					
Element	Atomic Number	Series	Weight %	Mole %	% Error
Carbon	6	K	79.06	83.41	6.28
Oxygen	8	K	20.94	16.59	23.72

Table 4: Elemental concentration at area 6 on *Brown Residue*					
Element	Atomic Number	Series	Weight %	Mole %	% Error
Carbon	6	K	17.62	33.42	15.62
Oxygen	8	K	32.11	45.72	15.43
Nickel	28	L	9.29	3.60	19.13
Sodium	11	K	0.27	0.27	42.12
Aluminum	13	K	0.26	0.22	25.74
Chlorine	17	K	1.13	0.73	12.12
Iron	26	K	39.31	16.04	4.26

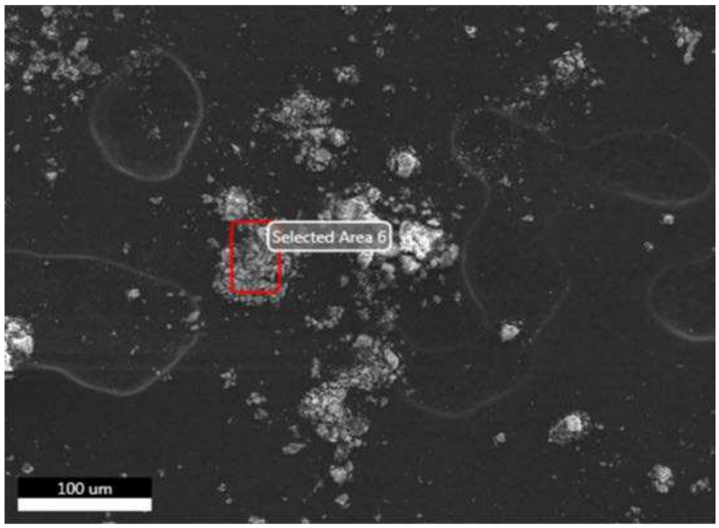

Figure 7: SEM-EDX image of all sampling locations analyzed of *Brown Residue*

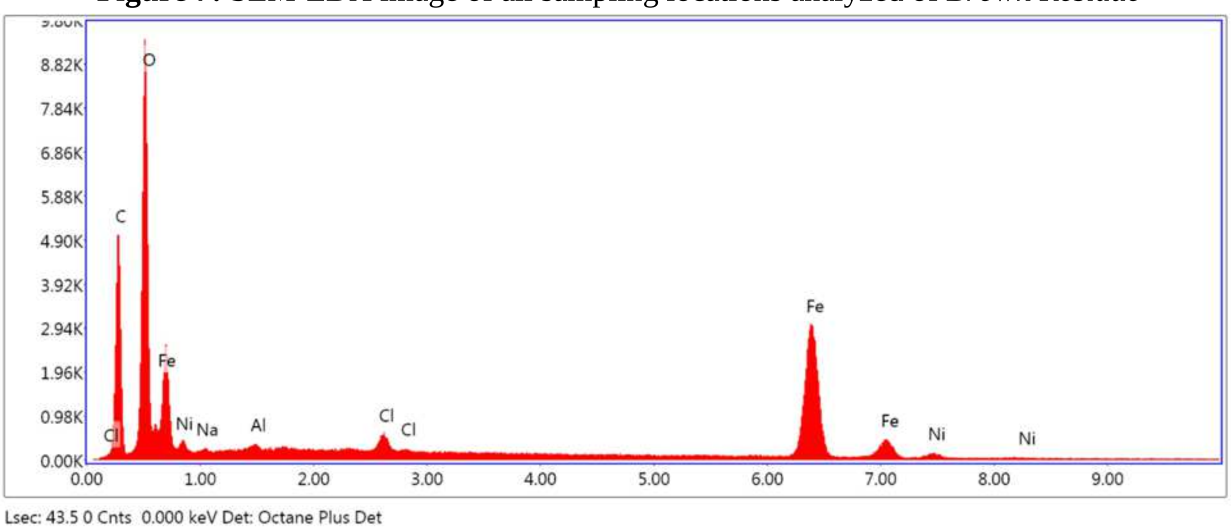

Figure 8: SEM-EDX elemental abundance of area 6 analyzed of *Brown Residue*

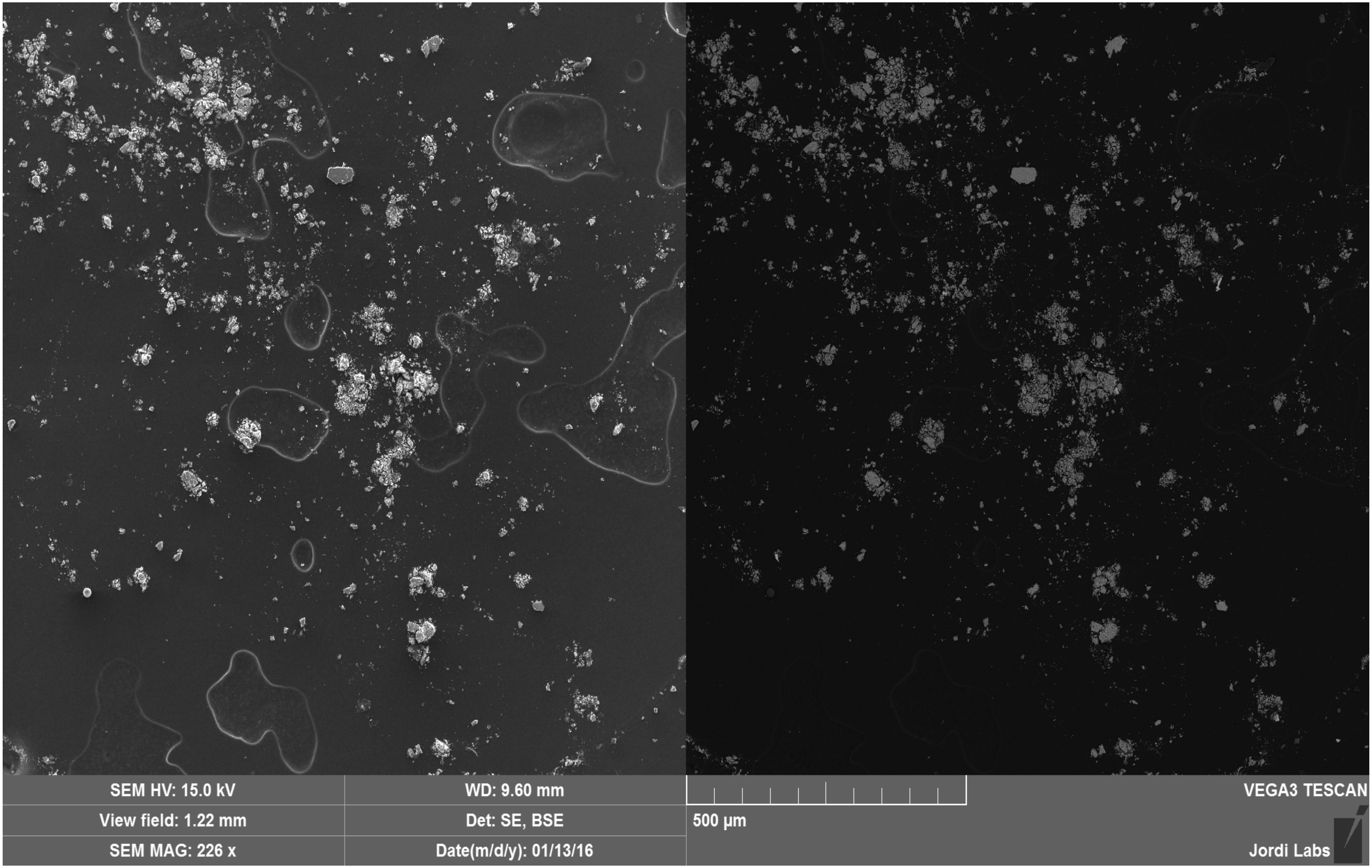

Figure 9: SEM SE (left) and BSE (right) images of *Brown Residue*

Brown Particle

FTIR-Microscopy

Brown Particle was received as a brown and white particle on a metal surface as seen in **Figure 1**. The particle was then placed on a sample cell as seen in **Figure 10**.

