

# Detecting Engineered Nanomaterials in Baby Formulas

Prepared by:

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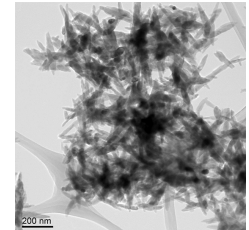
Prepared For:

Friends of the Earth

May 4, 2016

# Summary

- Two baby formulas from Friends of the Earth (ROUND 2 samples) were analyzed:
  - Gerber Good Start Soothe
  - Enfamil Baby Formula
  - Presence of engineered nanomaterials assessed using transmission electron microscopy (TEM) with energy dispersive x-ray (EDX) detection
    - Gerber Good Start Soothe Product:
      - A few other particles <100nm were detected and contained calcium & phosphate but were not needle-like in shape
    - Enfamil Product:
      - Samples are primarily composed of Ca, P, and O containing particles with some C, Na, and Cl containing particles
      - Calcium phosphate particles (nano HA) appeared to be a mixture of needle-like and non needle-like hydroxy-apatite
- Four formulas from FOE (ROUND 1 samples) were re-analyzed. General findings:
  - Needle-like calcium phosphate nanoparticles (nano HA) detected in Gerber Good Start Gentle & Well Beginning dry formulas, but not in the liquid formula
  - One sample (Similac Advance dry formula) appeared to contain silica oxide nanoparticles, but no HA.
  - Previous samples indicated titanium oxide nanoparticles
  - Attempts to repeat separation and analysis found only a few Si- and Ti-particles particles. Results highlight the challenges of extracting nanoparticles by centrifugation from complex organic matrices.
- Two hydroxy apatite (HA) reference food additive products procured
  - One was needle-like and the other was not
  - Provides positive confirmation that detected nano HA in baby formulas was likely added, rather than an artifact of any sample handling



Hydroxy apatite NPs

# Sample Preparation

The foods (~0.125 g each) were suspended in 40 mL ultrapure water and sonicated for 30 minutes to suspend particles. These samples were centrifuged at 15,000 G for 15 minutes to settle any particles present. The organics-rich supernatant was poured off, leaving a pellet of particulate matter in the centrifuge tube. This was re-suspended in 20 mL ultrapure water and sonicated for 5 minutes, then 100  $\mu$ L volumes were pipetted onto a copper/lacey carbon transmission electron microscopy grid and allowed to dry. Microscopy was performed on a Philips CM200 transmission electron microscope with energy dispersive spectroscopy as well as an XL30 ESEM-FEG scanning electron microscope with energy dispersive spectroscopy. Mean particle diameter was measured manually with ImageJ software. Particle number size distributions were developed and the percentage of particles less than 100 nm in width determined. Copper and Aluminum peaks are a result of the copper TEM grids and Aluminum SEM stubs used for analysis.

# Analysis of Particle Size

Sizing analysis was performed using ImageJ, a free image processing program available from the National Institute of Health. The number of primary particles was noted and were sized. The scale bar was used to set the scale for calculating each particle's diameter. In the case of high aspect ratio structures found in baby formula, both a width and length were measured. Error is reported as +/- 0.5 standard deviation. Percent of particles below 100nm was calculated based on the primary particles imaged.

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# FOE provided sample (Round 2) Gerber Baby Formula Powder



NUTRIENTS PER 100 CALORIES (5 FL OZ, PREPARED AS DIRECTED):		
PROTEIN	2.2	g
FAT	5.1	g
CARBOHYDRATE	11.2	g
WATER	131	g
LINOLEIC ACID	900	mg
<b>VITAMINS</b>		
A	300	IU
D	60	IU
E	2	IU
K	8	mcg
THIAMINE (B <sub>1</sub> )	100	mcg
RIBOFLAVIN (B <sub>2</sub> )	140	mcg
B <sub>6</sub>	75	mcg
B <sub>12</sub>	0.33	mcg
NIACIN	1050	mcg
FOLIC ACID (FOLACIN)	15	mcg
PANTOTHENIC ACID	450	mcg
BIOTIN	4.4	mcg
C (ASCORBIC ACID)	10	mg
CHOLINE	24	mg
INOSITOL	6	mg
<b>MINERALS</b>		
CALCIUM	72	mg
PHOSPHORUS	40	mg
MAGNESIUM	7	mg
IRON	1.5	mg
ZINC	0.8	mg
MANGANESE	15	mcg
COPPER	80	mcg
IODINE	12	mcg
SELENIUM	3	mcg
SODIUM	27	mg
POTASSIUM	108	mg
CHLORIDE	65	mg

## INGREDIENTS:

CORN MALTODEXTRIN, WHEY PROTEIN CONCENTRATE (FROM COW'S MILK, ENZYMATICALLY HYDROLYZED, REDUCED IN MINERALS), VEGETABLE OILS (PALM OLEIN, SOY, COCONUT, AND HIGH-OLEIC SAFFLOWER OR HIGH-OLEIC SUNFLOWER), AND LESS THAN 2% OF: POTASSIUM CITRATE, POTASSIUM PHOSPHATE, CALCIUM CHLORIDE, CALCIUM PHOSPHATE, SODIUM CITRATE, MAGNESIUM CHLORIDE, FERROUS SULFATE, ZINC SULFATE, COPPER SULFATE, POTASSIUM IODIDE, MANGANESE SULFATE, SODIUM SELENATE, M. ALPINA OIL\*, C. COHNII OIL\*\*, SODIUM ASCORBATE, INOSITOL, CHOLINE BITARTRATE, ALPHA-TOCOPHERYL ACETATE, NIACINAMIDE, CALCIUM PANTOTHENATE, RIBOFLAVIN, VITAMIN A ACETATE, PYRIDOXINE HYDROCHLORIDE, THIAMINE MONONITRATE, FOLIC ACID, PHYLLIQUINONE, BIOTIN, VITAMIN D<sub>3</sub>, VITAMIN B<sub>12</sub>, TAURINE, NUCLEOTIDES (CYTIDINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE, ADENOSINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE), ASCORBYL PALMITATE, MIXED TOCOPHEROLS, L-CARNITINE, SOY LECITHIN, *LACTOBACILLUS REUTERI* (DSM 17938) CULTURES.

\* A SOURCE OF ARACHIDONIC ACID (ARA)

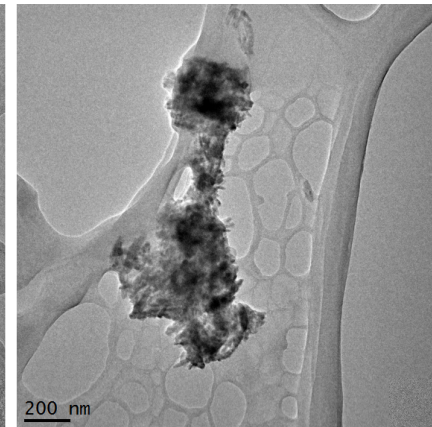
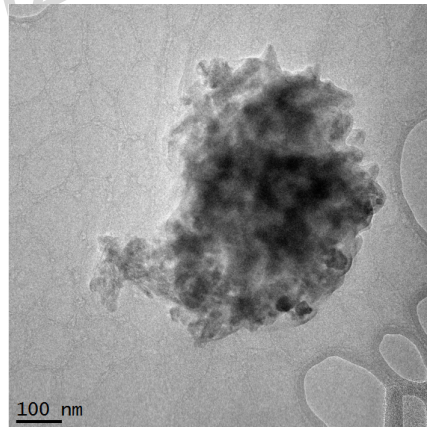
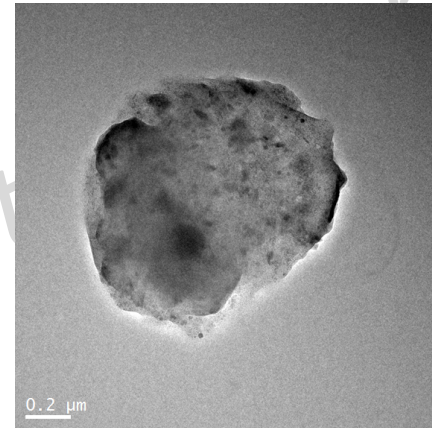
\*\* A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)

## Gerber Summary

- Images are organized from most persistent to least in the sample
- Samples are primarily composed of some particles containing carbon, calcium, potassium, phosphorous, silicon, and sulfur
- 30 particles counted with 100% below 100 nm in diameter
- X-ray Diffraction results were inconclusive in characterizing nanoparticles due to high organic content, or low content of crystalline metal

# Ca, O, P, Al, Ti, Si, S, P, K, and Fe containing particle

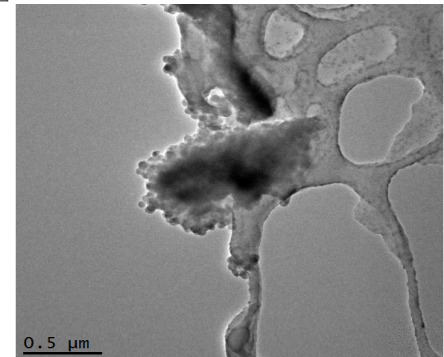
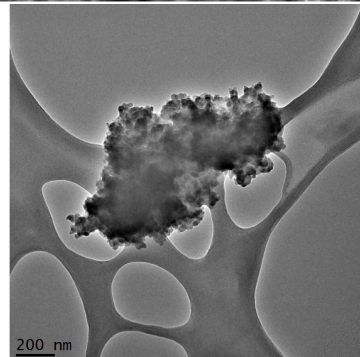
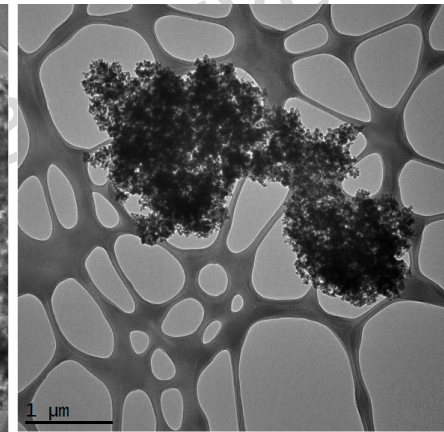
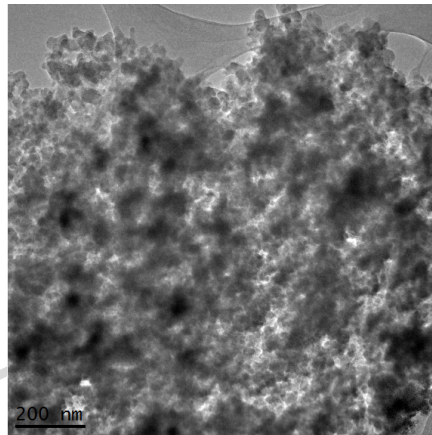
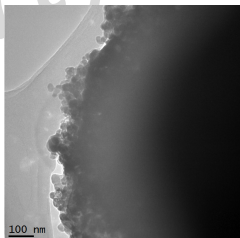
- 1,200 nm by 1,000 nm particle composed of 10 – 30 nm particles containing Ca, O, P, Al, Si, S, P, K, and Fe.
- 30 particles counted with 100% below 100 nm in diameter



# Silica, Titanium and oxygen detected

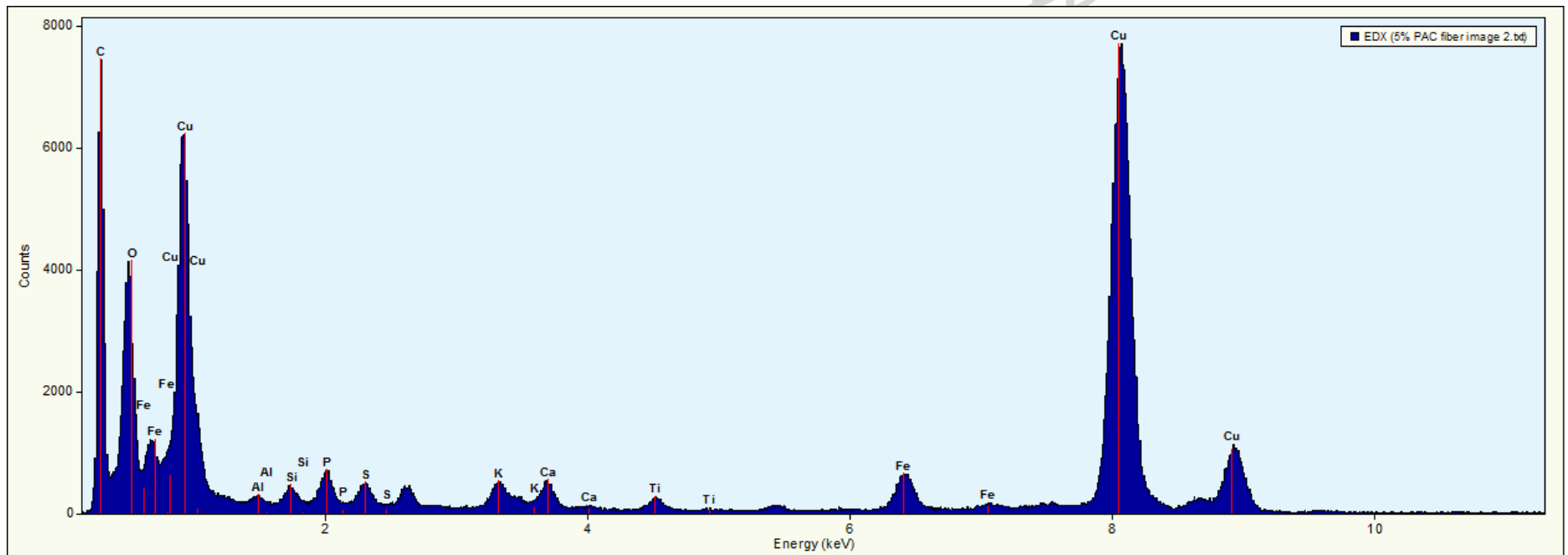
- Silica nanoparticles identified in sample (right images)
- Searched 20+ grids for materials
  - Only 4 Si- particles and 2 Ti- particles
- Titanium dioxide nanoparticles detected (below)

Ti and O on a  
Ca,P, O particle



# EDX of particle on slide 15

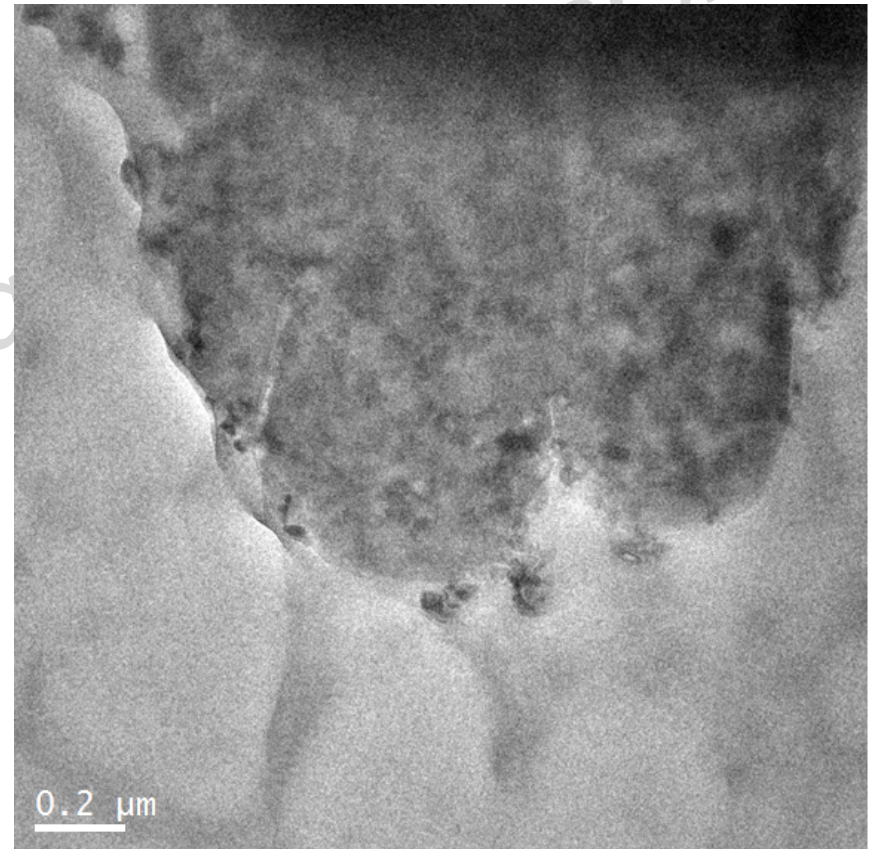
- Carbon and Cu from TEM grid





## C, P, and Ca containing particle

- Calcium and phosphorous containing particles in a carbon film
- Average Ca, P particles is 32 nm
- 24 particles counted with 100% of particles below 100nm



# FOE Provide sample (Round 2) Enfamil EnfaCare Baby Formula



## NUTRIENTS (Normal Dilution); per 100 Calories (4.5 fl oz)

PROTEIN	g	2.8	WATER	g	117
FAT	g	5.3	LINOLEIC ACID	mg	860
CARBOHYDRATE	g	10.4			

## VITAMINS

A	IU	450	NIACIN	mcg	1000
D	IU	70	FOLIC ACID (FOLACIN)	mcg	26
E	IU	4	PANTOTHENIC ACID	mcg	850
K	mcg	8	BIOTIN	mcg	5
THIAMIN (B1)	mcg	200	C (ASCORBIC ACID)	mg	16
RIBOFLAVIN (B2)	mcg	200	CHOLINE	mg	24
B6	mcg	60	INOSITOL	mg	30
B12	mcg	0.3			

## MINERALS

CALCIUM	mg	120	COPPER	mcg	120
PHOSPHORUS	mg	66	IODINE	mcg	21
MAGNESIUM	mg	8	SELENIUM	mcg	2.8
IRON	mg	1.8	SODIUM	mg	37
ZINC	mg	1	POTASSIUM	mg	105
MANGANESE	mcg	15	CHLORIDE	mg	78

**INGREDIENTS:** NONFAT MILK, WHEY PROTEIN CONCENTRATE, CORN SYRUP SOLIDS, LACTOSE, HIGH OLEIC VEGETABLE OIL (SUNFLOWER AND/OR SAFFLOWER OIL), SOY OIL, MEDIUM CHAIN TRIGLYCERIDES (MCT OIL), COCONUT OIL, AND LESS THAN 2%: MORTIERELLA ALPINA OIL\*, CRYPTHECODINIUM COHNII OIL\*\*, VITAMIN A PALMITATE, VITAMIN D3, VITAMIN E ACETATE, VITAMIN K1, THIAMIN HYDROCHLORIDE, RIBOFLAVIN, VITAMIN B6 HYDROCHLORIDE, VITAMIN B12, NIACINAMIDE, FOLIC ACID, CALCIUM PANTOTHENATE, BIOTIN, ASCORBIC ACID, CHOLINE CHLORIDE, INOSITOL, CALCIUM CARBONATE, CALCIUM PHOSPHATE, MAGNESIUM CHLORIDE, FERROUS SULFATE, ZINC SULFATE, MANGANESE SULFATE, CUPRIC SULFATE, POTASSIUM IODIDE, SODIUM SELENITE, SODIUM CHLORIDE, POTASSIUM CITRATE, TAURINE, L-CARNITINE, NUCLEOTIDES (ADENOSINE 5'-MONOPHOSPHATE, CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE).