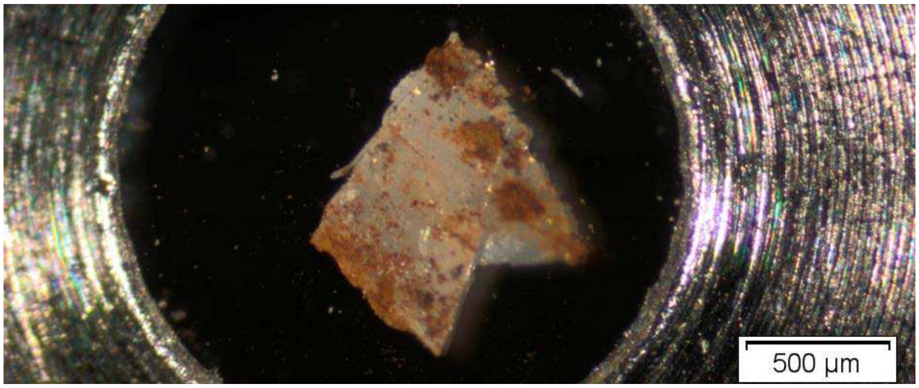

Figure 10: Optical Micrograph of *Brown Particle* on a sample cell

Results

It was found that *Brown Particle* was consistent with a mixture of PTFE and iron oxide. Specific absorbance assignments for *Brown Particle* are provided in **Table 2**. An overlay of the FTIR spectrum of the *Brown Particle in the Brown area,* the *Brown Particle in the White Area*, and the *Brown Residue* can be seen in **Figure 11**. An overlay of the FTIR spectrum of the *Brown Particle in the Brown area,* the *Brown Particle in the White Area* and PTFE can be seen in **Figure 12**.

It was also observed that the brown areas indicated stronger absorbances consistent with the spectrum from brown residue, while the white areas were more consistent with PTFE. An area map was acquired to demonstrate the distribution of iron oxide and PTFE in the *Brown Particle*. **Figures 13-14** show the resulting iron oxide and PTFE heat maps, which indicate *Brown Particle* is consistent with iron oxide embedded in a PTFE matrix.

Table 5
FTIR Peaks and Identifications Of Brown Particle

IR Frequency (cm^{-1})			**Possible Functional Group**	**Possible Source**
Brown Residue (shown in **Table 2**)	*Brown Particle Brown area*	*Brown Particle White area*		
3480	3371	N/O	O-H stretch	*Brown Residue*
1541, 1427, 1345	1557, 1426	1550, 1448	Fe-O comb/overtone	*Brown Residue*
N/O	1275-1150	1275-1150	C-F stretch	PTFE
804	781	776	Fe-OH stretch	*Brown Residue*
~675	~675	720	Fe-O stretch	*Brown Residue*

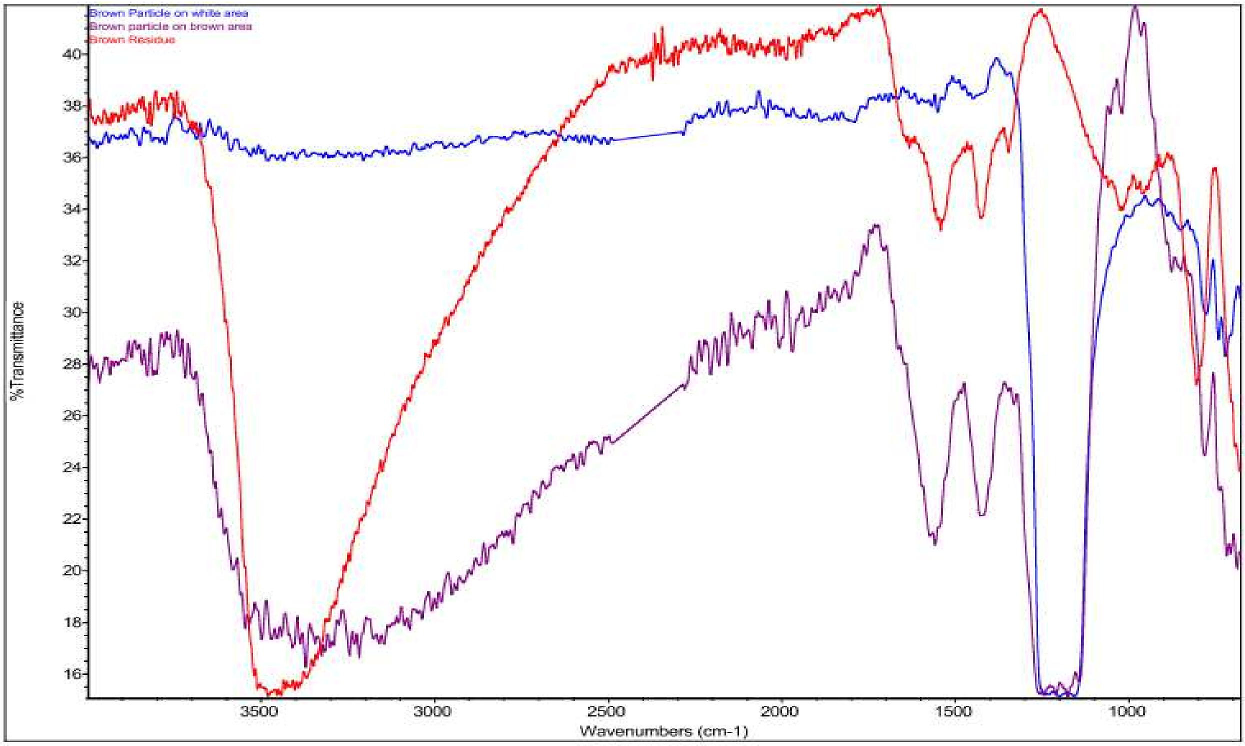

Figure 11: Overlay of *Brown Particle on white area* (blue), *Brown Particle on brown area* (purple), and *Brown Residue* (red)