\*A SOURCE OF ARACHIDONIC ACID (ARA)

\*\*A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)

# Enfamil EnfaCare Baby Formula Summary

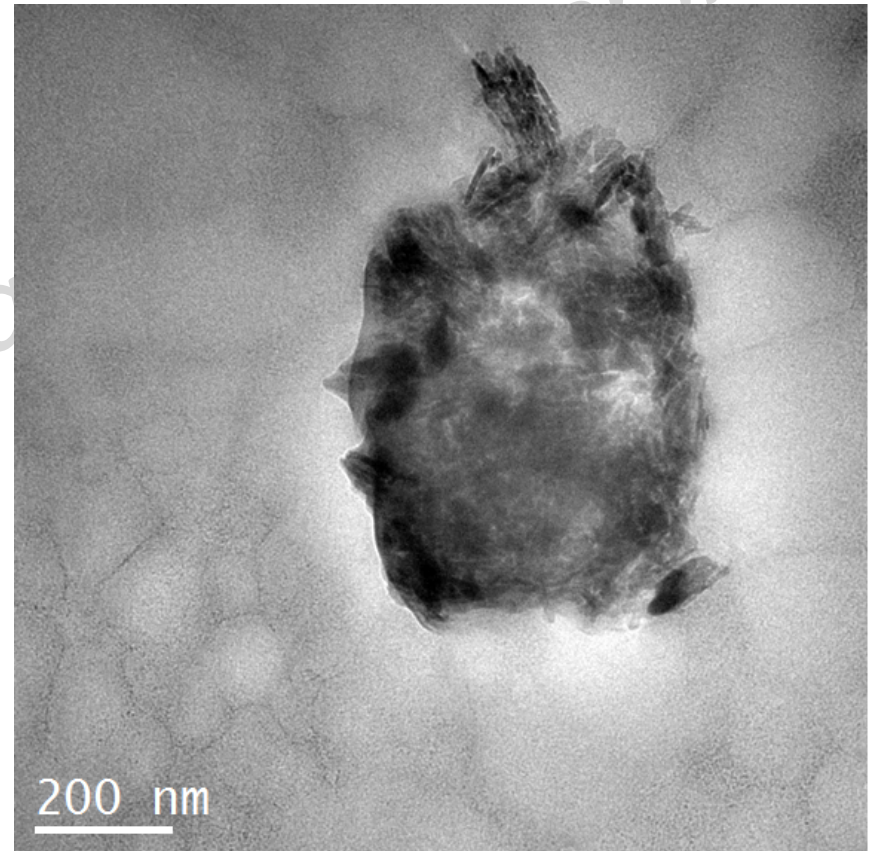
- Images are organized from most persistent to least in the sample
- Aggregates of high aspect ratio nanoparticles containing Ca, P, and O were observed

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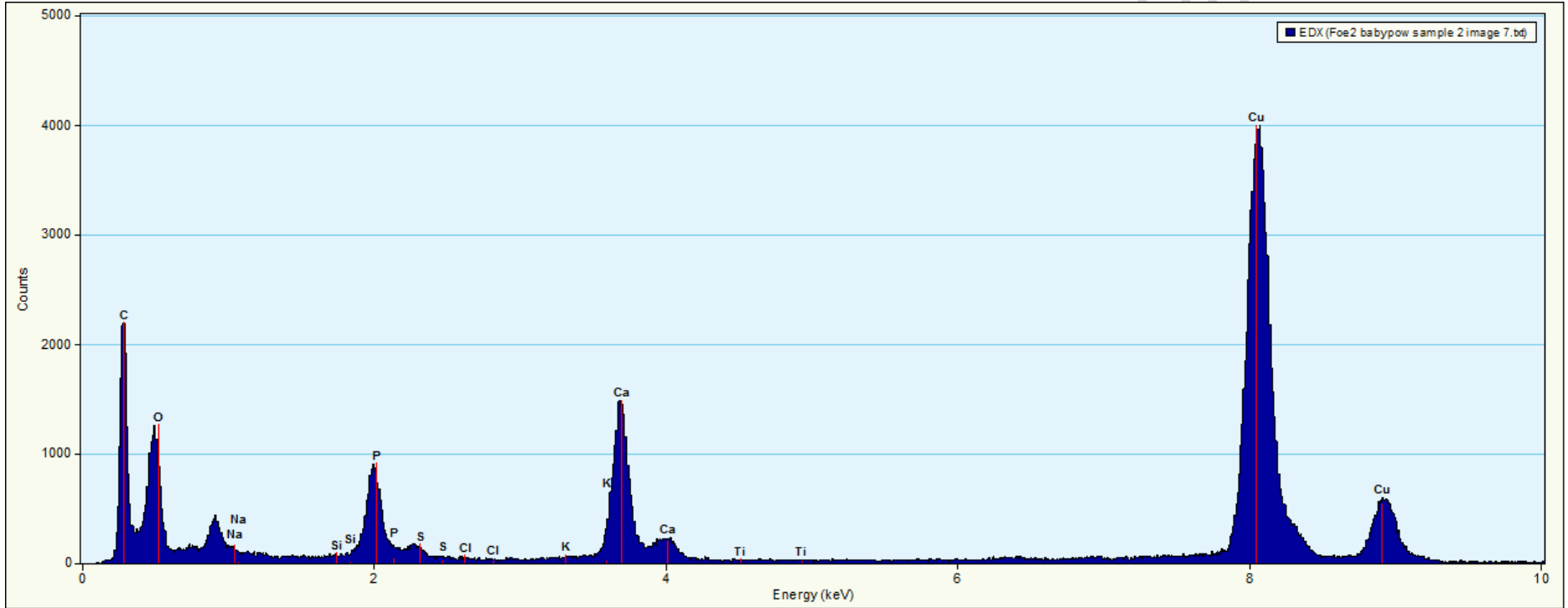
## Ca, P, and O containing particles

- Calcium phosphate particle inside a carbon film
- Aggregate particle is 756 nm by 515 nm
- Average particle size: 13 nm (width) and 110 nm (length)
- 56 particles counted with 100% of particles having one dimension less than 100 nm



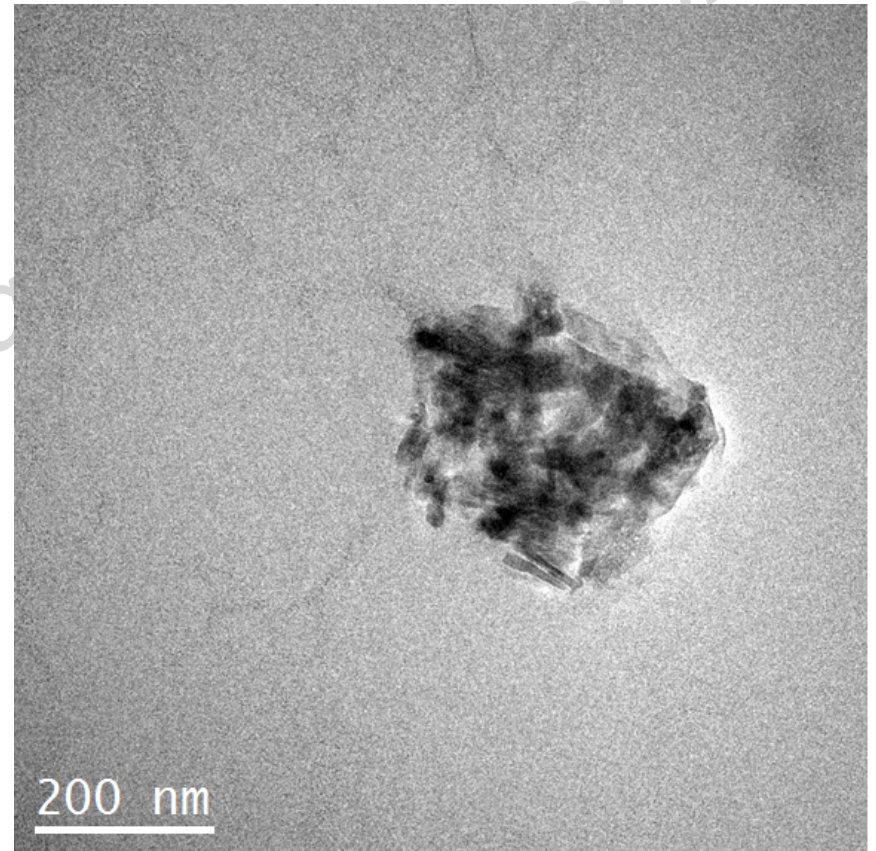
# EDX of particle on slide 20

- C and Cu peaks are from TEM grid



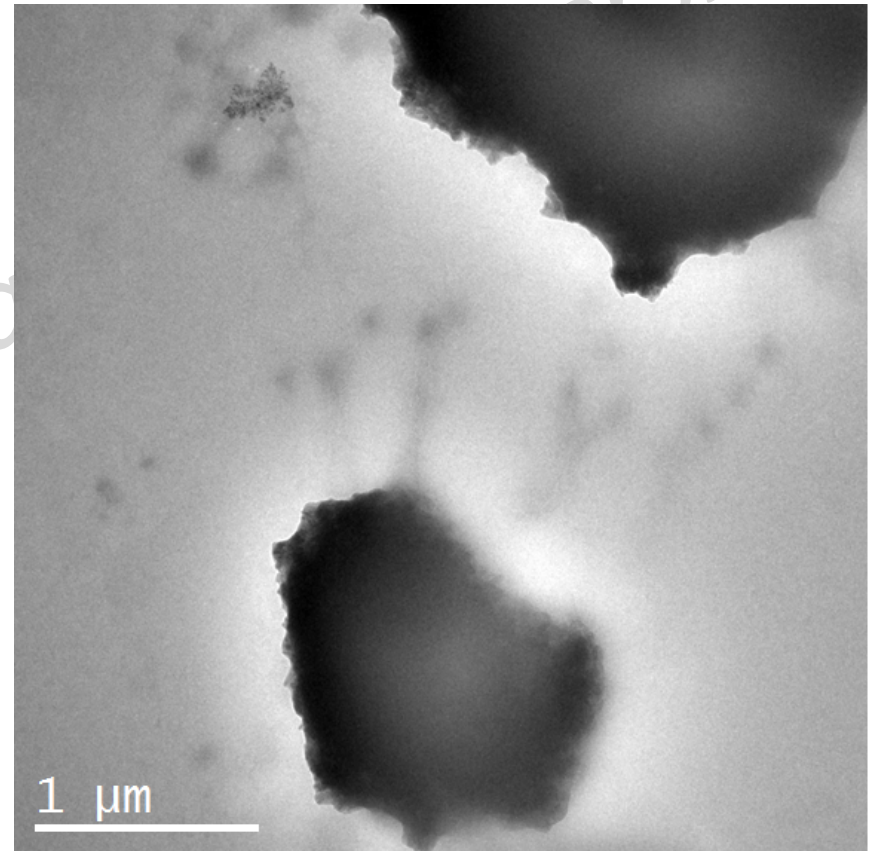
## Ca, P, and O containing particles

- Aggregate particle 340 nm by 320 nm
- Average particle size: 11 nm (width) and 75 nm (length)
- Perhaps needle-like shaped materials
- 26 particles counted with 100% of particles having one dimension less than 100nm



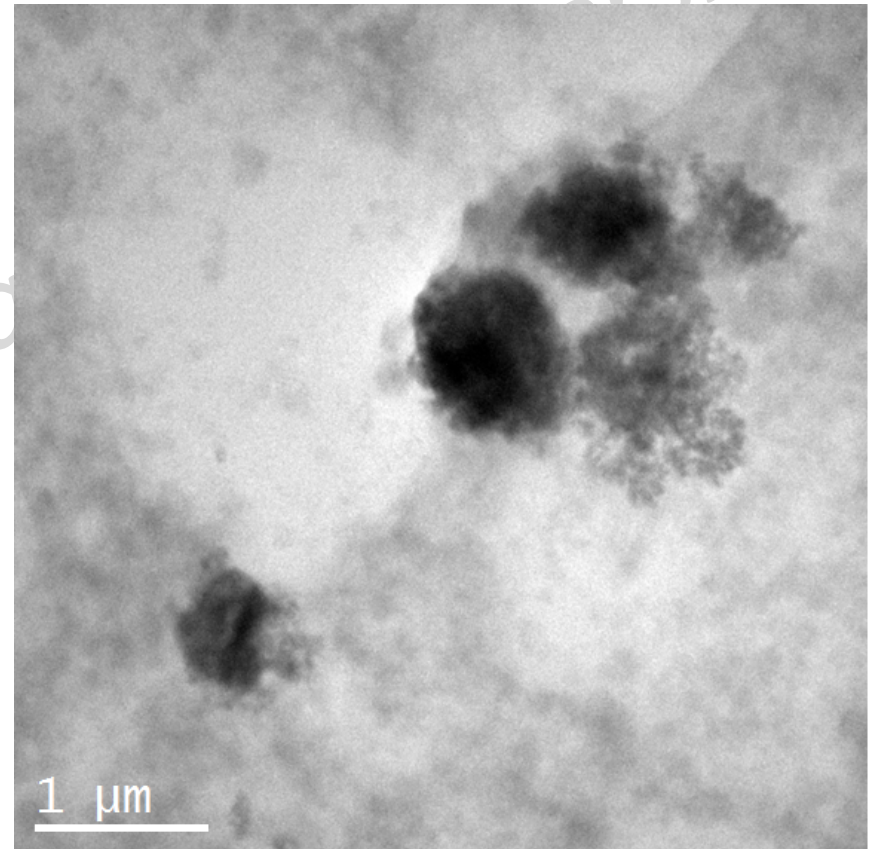
## Ca, P, and O containing particles

- Bottom particle is 1,627 nm by 1,065 nm composed of smaller particles which proved difficult to measure due to the contrast



## Ca, P, and O containing particles

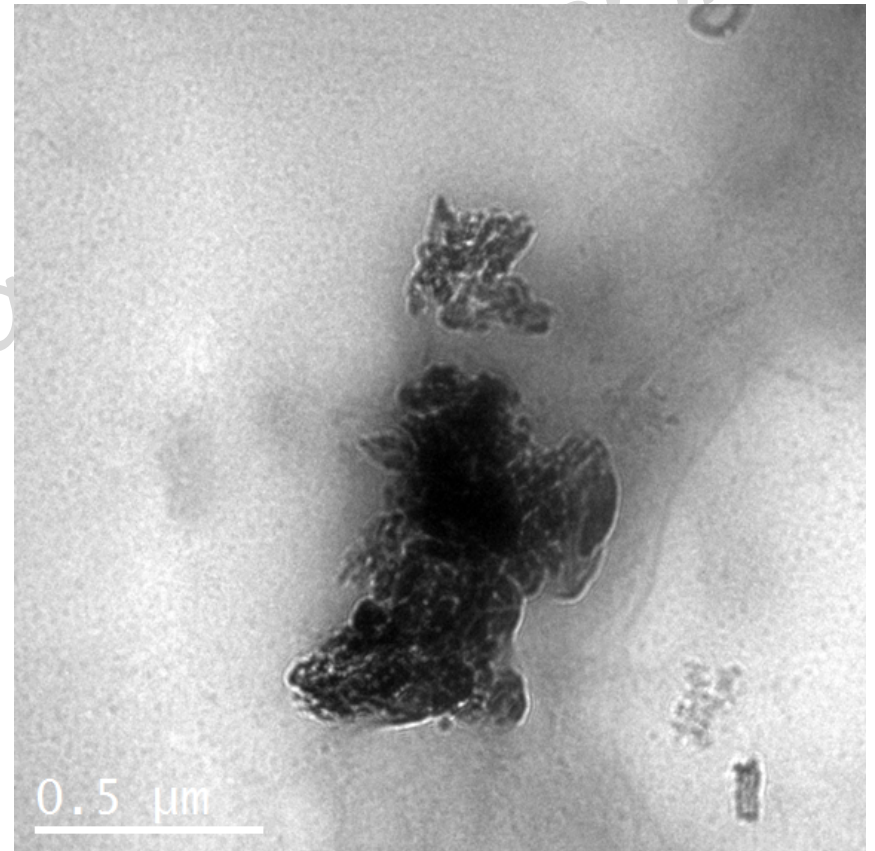
- Aggregate particles composed of Ca, P, and O with an average size of 870 nm
- Individuals difficult to size due to contrast
- Approximately 50 – 100 nm in size
- Not needle-like in structure





## Ca, P, and O containing particles

- Aggregate particle 790 nm by 500 nm containing Ca, P, and O
- Individual particles average: 27 nm (width) and 150 nm (length)
- 27 particles counted with 100% of particles with one dimension less than 100 nm



## Previous Baby Formula Study (FOE Round 1)

- 3 dry Baby Formulas were re-analyzed
  - Well Beginnings Advantage
  - Gerber Good Start Gentle
  - Similac Advance
- 1 liquid baby formula was re-analyzed (Similac Advance liquid)
- Presence of engineered nanomaterials using transmission electron microscopy (TEM) with energy dispersive x-ray (EDX) detection as well as scanning electron microscopy (SEM) with EDX