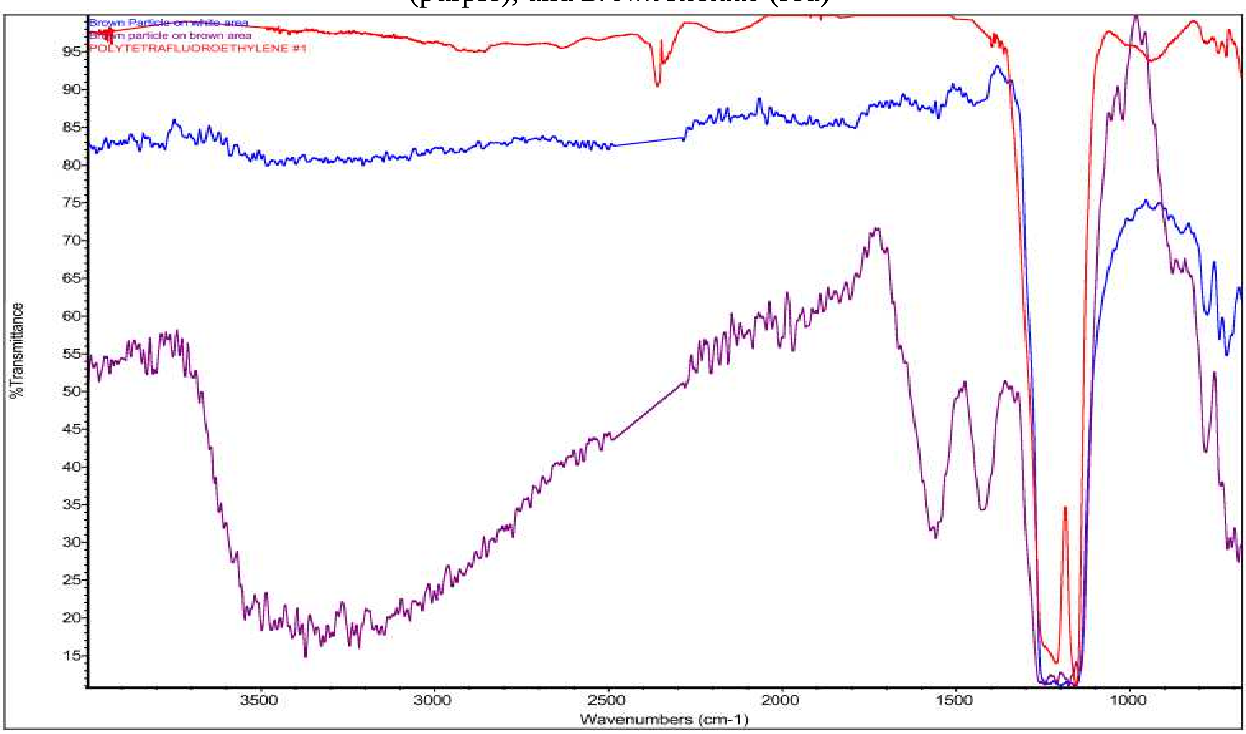

Figure 12: Overlay of *Brown Particle on white area* (blue), *Brown Particle on brown area* (purple), and PTFE (red)

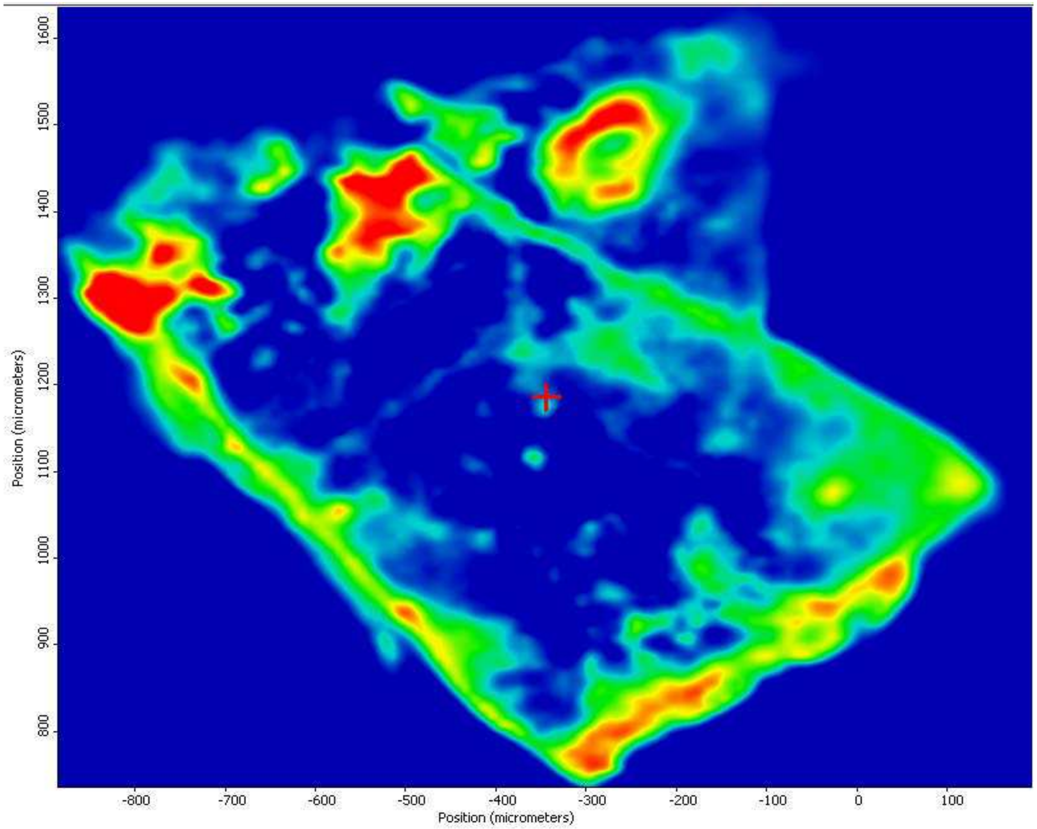

Figure 13: FTIR heat map of iron oxide absorbances (red is a strong absorbance, blue is weak)

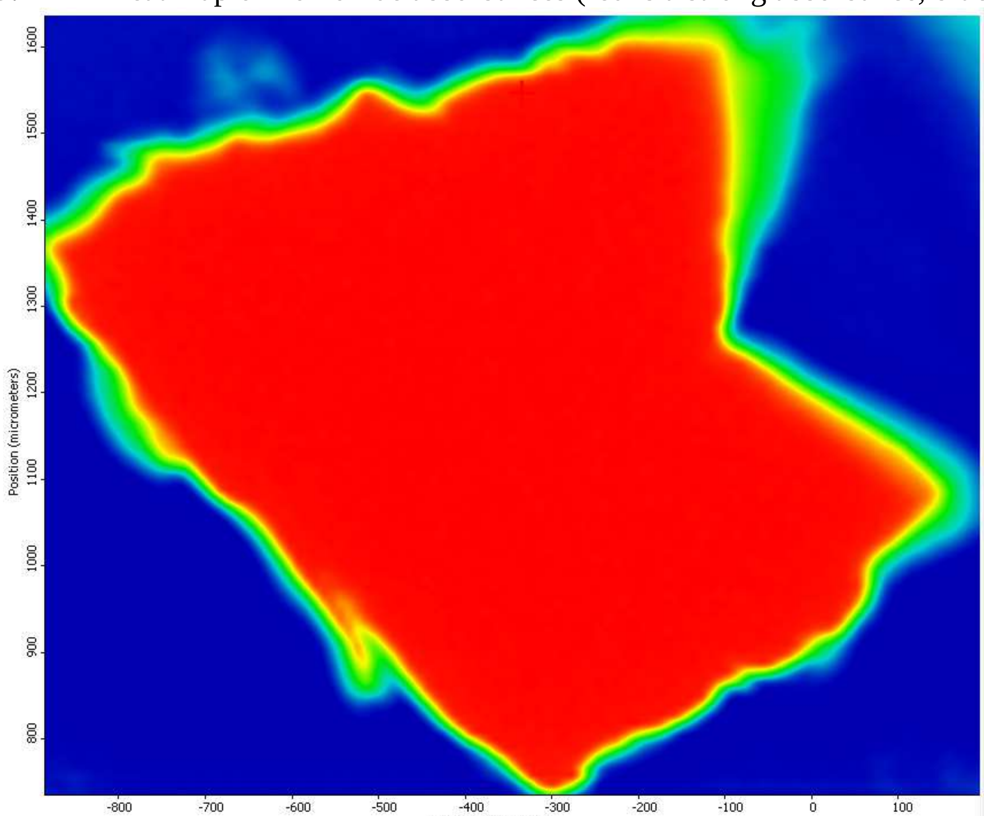

Figure 14: FTIR heat map of PTFE absorbances (red is a strong absorbance, blue is weak)

SEM-EDX

Brown Particle was analyzed by SEM-EDX at two sampling locations and a control location. A spectrum was taken over a large area to determine the general chemistry of the sample, and a smaller area was acquired on a brown portion. **Figure 15** shows the secondary electron (SE) and backscattered electron (BSE) images of the sample. It was also observed that one of the bright areas has moved off the particle after the acquisition of an area map as seen in **Figure 16**. This indicates that one of the bright particles (observed by FTIR to be iron oxide/hydroxide) was not embedded, but rather resting on the surface, and was forced off by electrostatic repulsion when exposed to an intense electron beam. Additionally, an EDX map was acquired. Individual elemental maps can be seen in **Figures 19-Figure 22.** **Figures 23-26** show the SEM-EDX images and the elemental composition of each sampling location is summarized in **Tables 6-8**.

It was observed that greater concentrations of F are localized where Fe is not observed as well as the inverse as seen in **Figure 17**. When this relationship is considered with the acquired FTIR map, it can be seen the surface of the PTFE particle is embedded with iron oxide particles because FTIR analyzes the chemistry of the entire thickness of the sample, while SEM-EDX is a surface sensitive technique. This is consistent with the results observed by FTIR microscopy. An overlay of oxygen and the iron distribution can also be seen in **Figure 18**, and generally are observed in the same areas of the image indicating iron is present as iron oxide/hydroxide.

Table 6: ***Elemental concentration at area 1 (control) on Brown Particle***

Element	Atomic Number	Series	Weight %	Mole %	% Error
Carbon	6	K	74.66	79.69	7.60
Oxygen	8	K	25.34	20.31	22.40

Table 7: ***Elemental concentration at area 3 on Brown Particle***

Element	Atomic Number	Series	Weight %	Mole %	% Error
Carbon	6	K	8.63	14.95	16.04
Oxygen	8	K	41.67	54.23	15.39
Fluorine	9	K	15.76	17.27	15.65
Sodium	11	K	1.40	1.27	7.68
Aluminium	13	K	0.14	0.11	34.83
Silicon	14	K	0.16	0.12	30.85
Sulfur	16	K	0.16	0.10	34.22
Chlorine	17	K	0.38	0.22	19.00
Iron	26	K	26.30	9.81	5.56
Nickel	28	K	5.40	1.92	11.52

Table 8: ***Elemental concentration at area 3 on Brown Particle***

Element	Atomic Number	Series	Weight %	Mole %	% Error
Carbon	6	K	24.06	35.02	23.86
Oxygen	8	K	18.59	20.32	25.75
Fluorine	9	K	44.06	40.55	16.94
Nickel	28	L	11.46	3.41	13.05
Aluminium	13	K	0.38	0.24	14.05
Iron	26	K	1.45	0.45	23.69

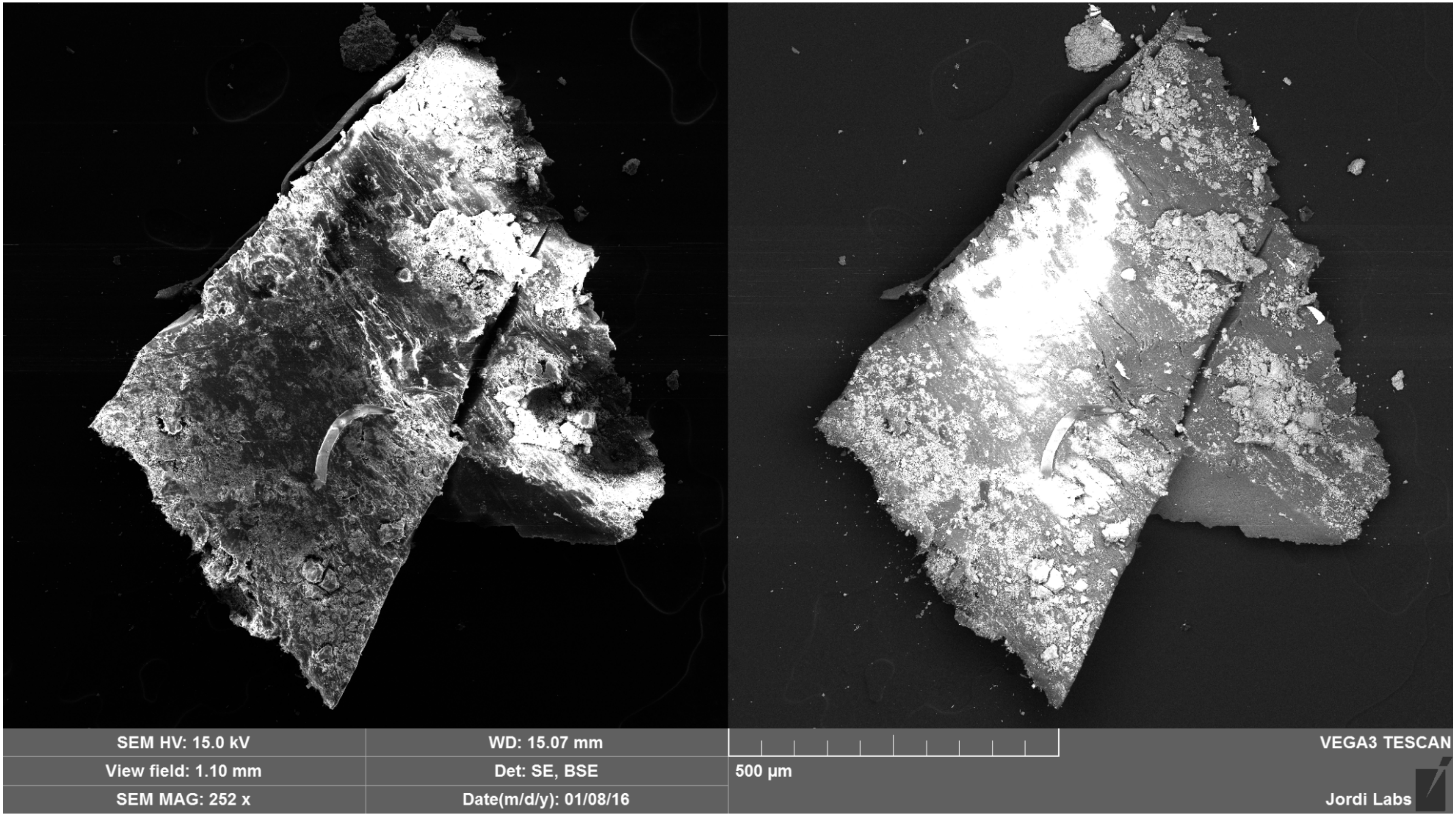

Figure 15: SEM SE (left) and BSE (right) images of *Brown Particle* before mapping