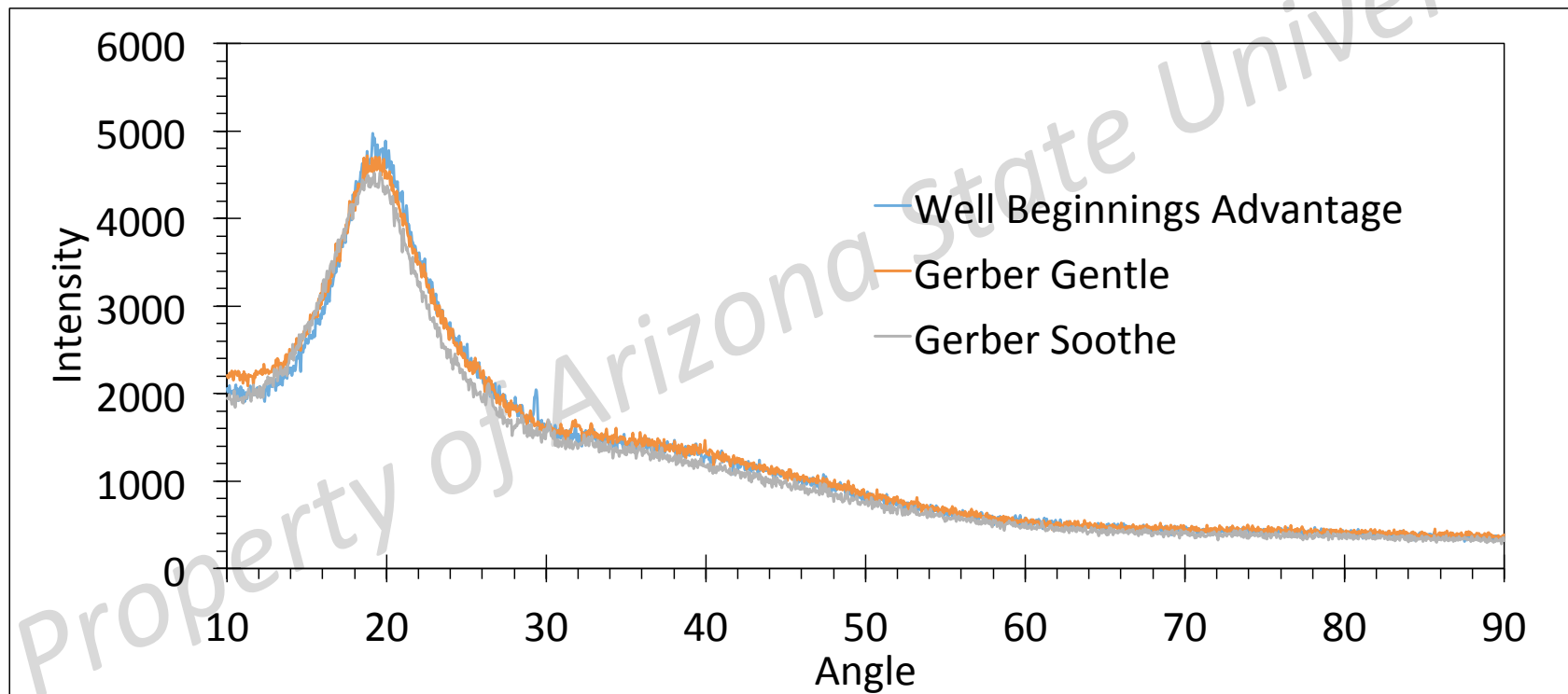
# Preparation

The foods (~0.125 g each) were suspended in 40 mL ultrapure water and sonicated for 30 minutes to suspend particles. These samples were centrifuged at 15,000 G for 15 minutes to settle any particles present. The organics-rich supernatant was poured off, leaving a pellet of particulate matter in the centrifuge tube. This was re-suspended in 20 mL ultrapure water and sonicated for 5 minutes, then 100 uL volumes were pipetted onto a copper/lacey carbon transmission electron microscopy grid and allowed to dry.

Microscopy was performed on a Philips CM200 transmission electron microscope with energy dispersive spectroscopy as well as an XL30 ESEM-FEG scanning electron microscope with energy dispersive spectroscopy. Mean particle diameter was measured manually with ImageJ software. Particle number size distributions were developed and the percentage of particles less than 100 nm in width determined. Copper and Aluminum peaks are a result of the copper TEM grids and Aluminum SEM stubs used for analysis.



# X-Ray Diffraction (XRD) Example spectra



## Summary of XRD

- Broad organic peak characteristic of amorphous carbon
- Inconclusive for measuring crystallinity of nanoparticles present in the baby formulas

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# Well Beginning Advantage



## Ingredients

INGREDIENTS: NONFAT MILK, LACTOSE, PALM OLEIN, WHEY PROTEIN CONCENTRATE, COCONUT OIL, SOY OIL, HIGH OLEIC (SAFFLOWER OR SUNFLOWER) OIL, GALACTO-OLIGOSACCHARIDES‡, LESS THAN 1%: CRYPTHECODINIUM COHNII OIL\*, MORTIERELLA ALPINA OIL\*\*, BETA-CAROTENE, LUTEIN, POTASSIUM BICARBONATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, MONOGLYCERIDES, CALCIUM CHLORIDE, CALCIUM HYDROXIDE, MAGNESIUM CHLORIDE, FERROUS SULFATE, CHOLINE BITARTRATE, ASCORBYL PALMITATE, SODIUM CITRATE, TAURINE, INOSITOL, ZINC SULFATE, MIXED TOCOPHEROL CONCENTRATE, VITAMIN E (d-ALPHA TOCOPHERYL ACETATE), NIACINAMIDE, CALCIUM PANTOTHENATE, L-CARNITINE, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, VITAMIN K (PHYTONADIONE), BIOTIN, SODIUM SELENITE, VITAMIN D3, CYANOCOBALAMIN, POTASSIUM PHOSPHATE, POTASSIUM IODIDE, POTASSIUM HYDROXIDE, NUCLEOTIDES (ADENOSINE-5'-MONOPHOSPHATE, CYTIDINE-5'-MONOPHOSPHATE, DISODIUM GUANOSINE-5'-MONOPHOSPHATE, DISODIUM URIDINE-5'-MONOPHOSPHATE).  
CONTAINS MILK AND SOY INGREDIENTS.

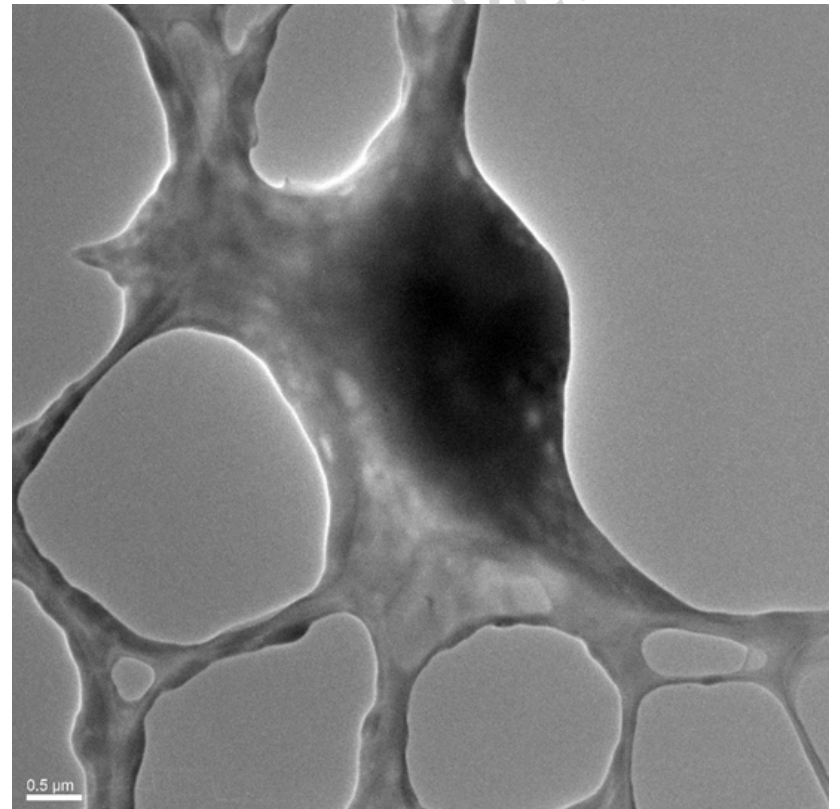
NUTRIENTS:	PER 100 CALORIES:
PROTEIN g	2.07
FAT g	5.6
CARBOHYDRATE g	10.7
WATER g	133
LINOLEIC ACID mg	860
<b>VITAMINS:</b>	
VITAMIN A IU	300
VITAMIN D IU	75
VITAMIN E IU	1.5
VITAMIN K mcg	8
THIAMINE (VITAMIN B1) mcg	100
RIBOFLAVIN (VITAMIN B2) mcg	160
VITAMIN B6 mcg	63
VITAMIN B12 mcg	0.26
NIACIN mcg	1100
FOLIC ACID (FOLACIN) mcg	16
PANTOTHENIC ACID mcg	470
BIOTIN mcg	4.6
VITAMIN C (ASCORBIC ACID) mg	9
CHOLINE mg	24
INOSITOL mg	4.9
<b>MINERALS:</b>	
CALCIUM mg	82
PHOSPHORUS mg	44
MAGNESIUM mg	6
IRON mg	1.9
ZINC mg	0.79
MANGANESE mcg	5
COPPER mcg	95
IODINE mcg	6
SELENIUM mcg	2
SODIUM mg	25
POTASSIUM mg	110
CHLORIDE mg	68

# Summary

- Carbon film based material
- Aggregates of high aspect ratio nanoparticles containing Ca, P, Si, and O were observed
- Average size  $28 \pm 5$  nm (width)  $160 \pm 30$  nm (length)
  - 250 particles counted with 100% of particles with one dimension less than 100 nm
- Nano needle-like HA observed