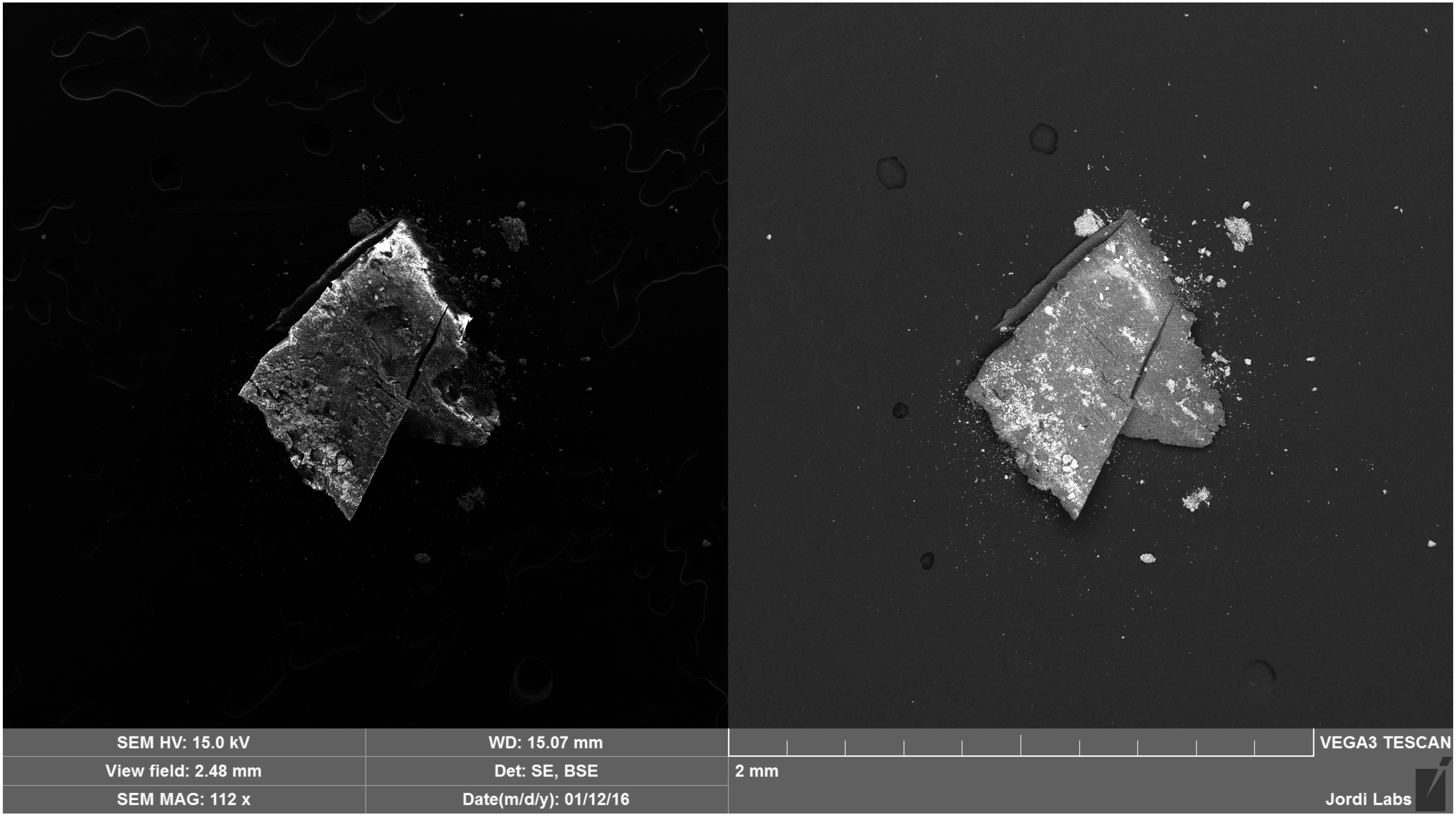

Figure 16: SEM SE (left) and BSE (right) images of *Brown Particle* after mapping

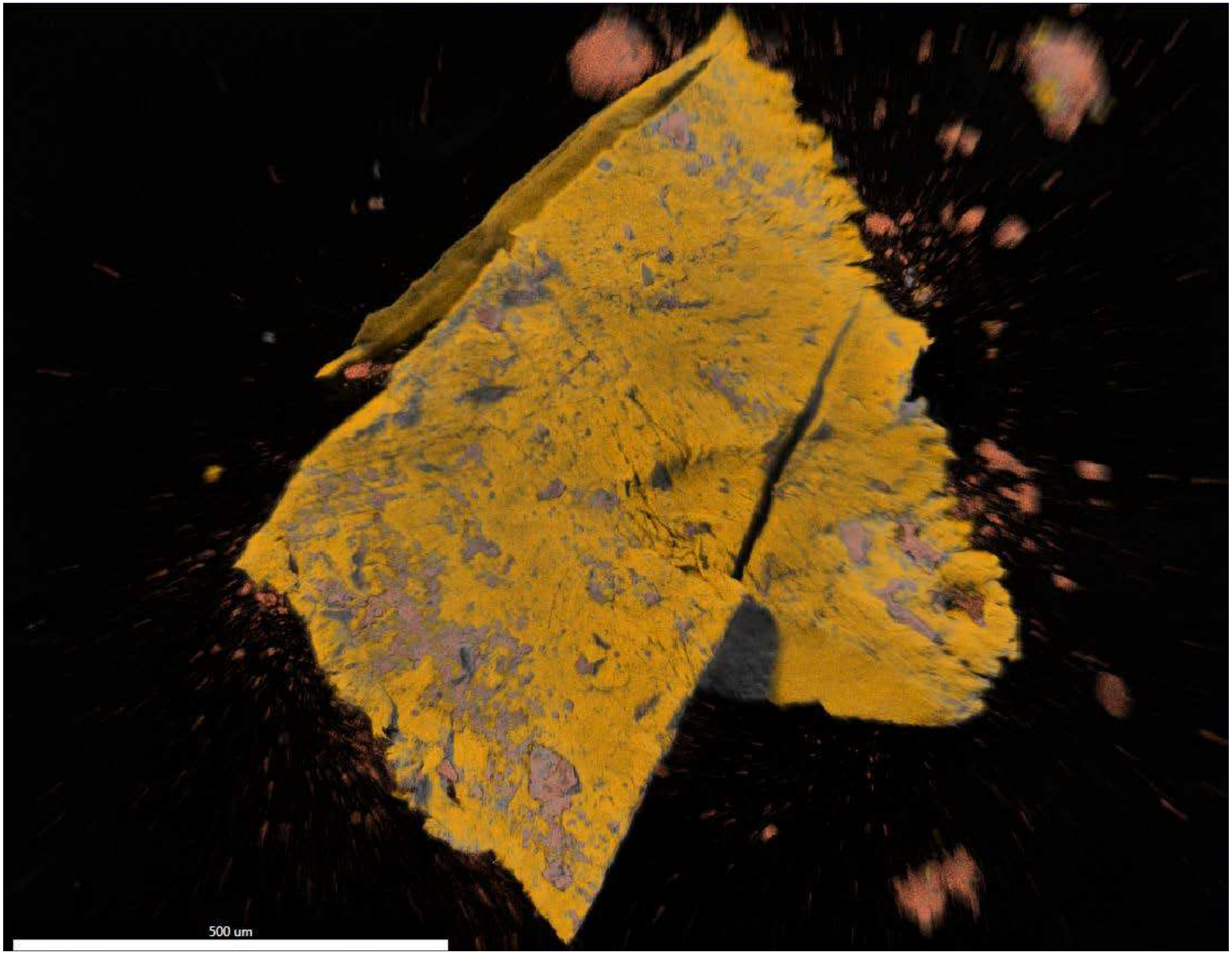

Figure 17: EDX map overlay of Fluorine (yellow) and Iron (reddish-orange)

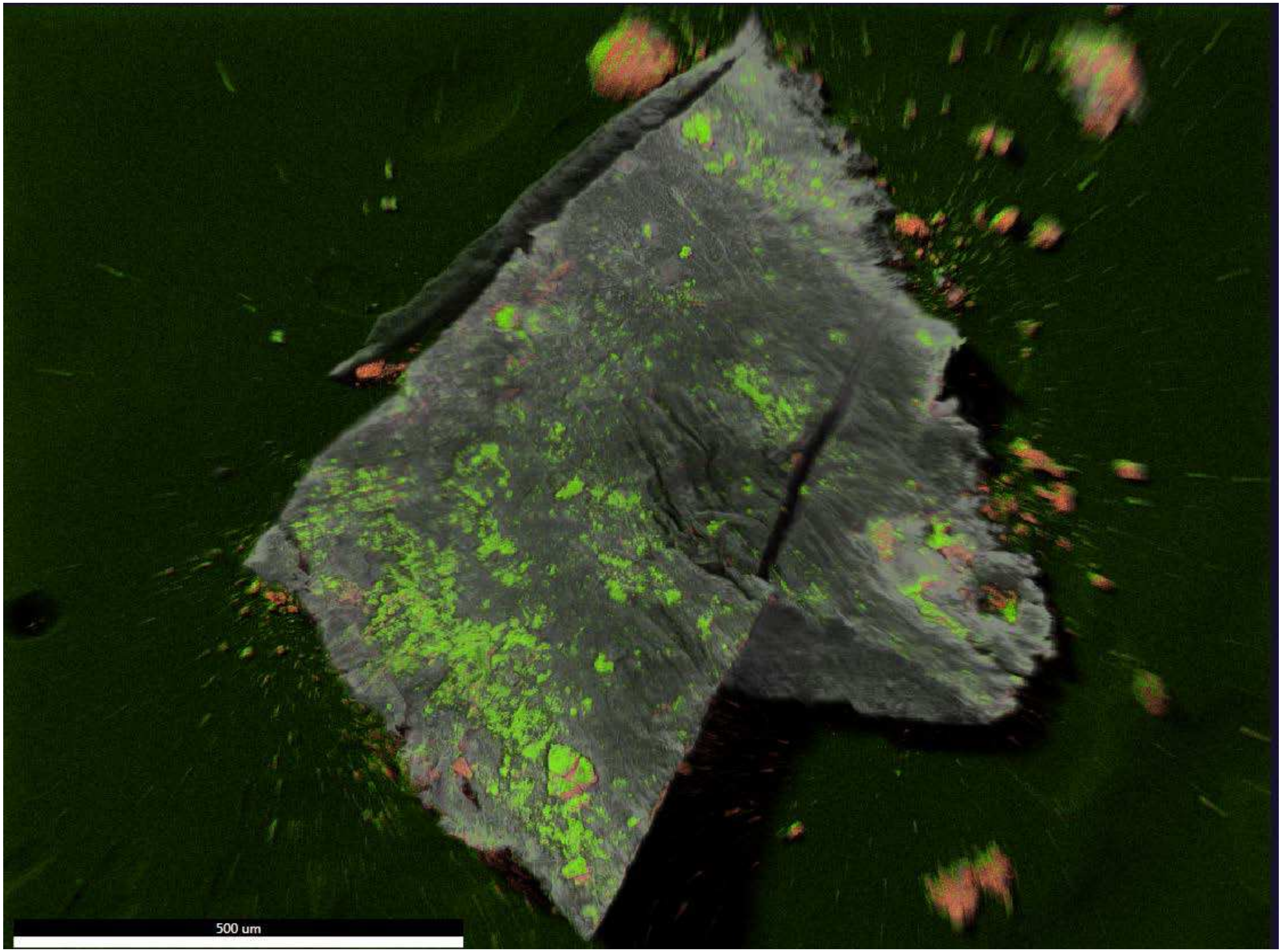

Figure 18: EDX map overlay of Oxygen (green) and Iron (reddish-orange)