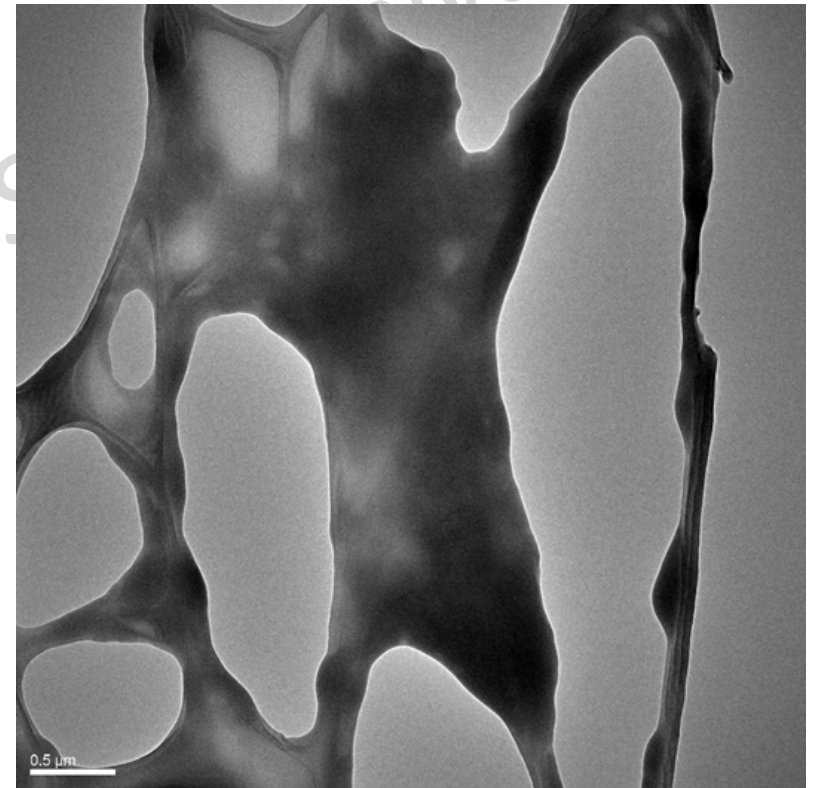
## C, O, P, Ca, Fe containing material

- Carbon film with Ca, P, and O elements within the film



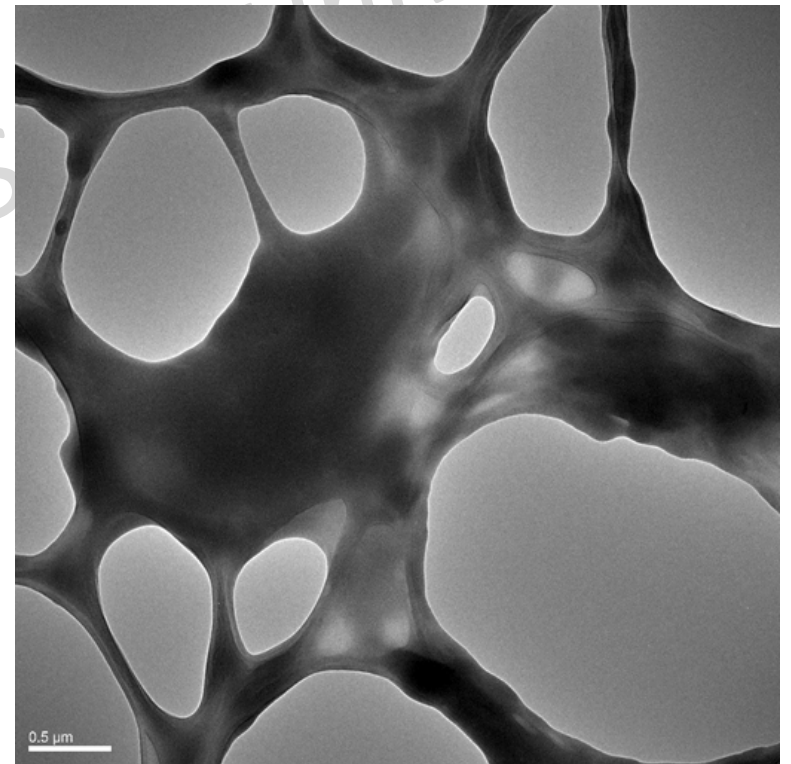
## C, O, P, and Ca containing material

- Carbon film with Ca, P, and O elements within the film



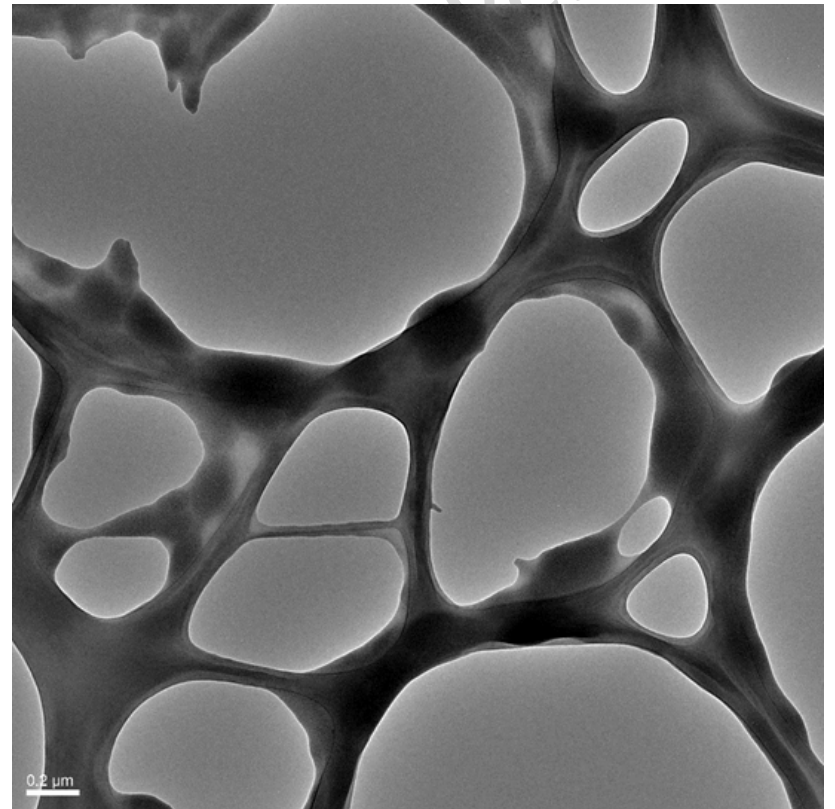
## C, O, P, Ca containing material

- Carbon film with Ca, P, and O elements within the film



## C, O, P, S, Cl, and Ca containing material

- Carbon film with Ca, P, S, Cl and O elements within the film



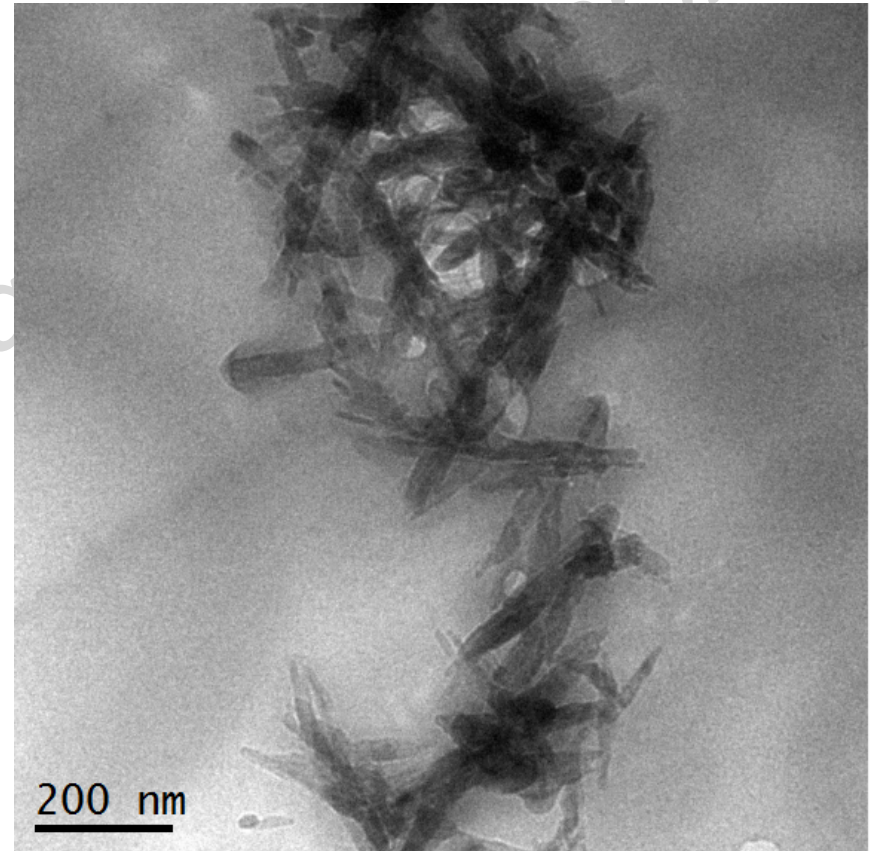
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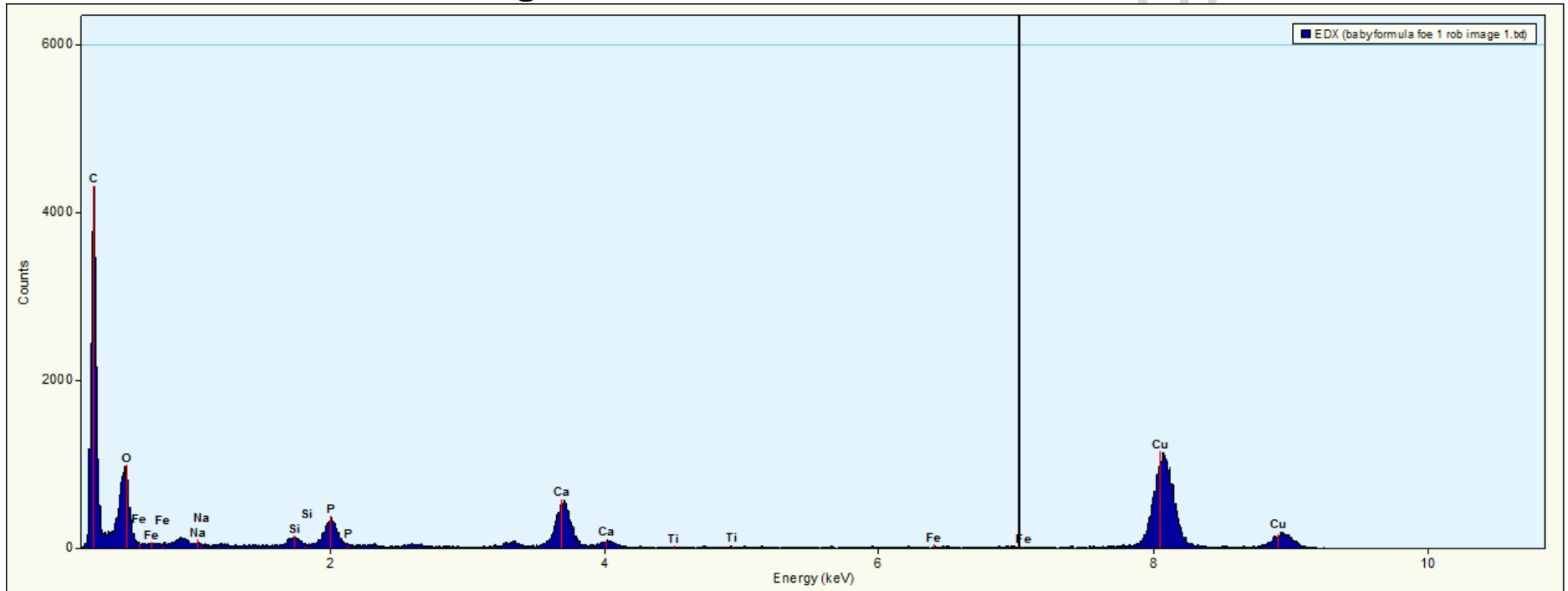
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 $160 \pm 30$  nm (length)
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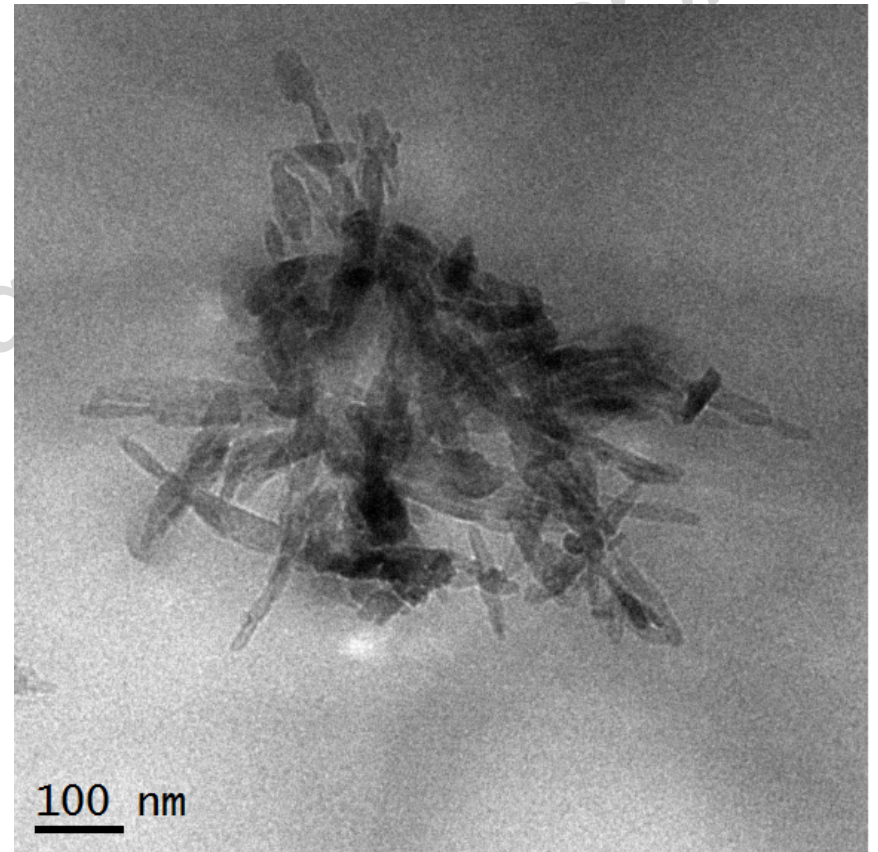
# EDX of particle on slide 34

- C and Cu from TEM grid



## Ca, P, Si, and O containing particles

- Average size  $28 \pm 5$  nm (width)  
 $160 \pm 30$  nm (length)

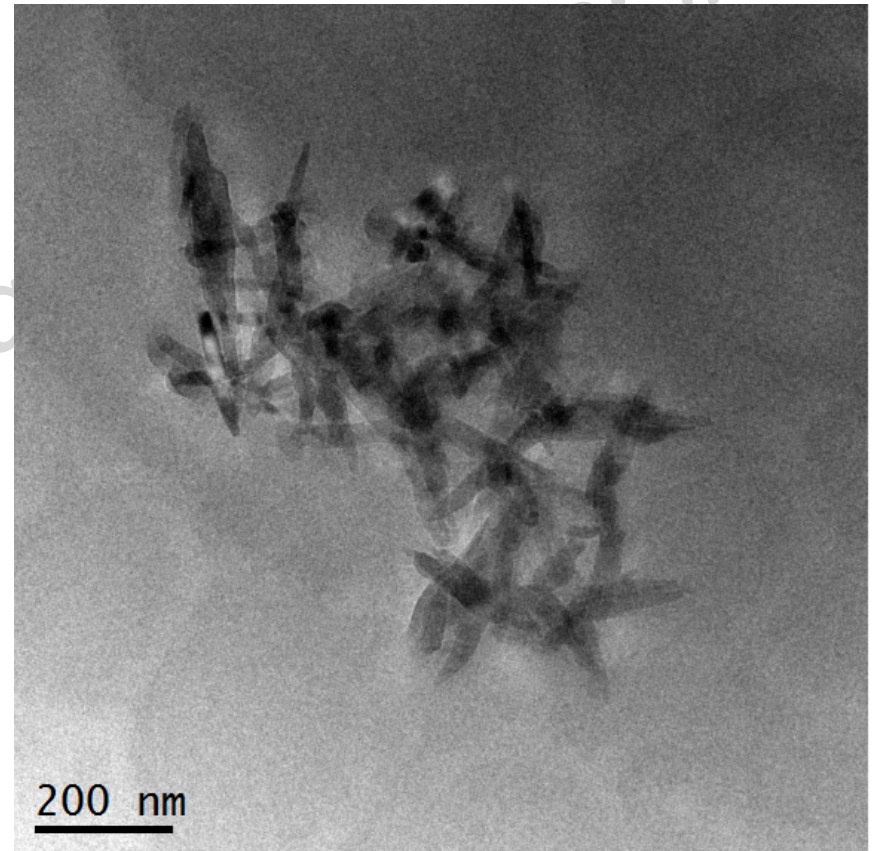


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## Ca, P, Si, and O containing particles

- Average size  $28 \pm 5$  nm (width)  
 $160 \pm 30$  nm (length)



# Gerber Gentle



NUTRIENTS PER 100 CALORIES (5 FL OZ, PREPARED AS DIRECTED):		
PROTEIN	2.2	g
FAT	5.1	g
CARBOHYDRATE	11.6	g
WATER	134	g
LINOLEIC ACID	900	mg
<b>VITAMINS</b>		
A	300	IU
D	60	IU
E	2	IU
K	8	mcg
THIAMINE (B <sub>1</sub> )	100	mcg
RIBOFLAVIN (B <sub>2</sub> )	140	mcg
B <sub>5</sub>	75	mcg
B <sub>12</sub>	0.33	mcg
NIACIN	1050	mcg
FOLIC ACID (FOLACIN)	15	mcg
PANTOTHENIC ACID	450	mcg
BIOTIN	4.4	mcg
C (ASCORBIC ACID)	10	mg
CHOLINE	24	mg
INOSITOL	6	mg
<b>MINERALS</b>		
CALCIUM	67	mg
PHOSPHORUS	38	mg
MAGNESIUM	7	mg
IRON	1.5	mg
ZINC	0.8	mg
MANGANESE	15	mcg
COPPER	80	mcg
IODINE	12	mcg
SELENIUM	3	mcg
SODIUM	27	mg
POTASSIUM	108	mg
CHLORIDE	65	mg

## INGREDIENTS:

WHEY PROTEIN CONCENTRATE (FROM COW'S MILK, ENZYMATICALLY HYDROLYZED, REDUCED IN MINERALS), VEGETABLE OILS (PALM OLEIN, SOY, COCONUT, AND HIGH-OLEIC SAFFLOWER OR HIGH-OLEIC SUNFLOWER), CORN MALTODEXTRIN, LACTOSE, GALACTO-OLIGOSACCHARIDES\*, AND LESS THAN 2% OF: POTASSIUM CITRATE, POTASSIUM PHOSPHATE, CALCIUM CHLORIDE, CALCIUM PHOSPHATE, SODIUM CITRATE, MAGNESIUM CHLORIDE, FERROUS SULFATE, ZINC SULFATE, COPPER SULFATE, POTASSIUM IODIDE, MANGANESE SULFATE, SODIUM SELENATE, M. ALPINA OIL\*\*, C. COHNII OIL\*\*\*, SODIUM ASCORBATE, INOSITOL, CHOLINE BITARTRATE, ALPHA-TOCOPHERYL ACETATE, NIACINAMIDE, CALCIUM PANTOTHENATE, RIBOFLAVIN, VITAMIN A ACETATE, PYRIDOXINE HYDROCHLORIDE, THIAMINE MONONITRATE, FOLIC ACID, PHYLLIQUINONE, BIOTIN, VITAMIN D3, VITAMIN B12, TAURINE, NUCLEOTIDES (CYTIDINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE, ADENOSINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE), ASCORBYL PALMITATE, MIXED TOCOPHEROLS, L-CARNITINE, SOY LECITHIN.

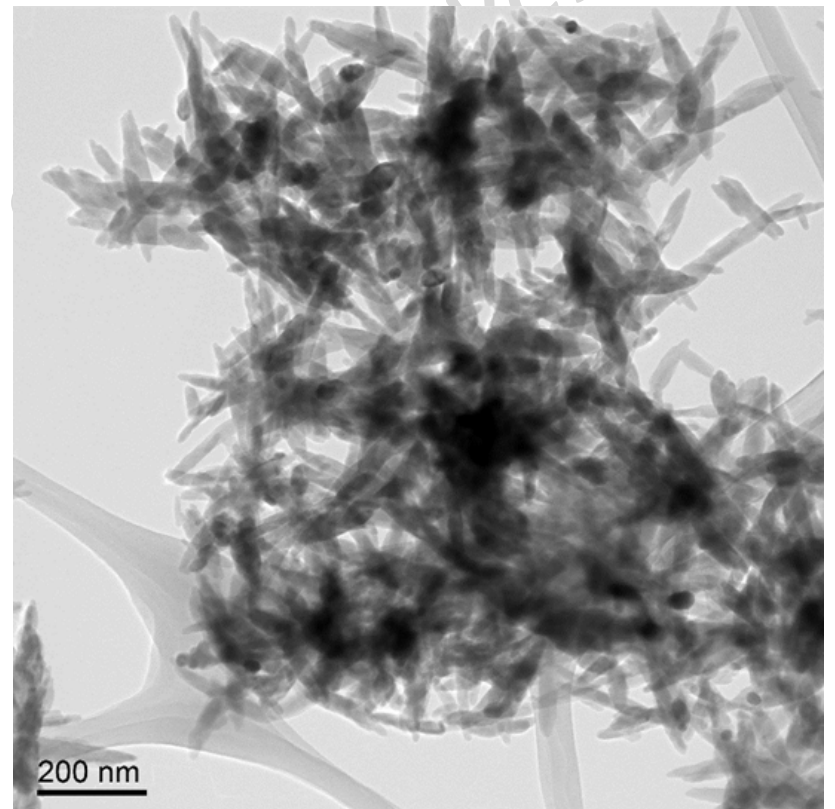
# Summary

- Aggregates of high aspect ratio nanoparticles containing Ca, P, and O were observed
- Average size  $28 \pm 7$  nm (width)  $237 \pm 119$  nm (length)
  - 250 particles counted with 100% of particles with one dimension less than 100nm
- Nano needle-like HA observed

Property of Arizona State University

## Ca, P, and O containing particles

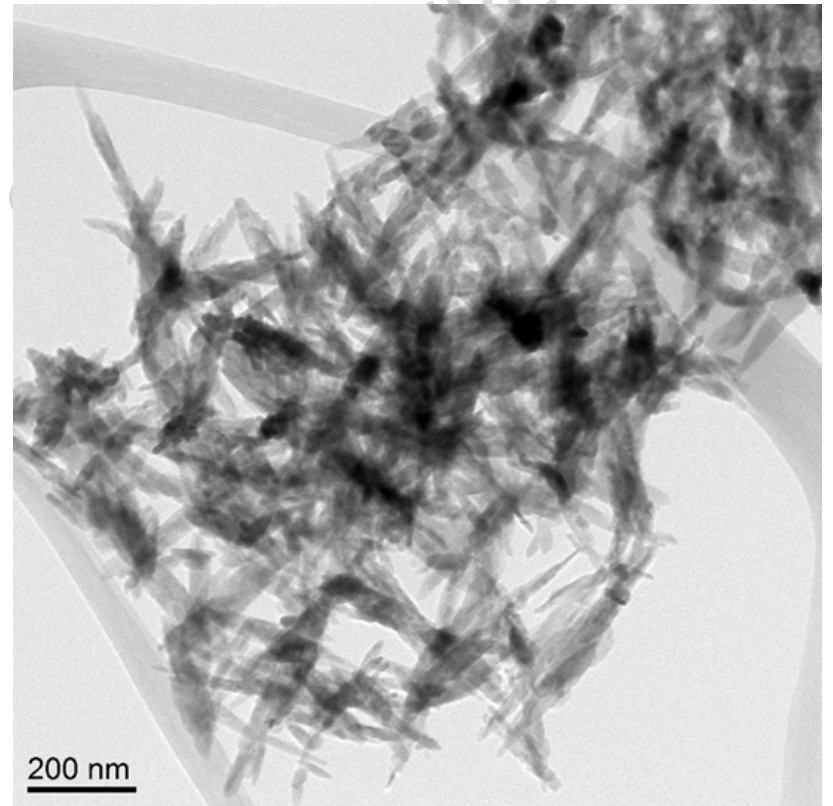
- Aggregate particles composed of Ca, P, and O with an average individual particle size of  $28 \pm 7$  nm (width)  $237 \pm 119$  nm (length)





## Ca, P, and O containing particles

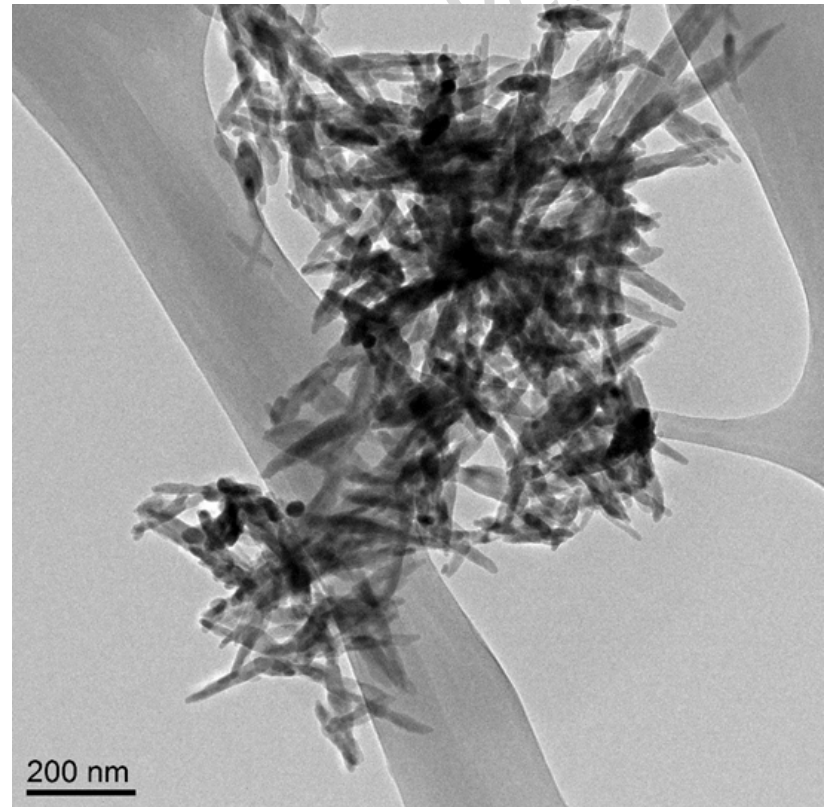
- Aggregate particles composed of Ca, P, and O with an average individual particle size of  $28 \pm 7$  nm (width)  $237 \pm 119$  nm (length)





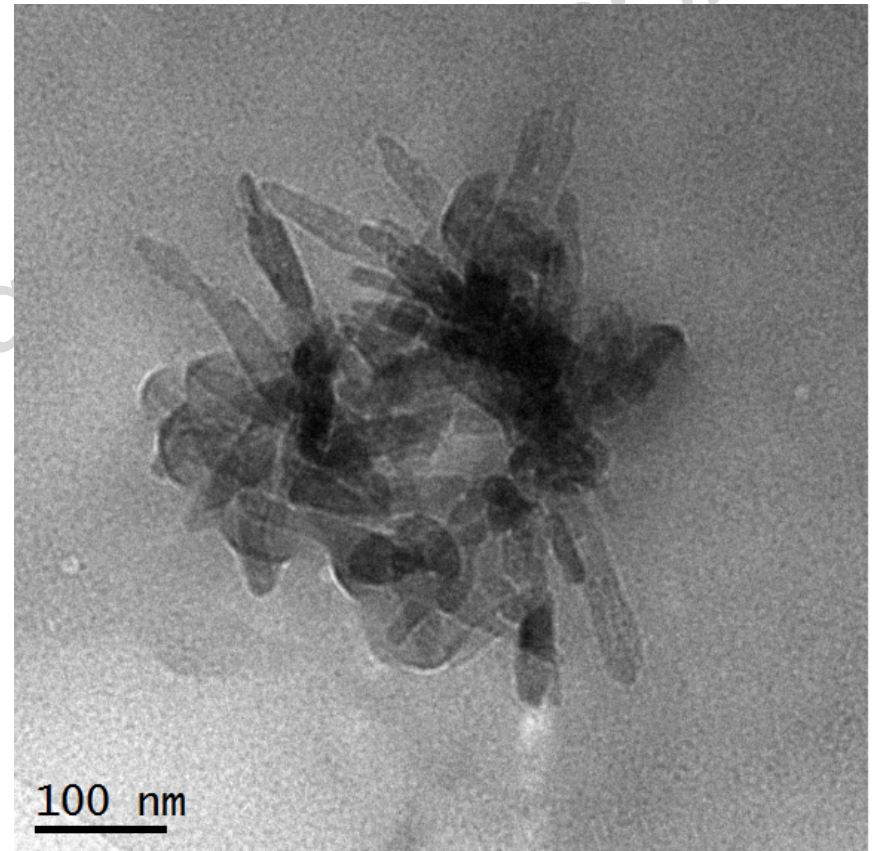
## Ca, P, and O containing particles

- Aggregate particles composed of Ca, P, and O with an average individual particle size of  $28 \pm 7$  nm (width)  $237 \pm 119$  nm (length)
- Nano needle-like HA observed



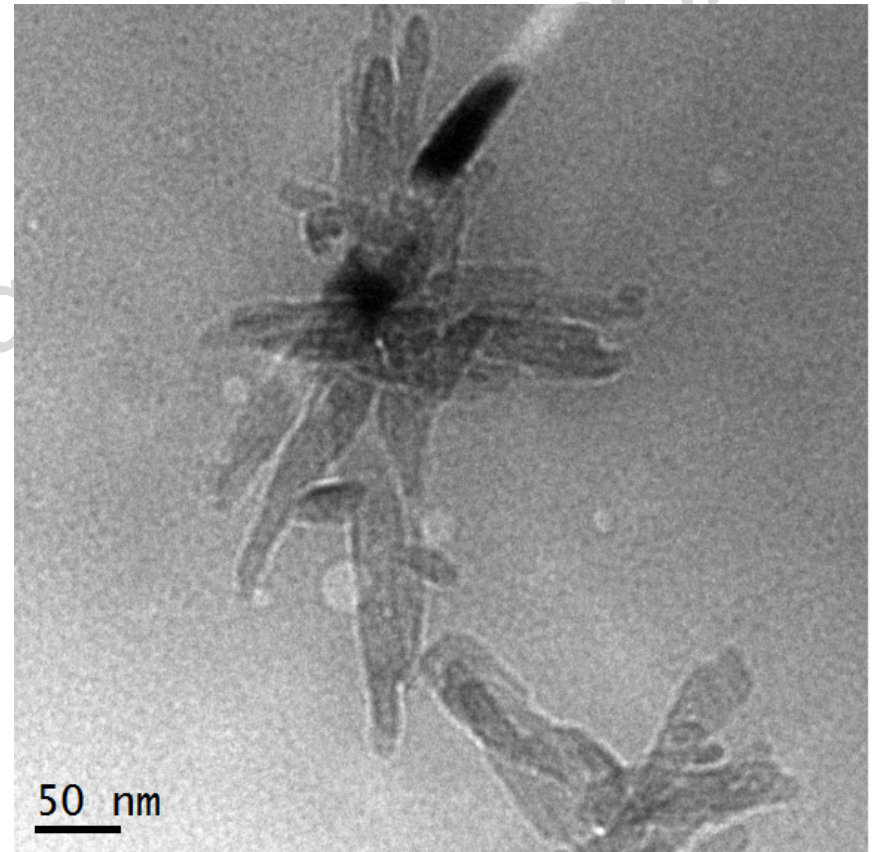
## Ca, P, and O containing particles

- Average size  $31 \pm 4$  nm (width)  
 $146 \pm 29$  nm (length)



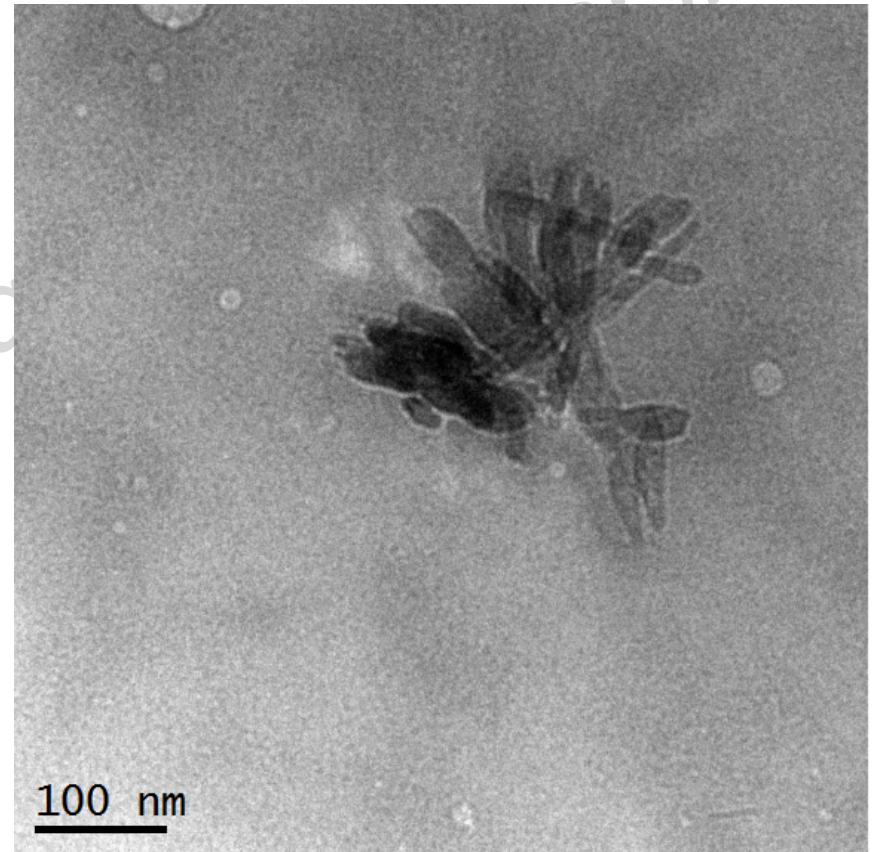
## Ca, P, and O containing particles

- Average size  $31 \pm 4$  nm (width)  
 $146 \pm 29$  nm (length)



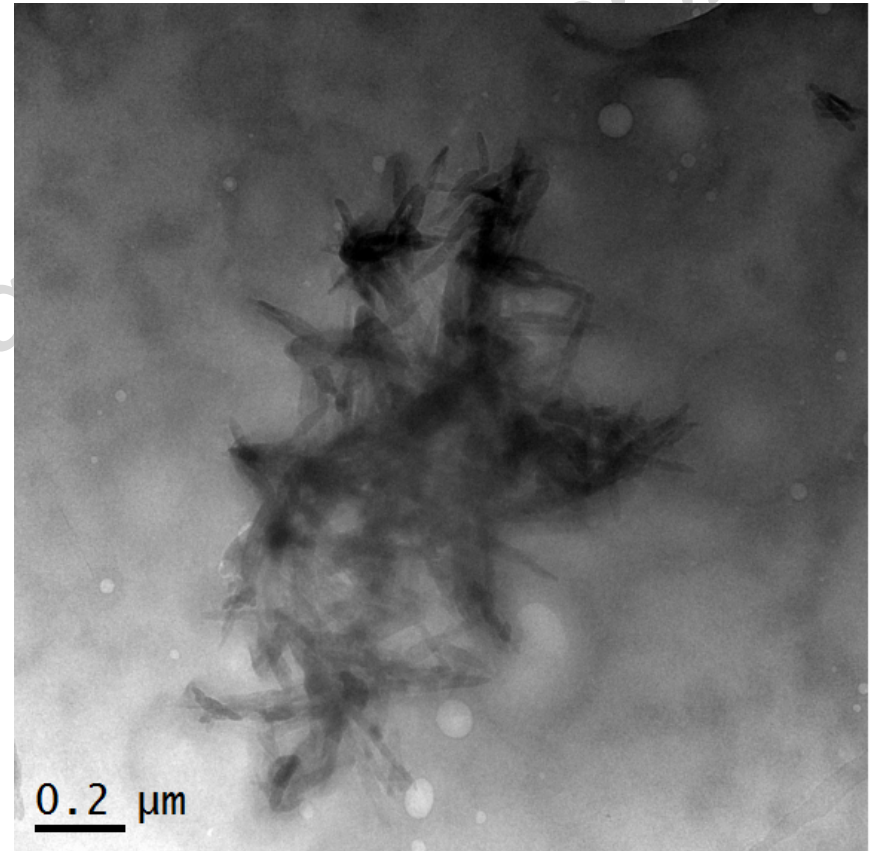
## Ca, P, and O containing particles

- Average size  $31 \pm 4$  nm (width)  
 $146 \pm 29$  nm (length)



## Ca, P, and O containing particles

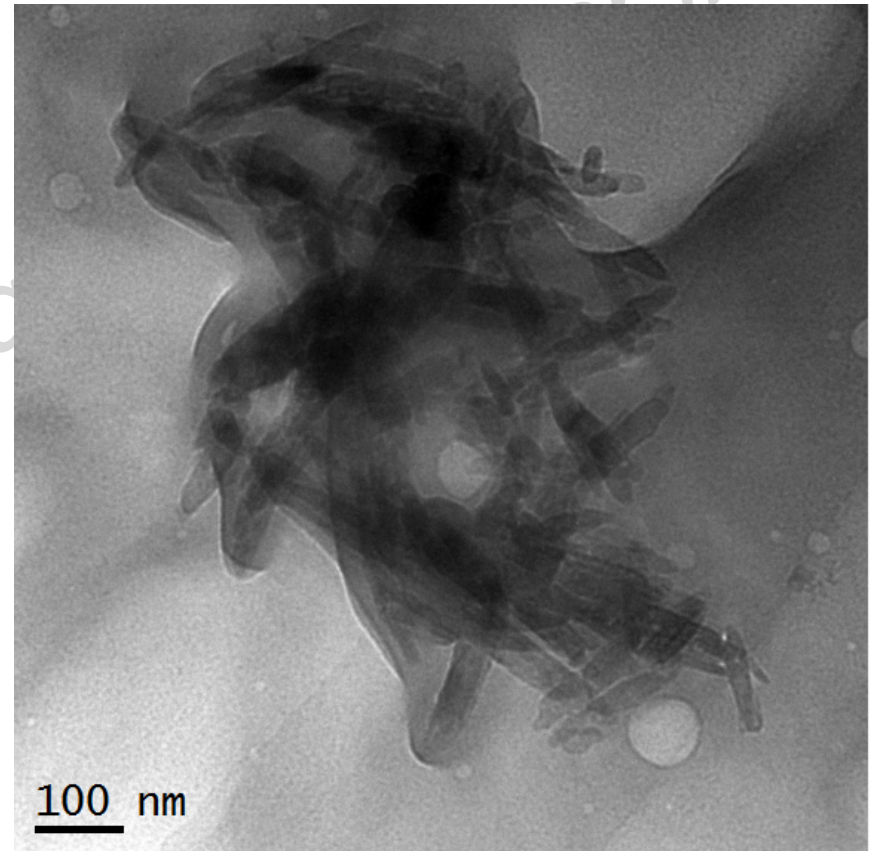
- Average size  $31 \pm 4$  nm (width)  
 $146 \pm 29$  nm (length)





## Ca, P, and O containing particles

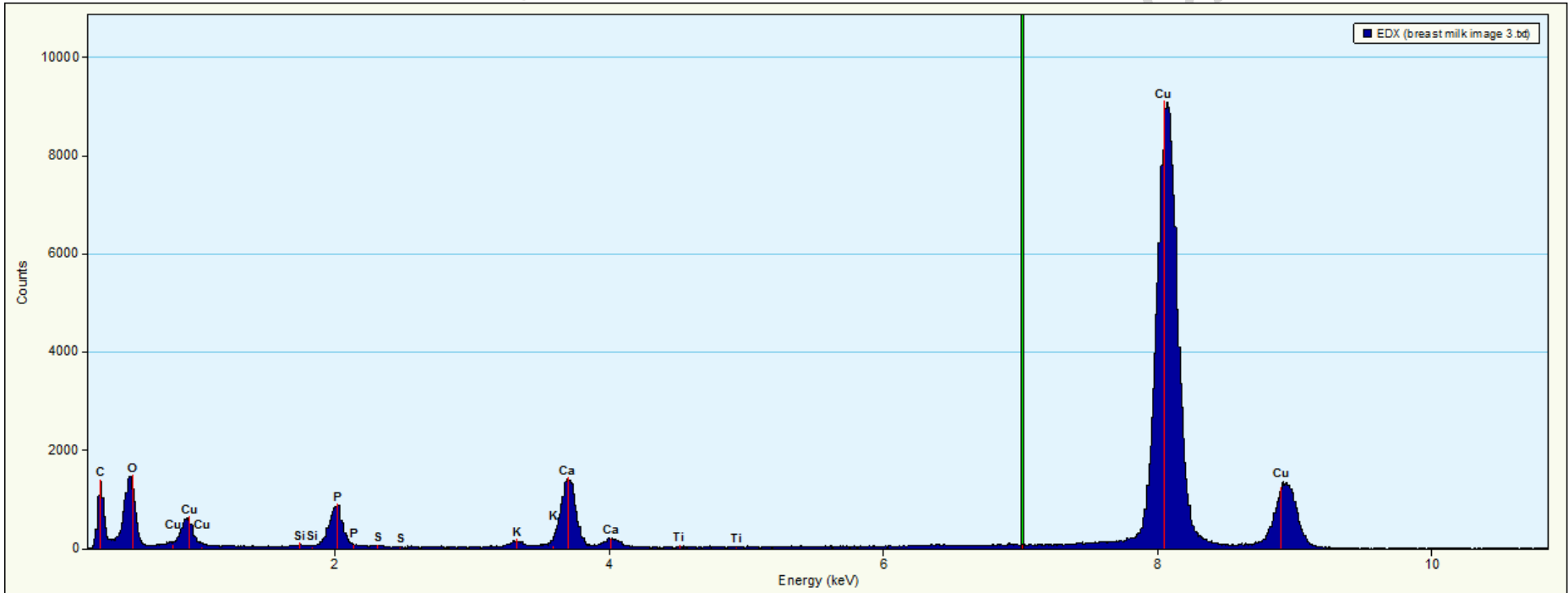
- Average size  $31 \pm 4$  nm (width)  
 $146 \pm 29$  nm (length)



# EDX of Gerber Gentle Baby Formula

- C and Cu from TEM grid

University



# Similac Advance



## Ingredients:

Nonfat Milk, Lactose, Whey Protein Concentrate, High Oleic Safflower Oil, Soy Oil, Coconut Oil, Galactooligosaccharides. Less than 2% of the Following: C. Cohnii Oil, M. Alpina Oil, Beta-Carotene, Lutein, Lycopene, Potassium Citrate, Calcium Carbonate, Ascorbic Acid, Soy Lecithin, Potassium Chloride, Magnesium Chloride, Ferrous Sulfate, Choline Bitartrate, Choline Chloride, Ascorbyl Palmitate, Salt, Taurine, m-Inositol, Zinc Sulfate, Mixed Tocopherols, d-Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, L-Carnitine, Vitamin A Palmitate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Riboflavin, Pyridoxine Hydrochloride, Folic Acid, Manganese Sulfate, Phylloquinone, Biotin, Sodium Selenate, Vitamin D3, Cyanocobalamin, Calcium Phosphate, Potassium Phosphate, Potassium Hydroxide, and Nucleotides (Adenosine 5'-Monophosphate, Cytidine 5'-Monophosphate, Disodium Guanosine 5'-Monophosphate, Disodium Uridine 5'-Monophosphate).

Contains milk ingredients.

Serving Size: 5.3fl oz  
Serving Per Container:

## Amount Per Serving

Calories: 100

%Daily Value\*

**Total Fat** 5.6g  
**Sodium** 25mg  
**Potassium** 110mg  
**Total Carbohydrate** 10.7g  
**Protein** 2.07g  
**Vitamin A** 300IU  
**Calcium** 82mg  
**Iron** 1.9mg

Vitamin D	Vitamin E
Biotin	Chloride
Copper	Iodine
Magnesium	Manganese
Niacin	Pantothenic Acid
Phosphorus	Selenium
Vitamin B12	Vitamin B6
Zinc	Vitamin C (Ascorbic Acid)
Inositol	Choline
Linoleic Acid	Folic Acid (Folacin)
Water	Riboflavin (Vit. B2)
Thiamin (Vit. B1)	Vitamin K

\* Percentage of Daily Values are based on a 2,000 calorie diet.