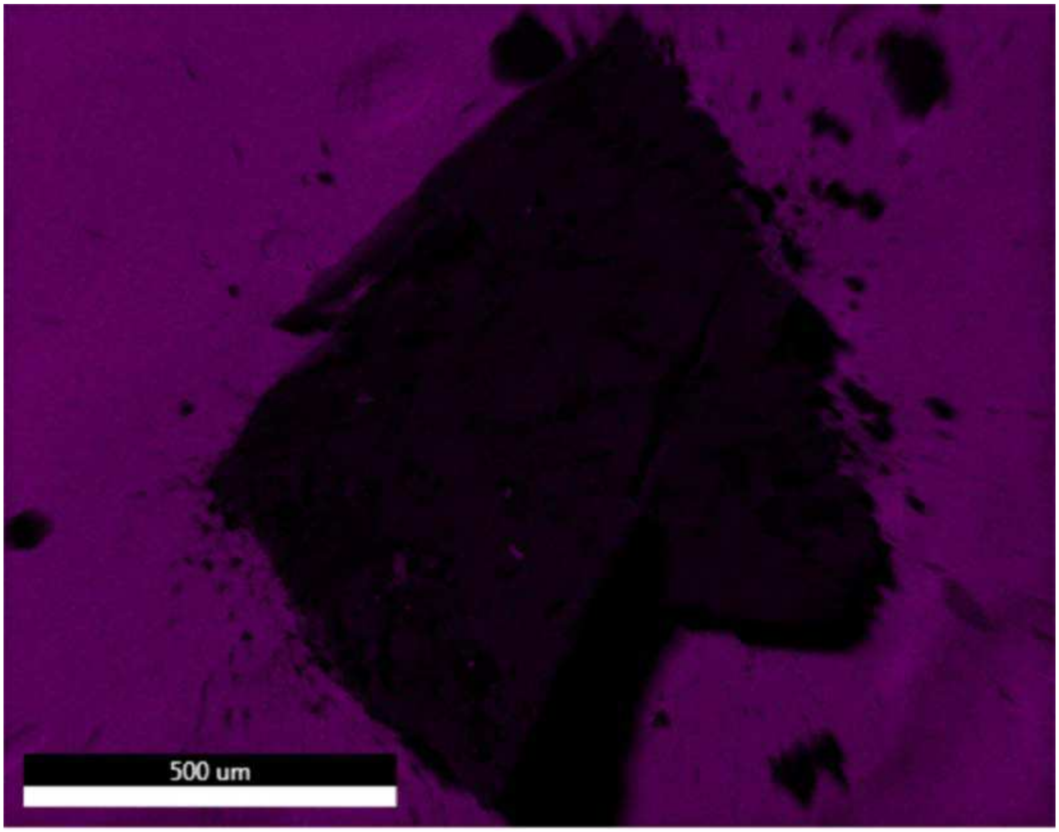

Figure 19: EDX map of Carbon

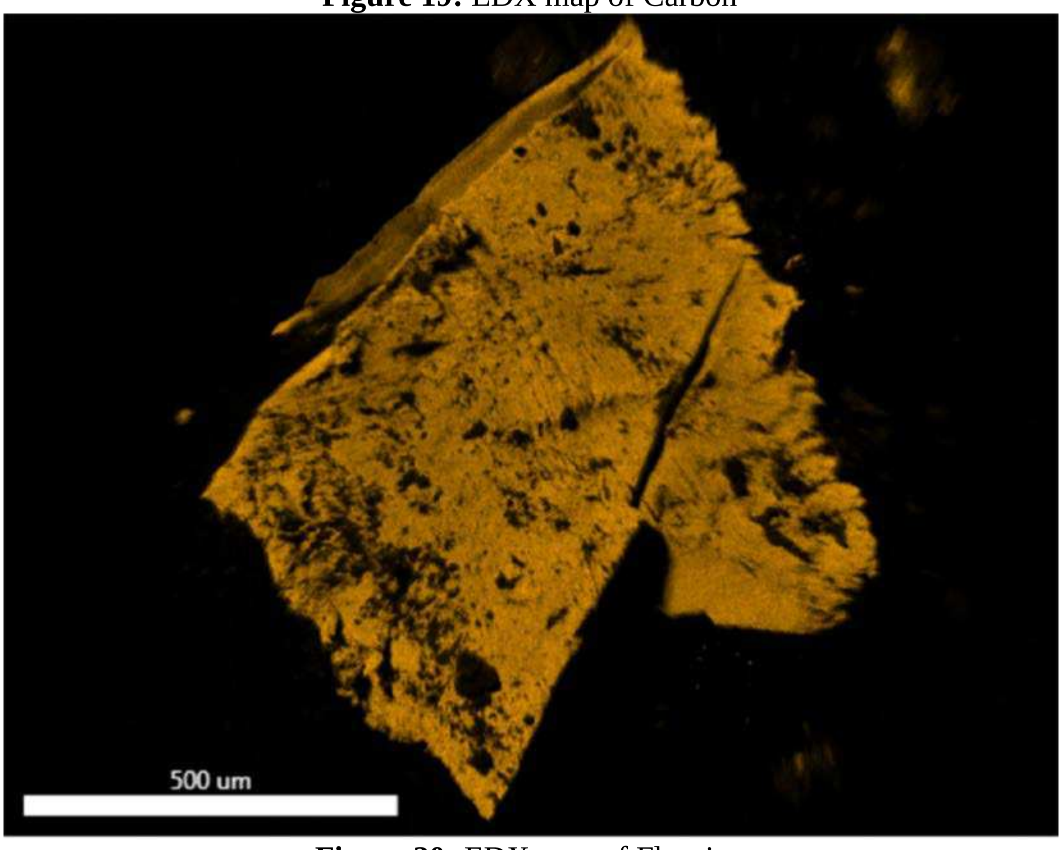

Figure 20: EDX map of Fluorine

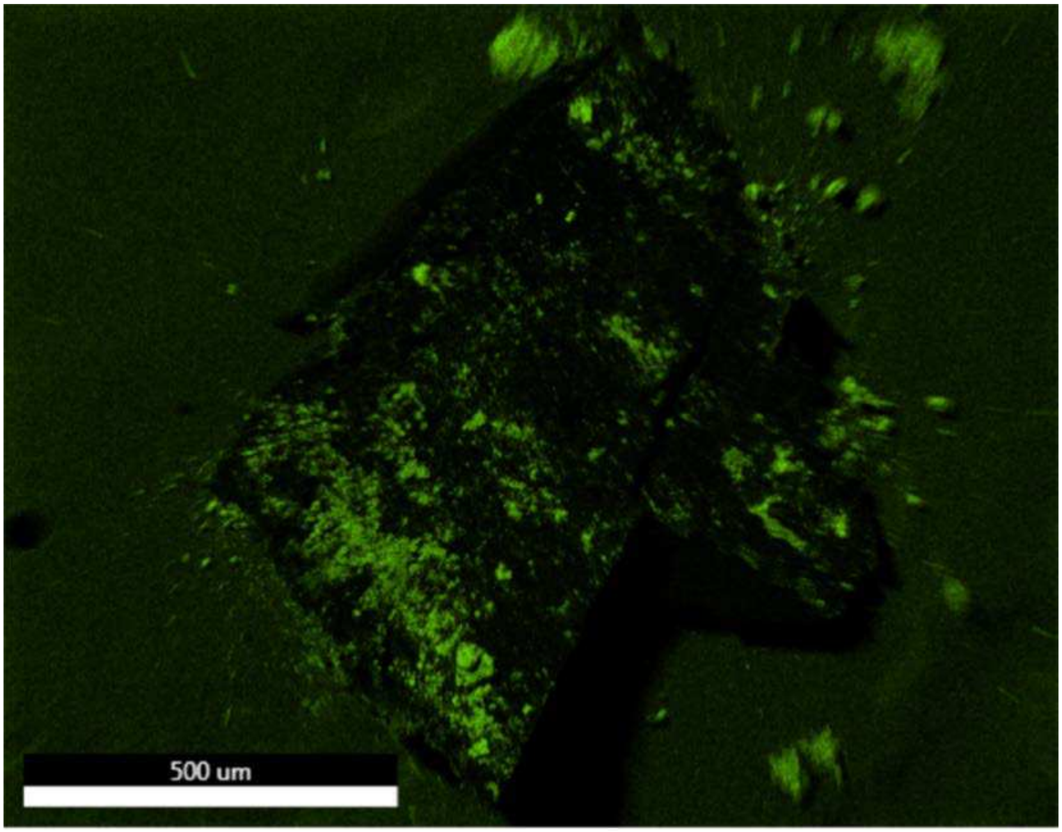

Figure 21: EDX map of Oxygen

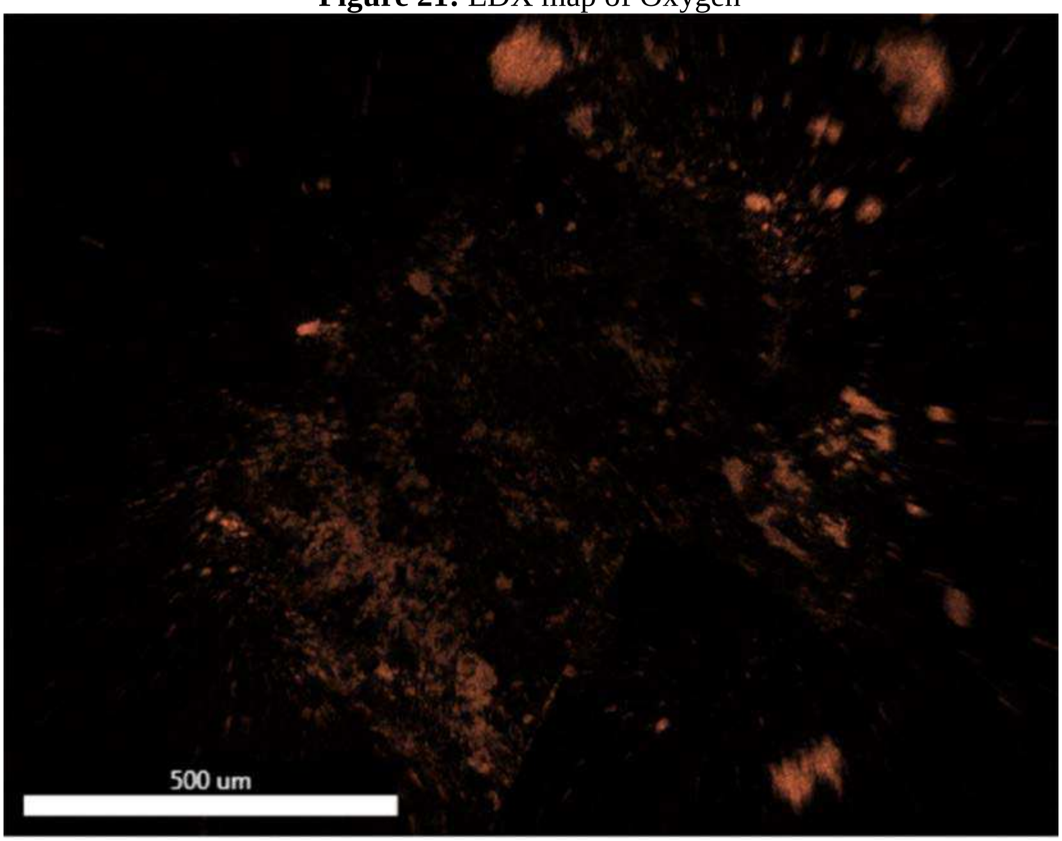

Figure 22: EDX map of Iron

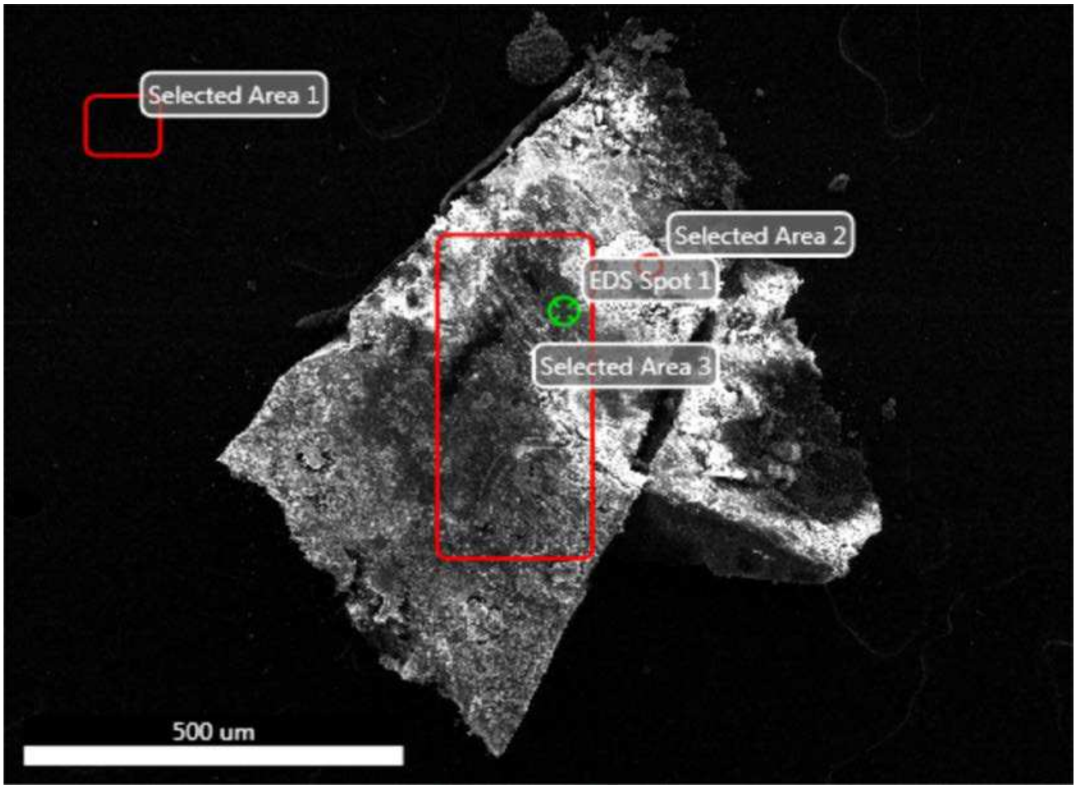

Figure 23: SEM-EDX image of all sampling locations analyzed of *Brown Particle*

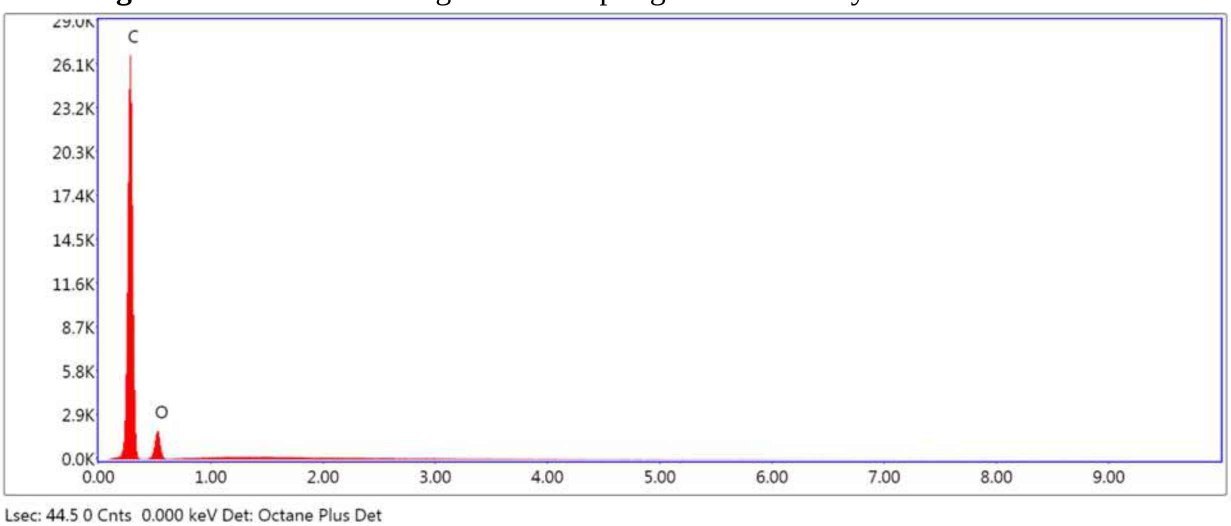

Figure 24: SEM-EDX elemental abundance of area 1 (control) analyzed of *Brown Particle*

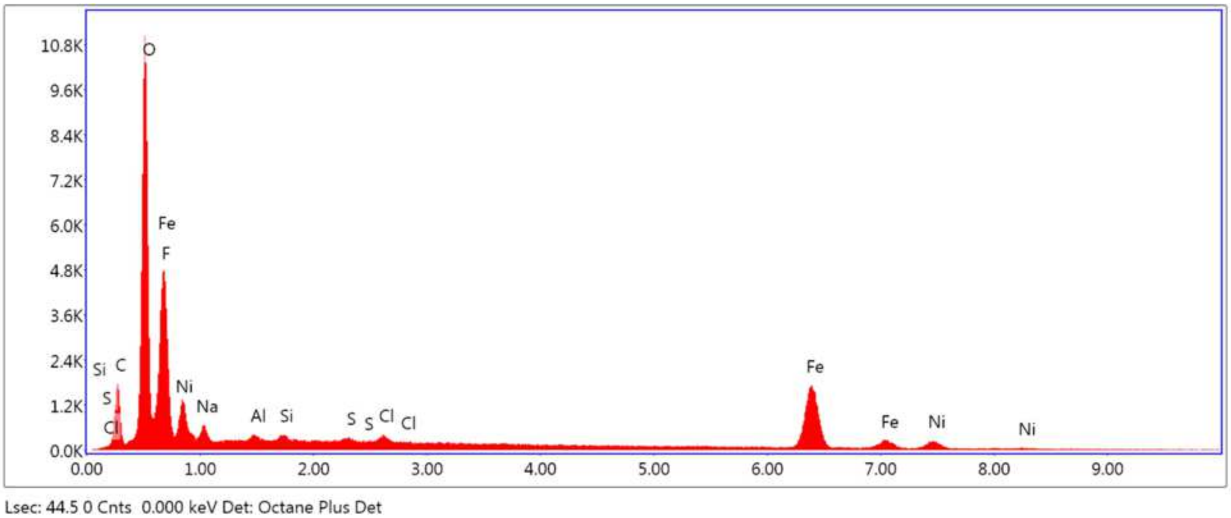

Figure 25: SEM-EDX elemental abundance of area 2 analyzed of *Brown Particle*

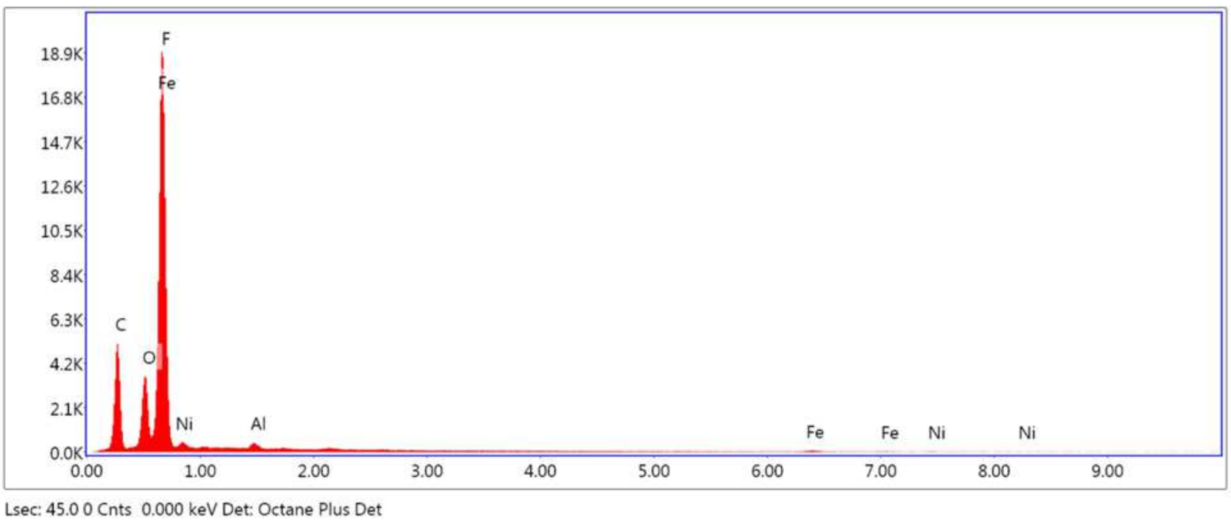

Figure 26: SEM-EDX elemental abundance of area 3 analyzed of *Brown Particle*

Analysis Conditions

This section of a Jordi report provides information on the methods used including instrument type, temperatures, solvents, sample preparation, etc. The specific conditions have been removed for this case study.

Closing Comments

Deformulation of an unknown material is intended to provide a best estimate of the chemical nature of the sample. All chemical structures are supported by the evidence presented but are subject to revision upon receipt of additional evidence. Additional factors such as material processing conditions may also affect final material properties.

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Jordi Labs specializes in polymer testing and has 30 years experience doing complete polymer deformulations. We are one of the few labs in the country specialized in this type of testing. We will work closely with you to help explain your test results and solve your problem. We appreciate your business and are looking forward to speaking with you concerning these results.

Sincerely,

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