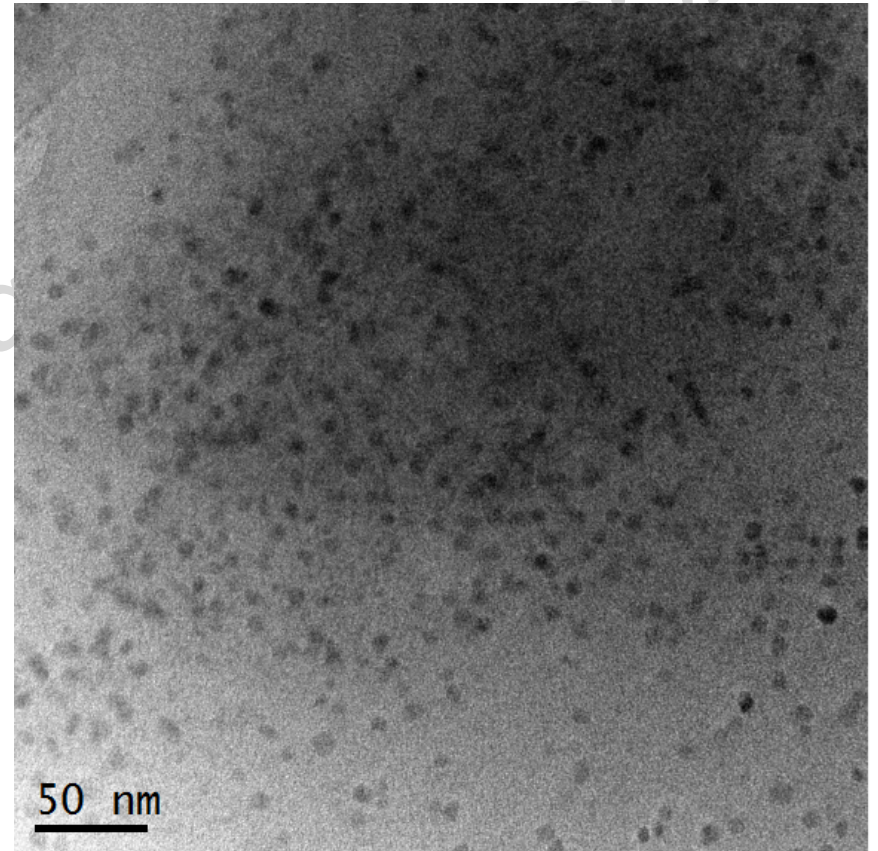
## Summary

- Nanoparticles containing Ca, P, K, Si and O were observed
- Average diameter of  $7 \pm 1$  nm (probably silica dioxide)
  - 250 particles counted with 100% of particles with a diameter less than 100nm
- No needle-like HA observed

Property of Arizona State University

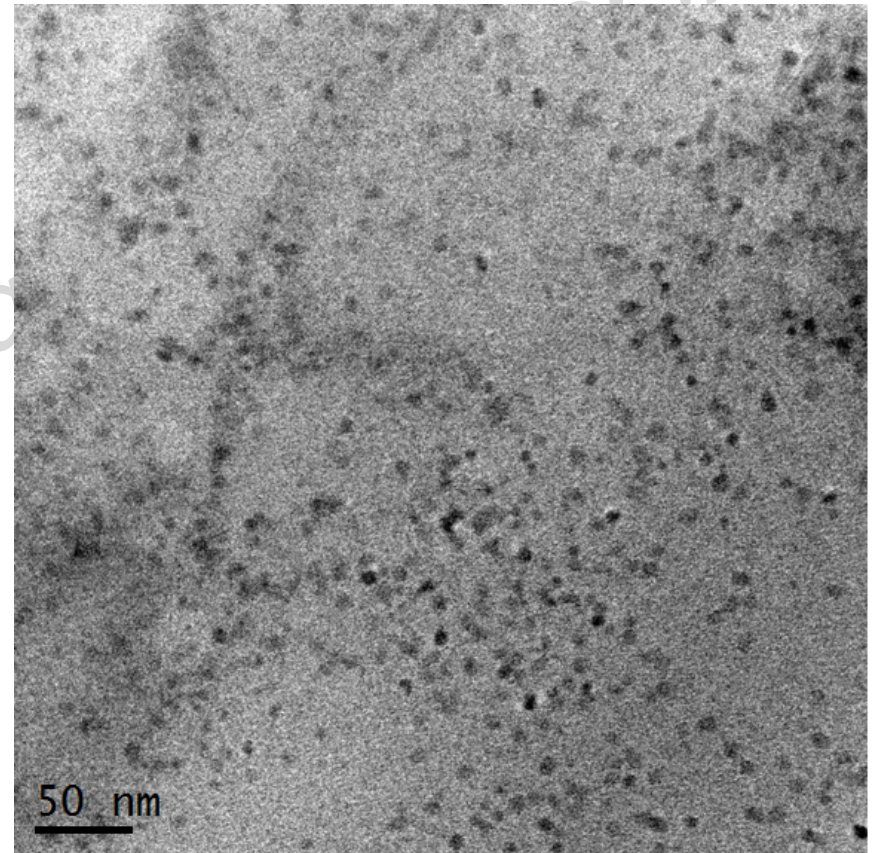
## Ca, P, K, Si and O containing particles

- Average diameter of  $7 \pm 1$  nm



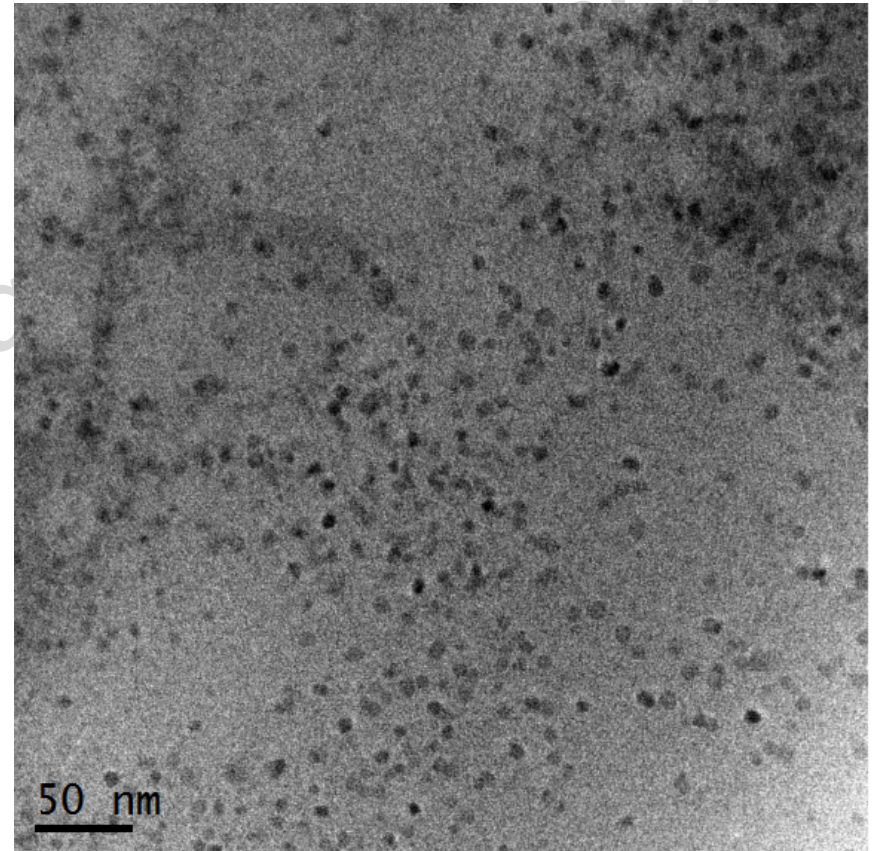
## Ca, P, K, Si and O containing particles

- Average diameter of  $7 \pm 1$  nm



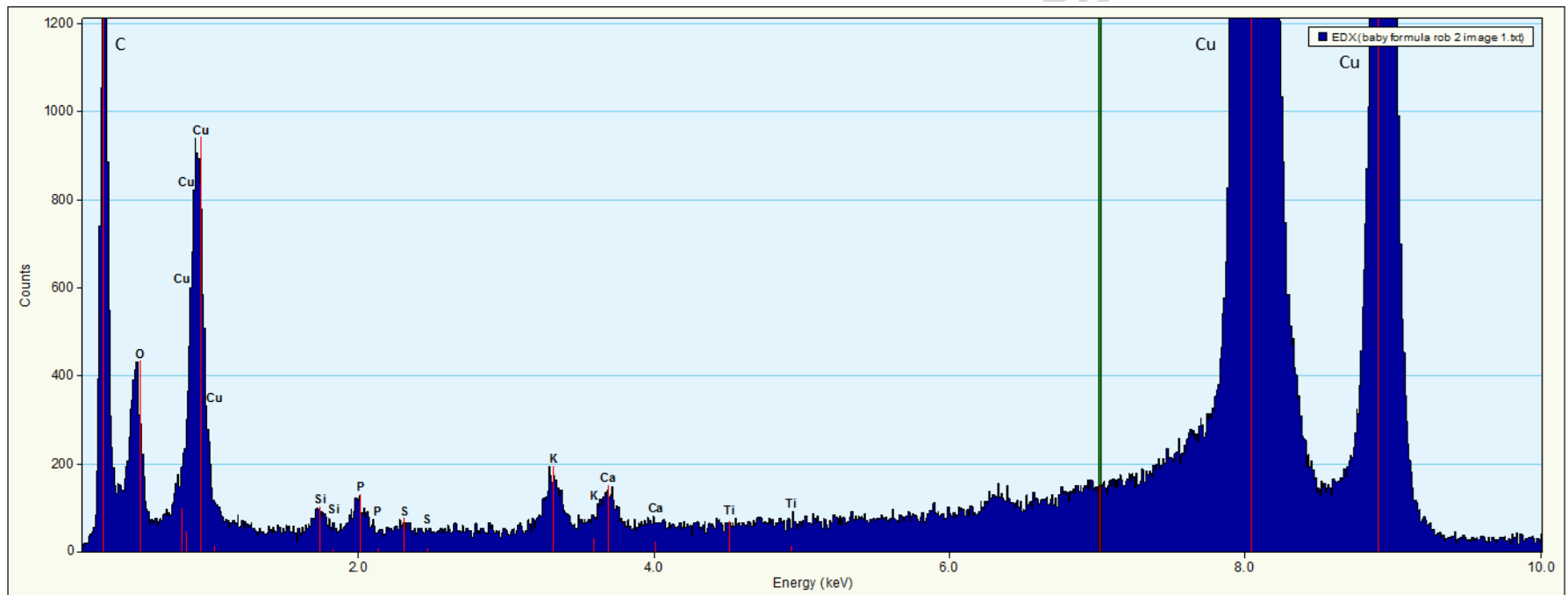
## Ca, P, K, Si and O containing particles

- Average diameter of  $7 \pm 1$  nm



# EDX of Similac Advance Baby Formula

- Carbon and Cu peaks are from TEM grid



# Similac Advance Liquid Formula



## Ingredients:

Water, Nonfat Milk, Lactose, High Oleic Safflower Oil, Soy Oil, Coconut Oil, Galacto-Oligosaccharides, Whey Protein Concentrate; Less than 0.5% of: C. Cohnii Oil, M, Alpina Oil, Beta-Carotene, Lutein, Lycopene, Ascorbic Acid, Calcium Carbonate, Potassium Citrate, Soy Lecithin, Monoglycerides, Potassium Chloride, Carrageenan, Magnesium Chloride, Ferrous Sulfate, Choline Bitartrate, Choline Chloride, Taurine, Calcium Phosphate, Potassium Phosphate, M-Inositol, Zinc Sulfate, Niacinamide, D-Alpha-Tocopheryl Acetate, Calcium Pantothenate, L-Carnitine, Riboflavin, Vitamin A Palmitate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Folic Acid, Manganese Sulfate, Phylloquinone, Biotin, Sodium Selenate, Vitamin D3, Cyanocobalamin, Salt, Potassium Hydroxide and Nucleotides (Adenosine 5-Mono-Phosphate, Cytidine 5-Monophosphate, Disodium Guanosine 5-Monophosphate, Disodium Uridine 5-Monophosphate).

Serving Size: 5fl oz  
Serving Per Container:

## Amount Per Serving

Calories: 100

%Daily Value\*

Total Fat 5.4g

Sodium 24mg

Potassium 105mg

Total Carbohydrate 11.2g

Protein 2.07g

Vitamin A 300IU

Calcium 78mg

Iron 1.8mg

Vitamin D

Vitamin E

Biotin

Chloride

Copper

Iodine

Magnesium

Manganese

Niacin

Pantothenic Acid

Phosphorus

Selenium

Vitamin B12

Vitamin B6

Zinc

Vitamin C (Ascorbic Acid)

Inositol

Choline

Linoleic Acid

Folic Acid (Folacin)

Water

Riboflavin (Vit. B2)

Thiamin (Vit. B1)

Vitamin K

\* Percentage of Daily Values are based on a 2,000 calorie diet.

# Summary

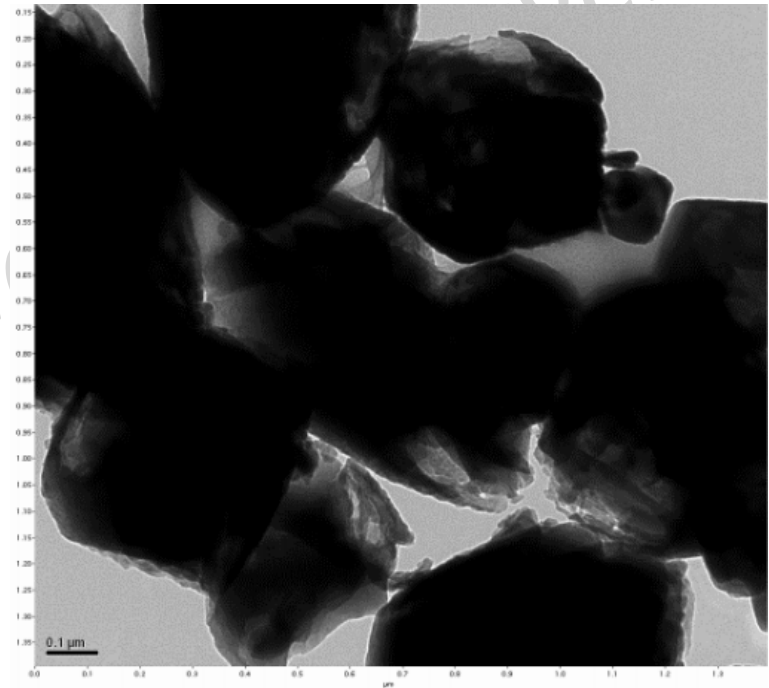
- Aggregates of Ti containing nanoparticles were observed
  - Nanoparticles containing Ti varied in size from 16 nm to 530 nm
- Calcium Phosphate particles were observed
  - Average particle size:  $590 \pm 126$  nm
  - None were below 100nm
  - No needle-like structures

Property of Arizona State University



## Ti containing particle

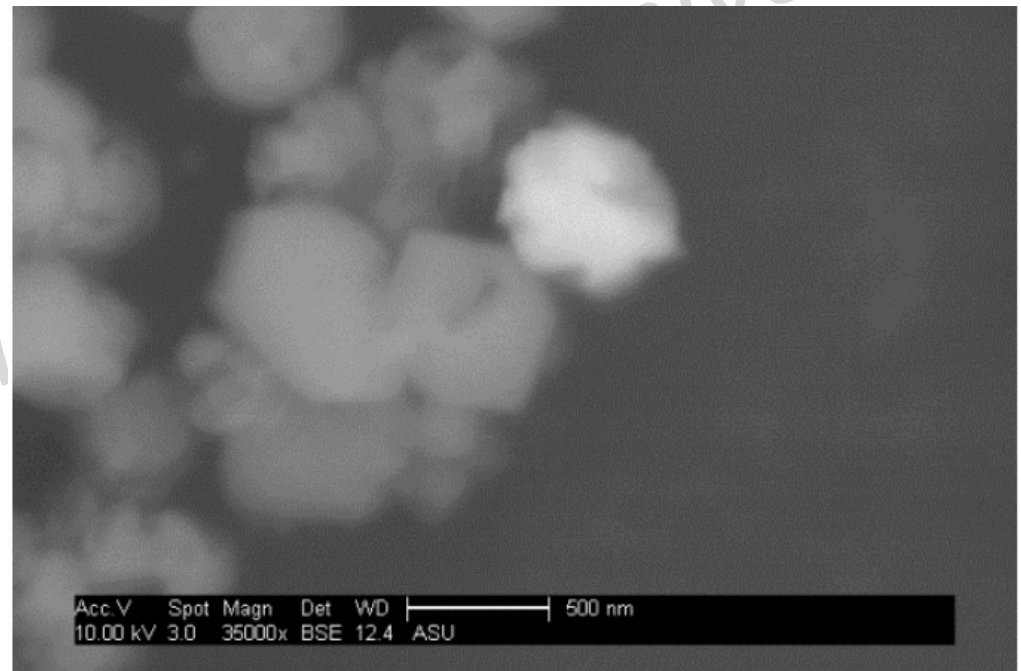
- Spherical nanoparticle
- Diameter between 300 and 400 nm





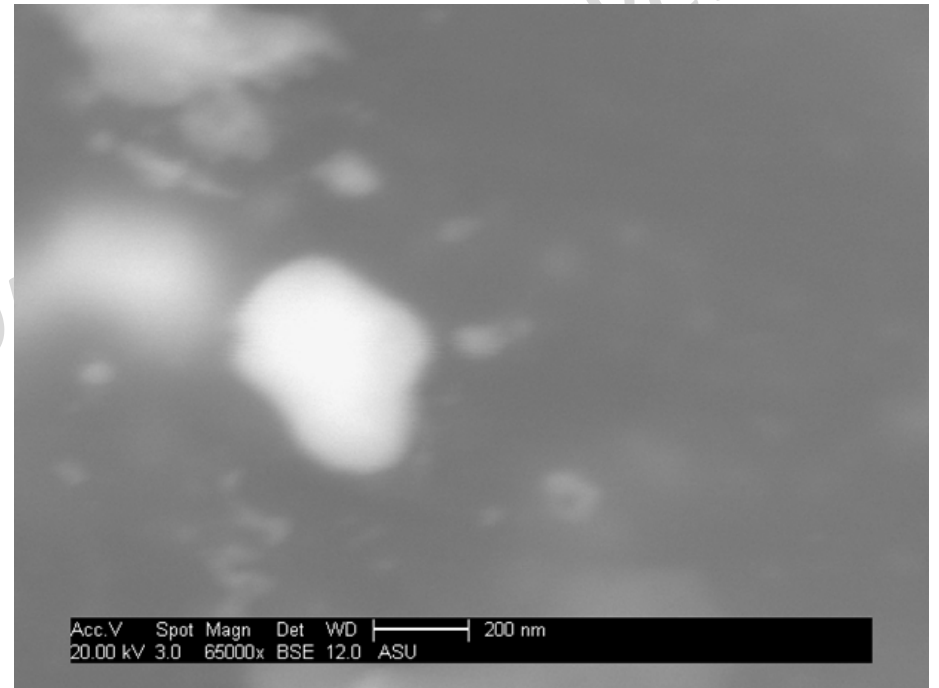
## Ti containing particle

- Spherical nanoparticle
- Diameter between 300 and 500 nm



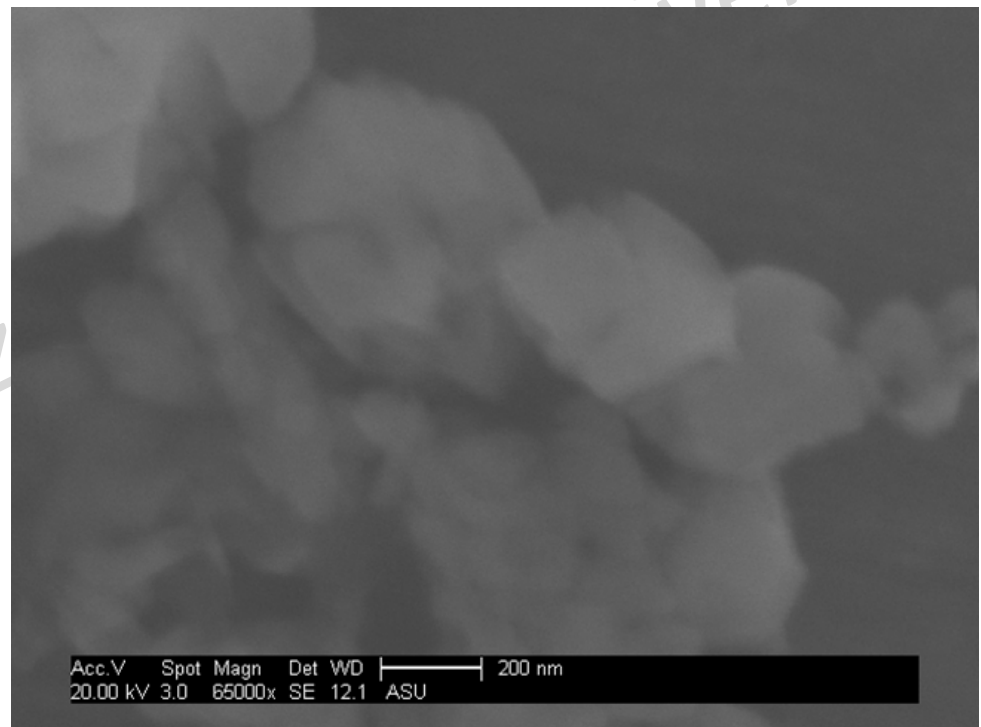
## Ti containing particle

- Spherical nanoparticle
- Diameter between 16 and 400 nm



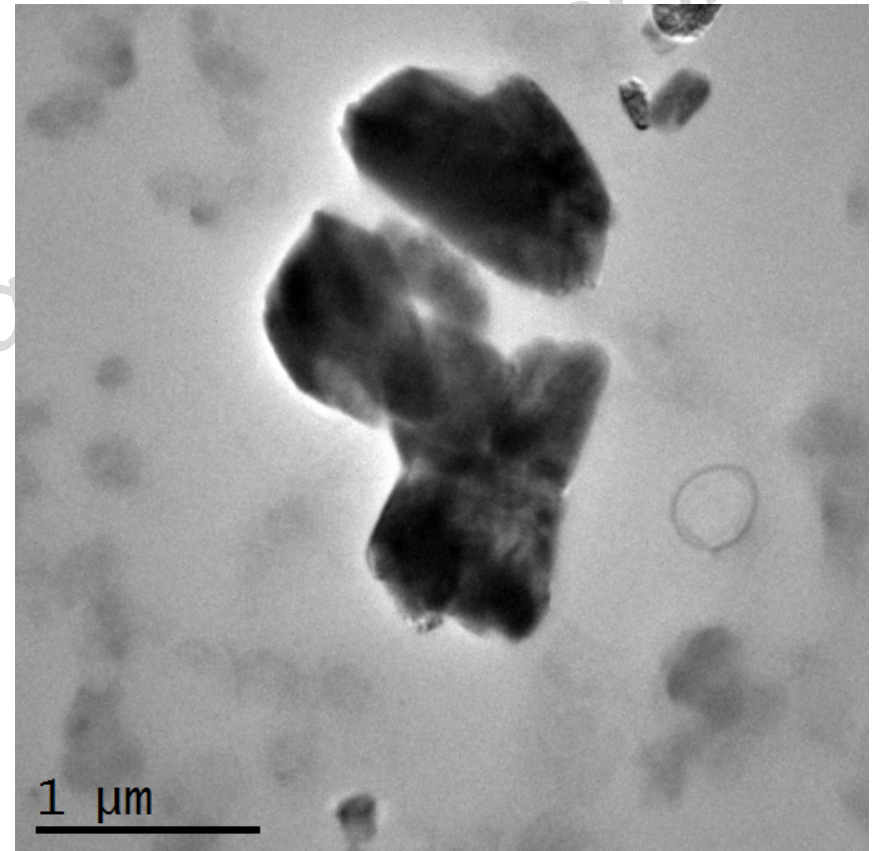
## Ti containing particle

- Spherical nanoparticle
- Diameter between 50 and 530 nm



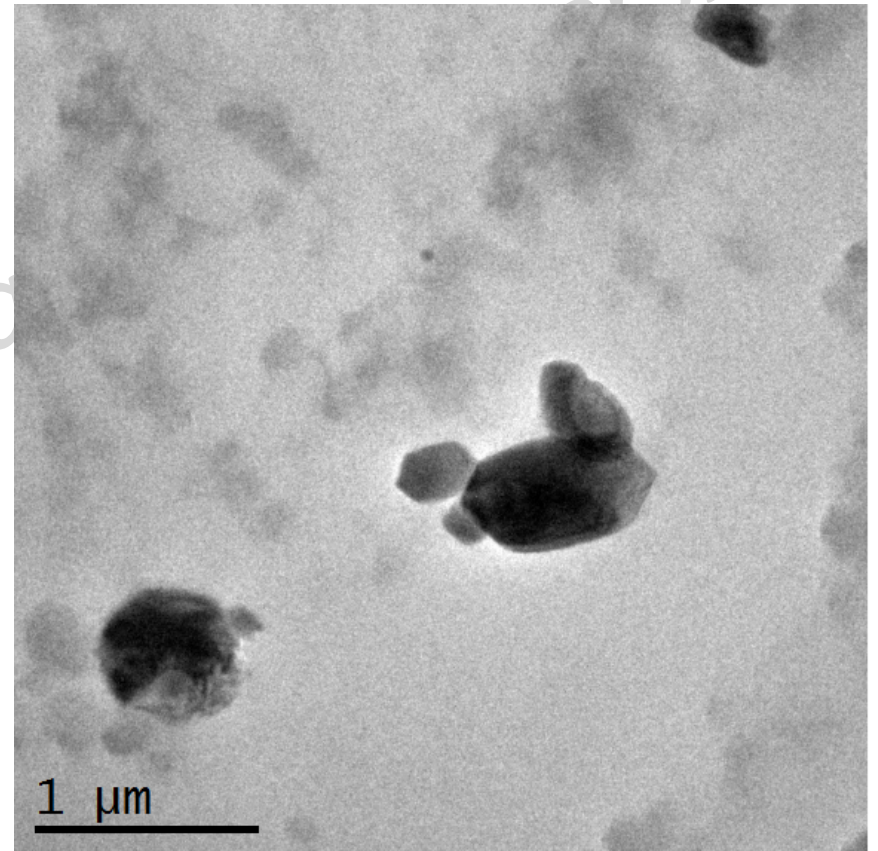
## Ca and O containing particles

- Average particle size:  $590 \pm 126$  nm



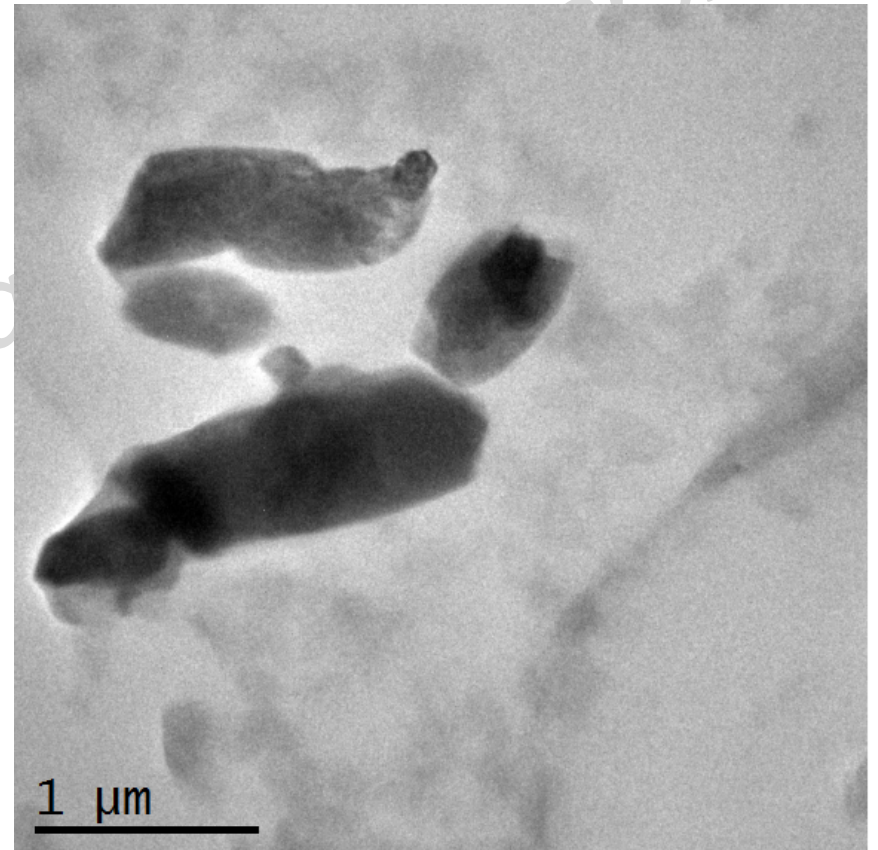
## Ca and O containing particles

- Average particle size:  $590 \pm 126$  nm



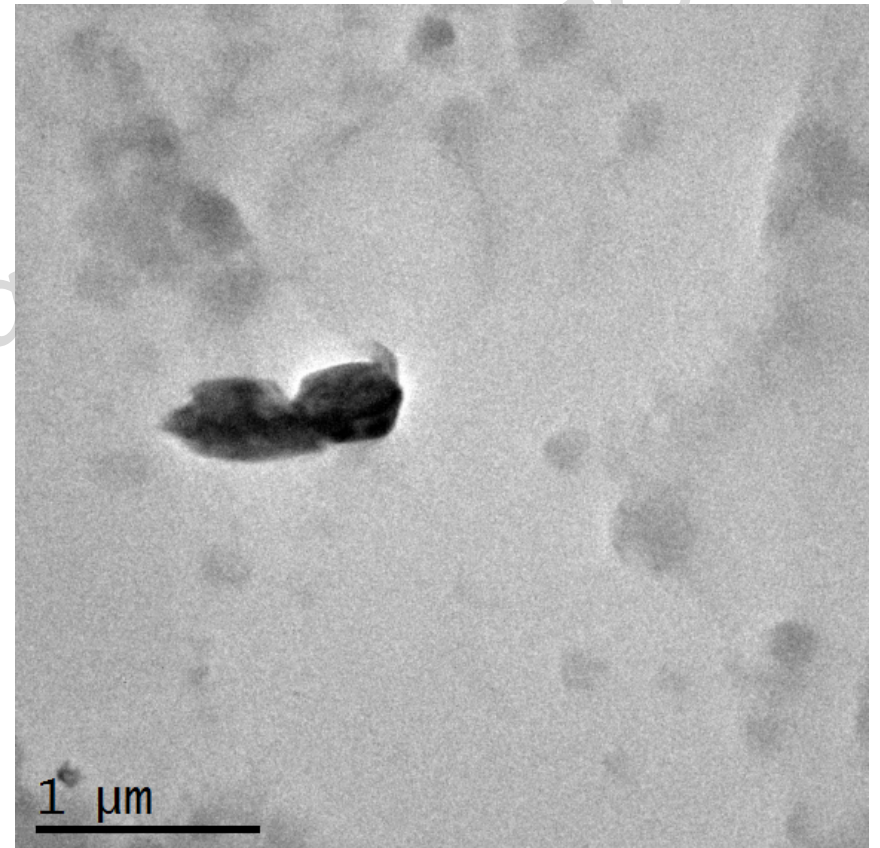
## Ca and O containing particles

- Average particle size:  $590 \pm 126$  nm



## Ca and O containing particles

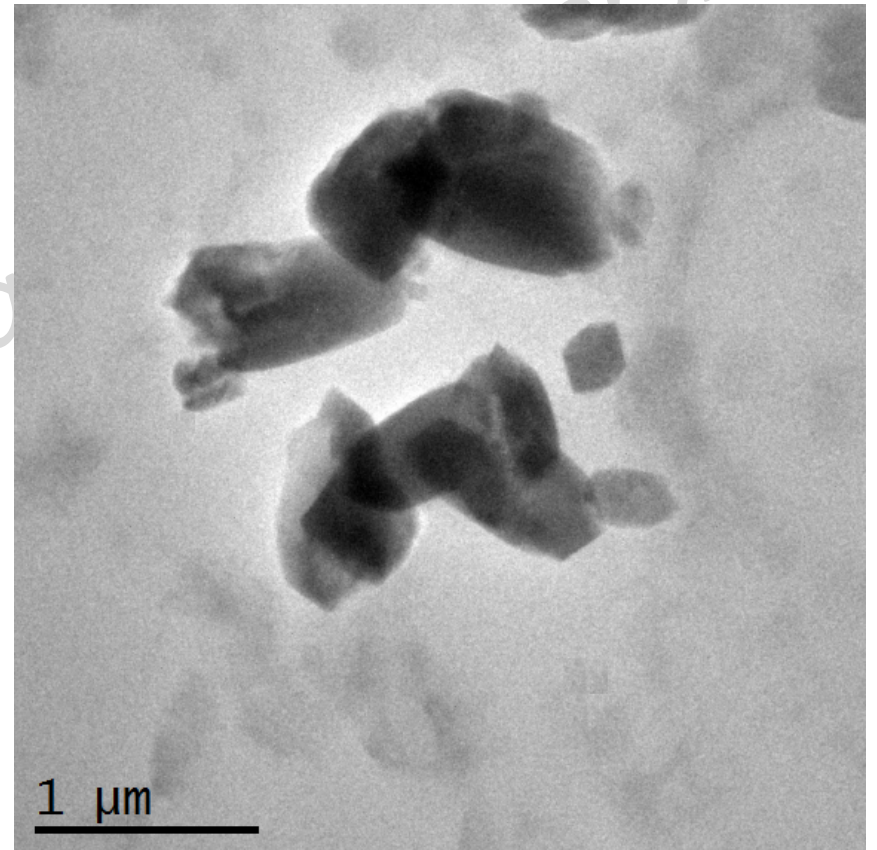
- Average particle size:  $590 \pm 126$  nm





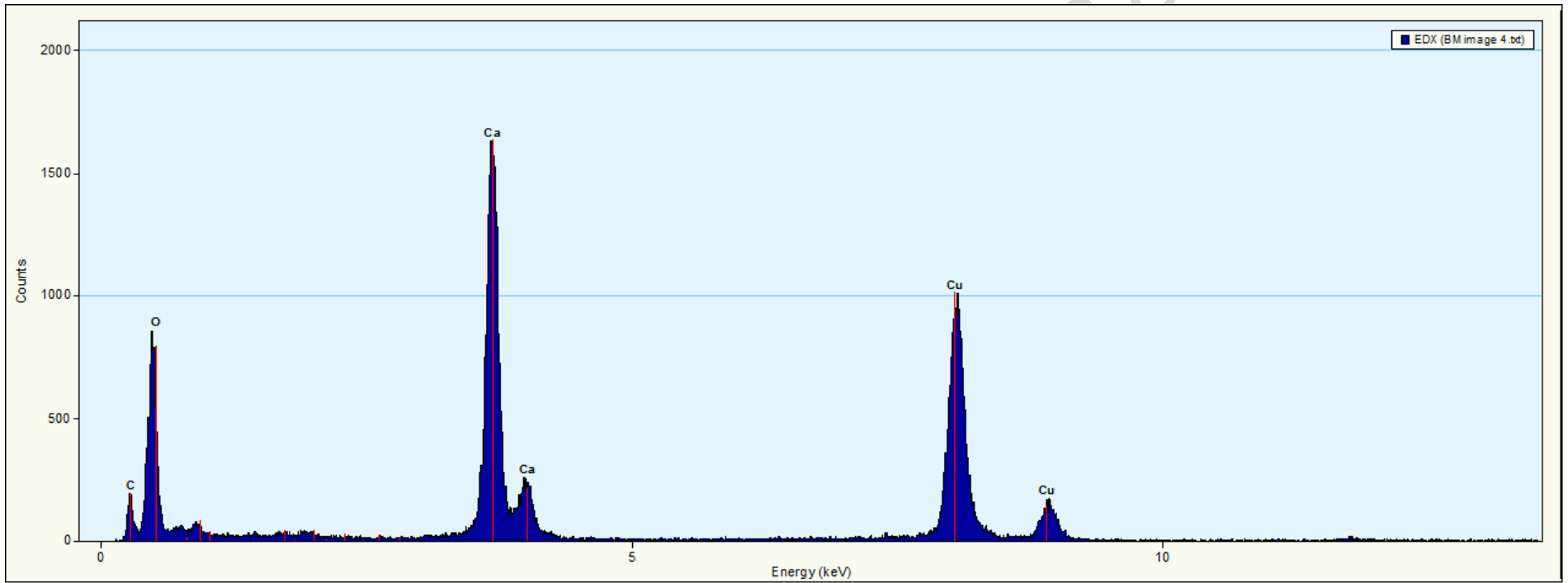
## Ca and O containing particles

- Average particle size:  $590 \pm 126$  nm





# EDX of Similar Advance Liquid Formula



## Two Reference Food Grade Hydroxyapatite Products

- Research question: Is hydroxyapatite (HA) formed in the sample preparation process for TEM or an additive to the baby formula?
- Two food grade HA products were analyzed
- Sample preparation
  - Maintained same sample preparation as with the six baby formulas (Slide 3)

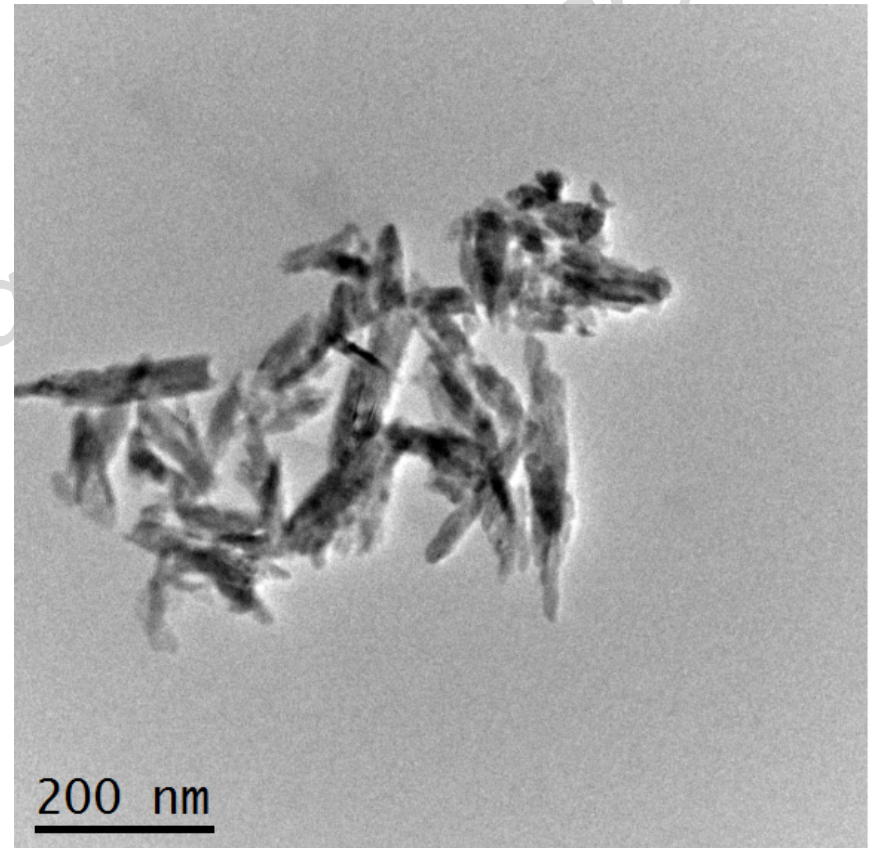
Property of Arizona State University

# Hydroxyapatite Food-Grade Product 1

- 99% pure food-grade product purchased from USA manufacturer (American Elemental, CA; <https://www.americanelements.com/hydroxyapatite-nanopowder-1306-06-5> ) – Product code **CA-PATOH-02-NP**
- Needle-like hydroxyapatite structures dominated the product
- 250 particles counted with 100% of particles with one dimension less than 100nm

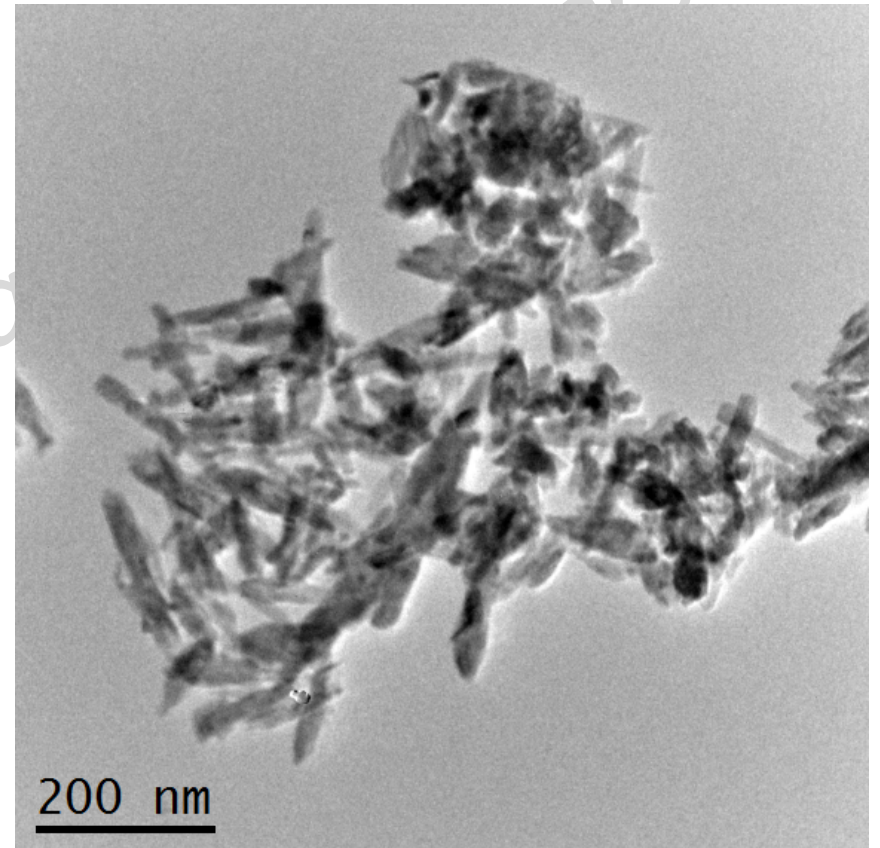
## Ca, P, and O containing particles

- Average particle size:  $131 \pm 25$  nm (width) and  $30 \pm 5$  nm (length)



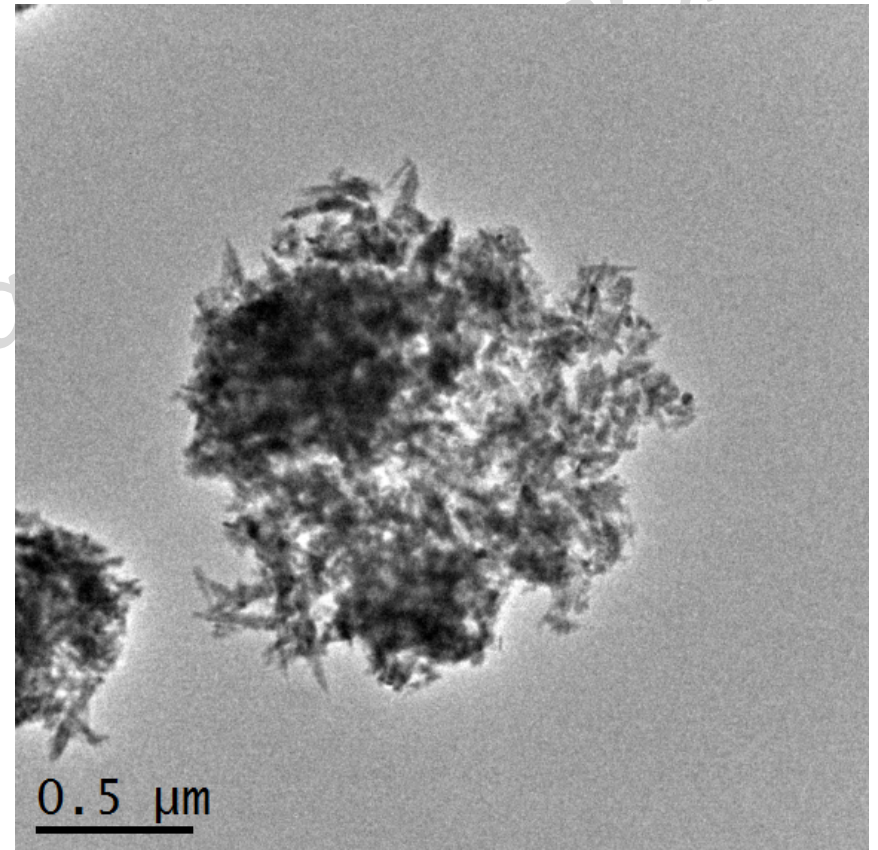
## Ca, P, and O containing particles

- Average particle size:  $131 \pm 25$  nm (width) and  $30 \pm 5$  nm (length)



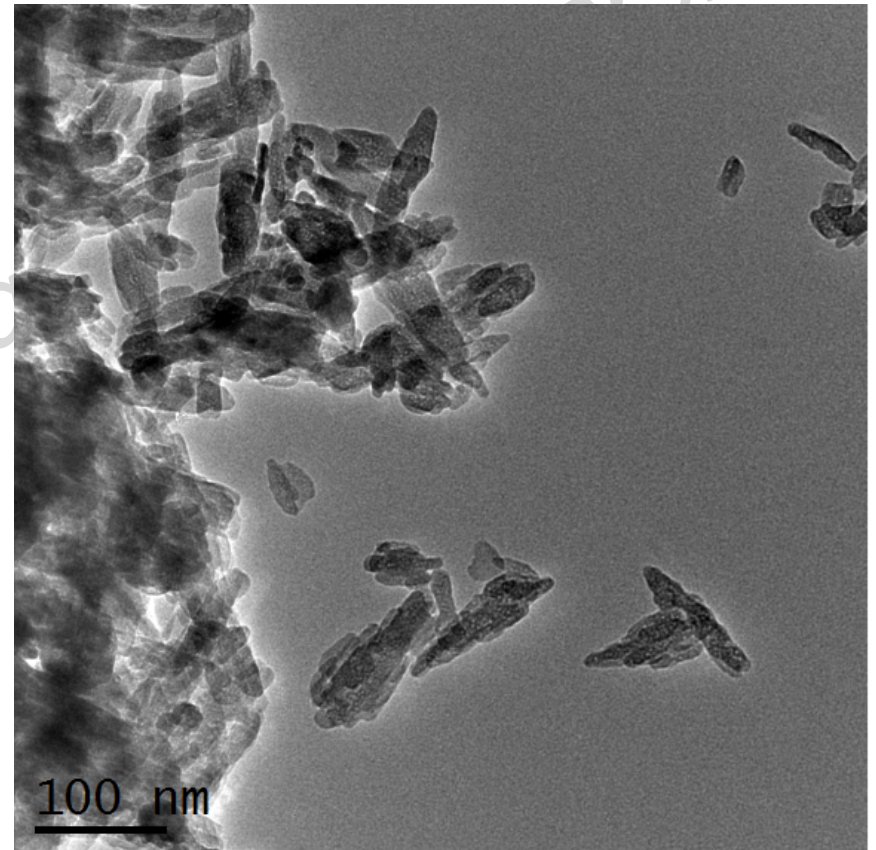
## Ca, P, and O containing particles

- Average particle size:  $131 \pm 25$  nm (width) and  $30 \pm 5$  nm (length)



## Ca, P, and O containing particles

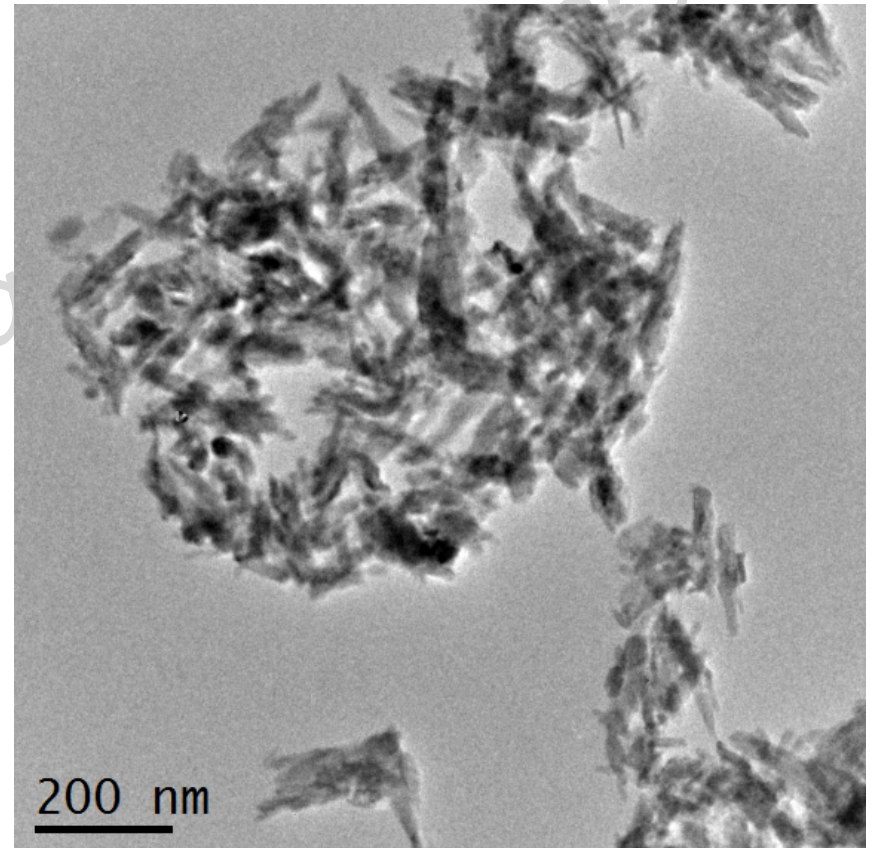
- Average particle size:  $131 \pm 25$  nm (width) and  $30 \pm 5$  nm (length)





## Ca, P, and O containing particles

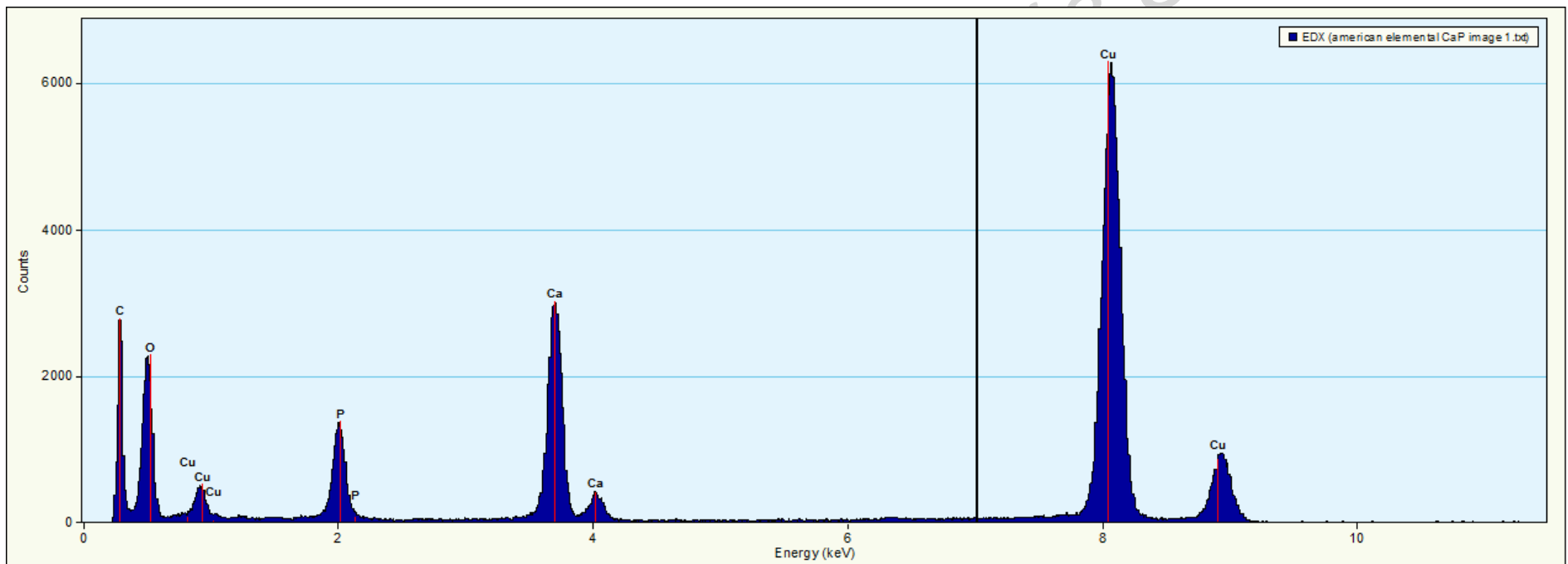
- Average particle size:  $131 \pm 25$  nm (width) and  $30 \pm 5$  nm (length)



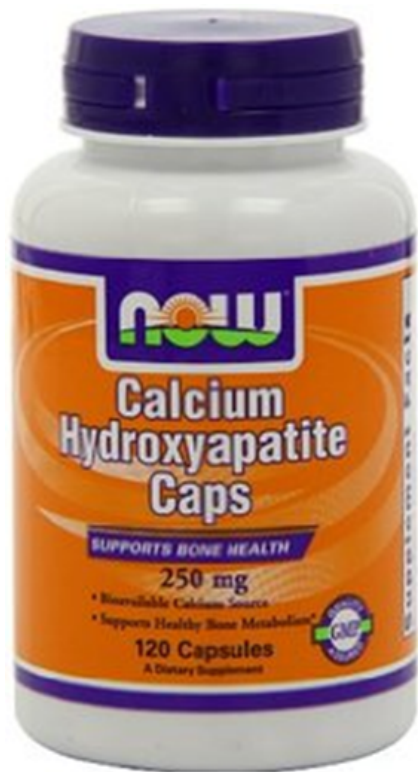


# EDX of Food-Grade HA Product 1

- C and Cu from TEM grid



# Hydroxyapatite Food-Grade Product 2



**SUGGESTED USAGE:** As a dietary supplement, take 4 capsules daily in divided doses, with meals.

Calcium-rich, high collagen microcrystalline hydroxyapatite (MCH) forms the basis for this unique calcium supplement. MCH is the actual form of calcium found naturally in bone tissue. It provides both the organic and inorganic constituents found in the skeletal structure. Look for this and other fine NOW® products in health food stores nationwide.

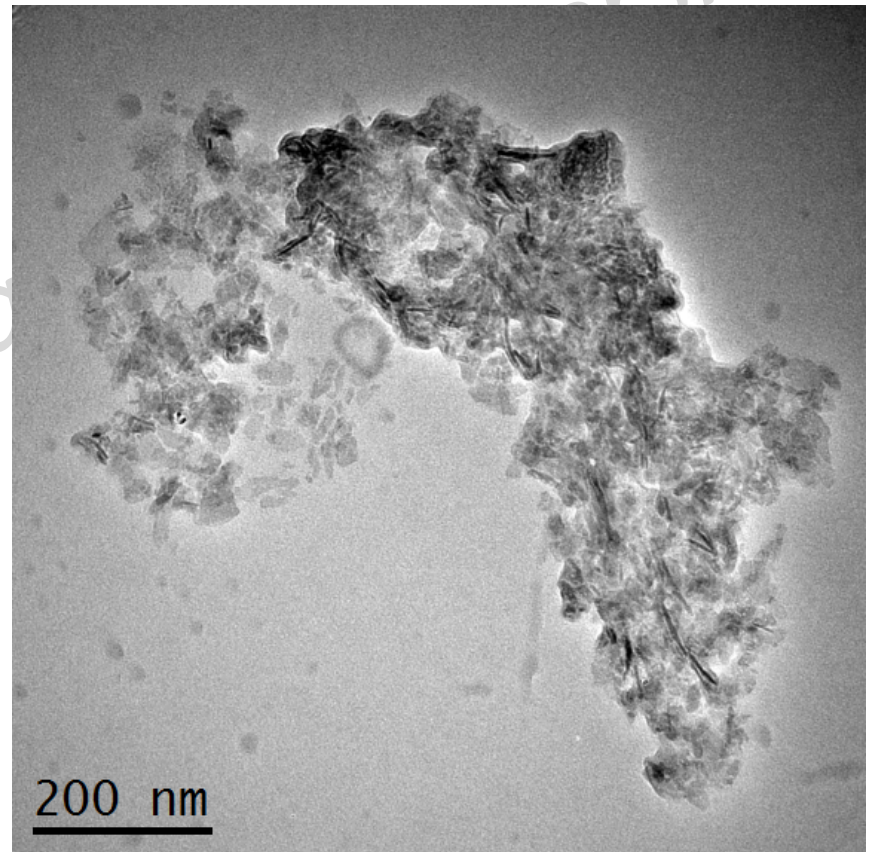
<b>Supplement Facts</b>		
Serving Size 4 Capsules		
Servings per container 30		
	<b>Amount Per Serving</b>	<b>%Daily Value</b>
Calcium (Hydroxyapatite)	1.0g	100%
Phosphorus	500 mg	50%
(From Calcium Hydroxyapatite)		

Other Ingredients: Magnesium Stearate.

Contains no yeast, wheat, gluten, corn, soy, milk, sugar, salt, preservatives or synthetics.

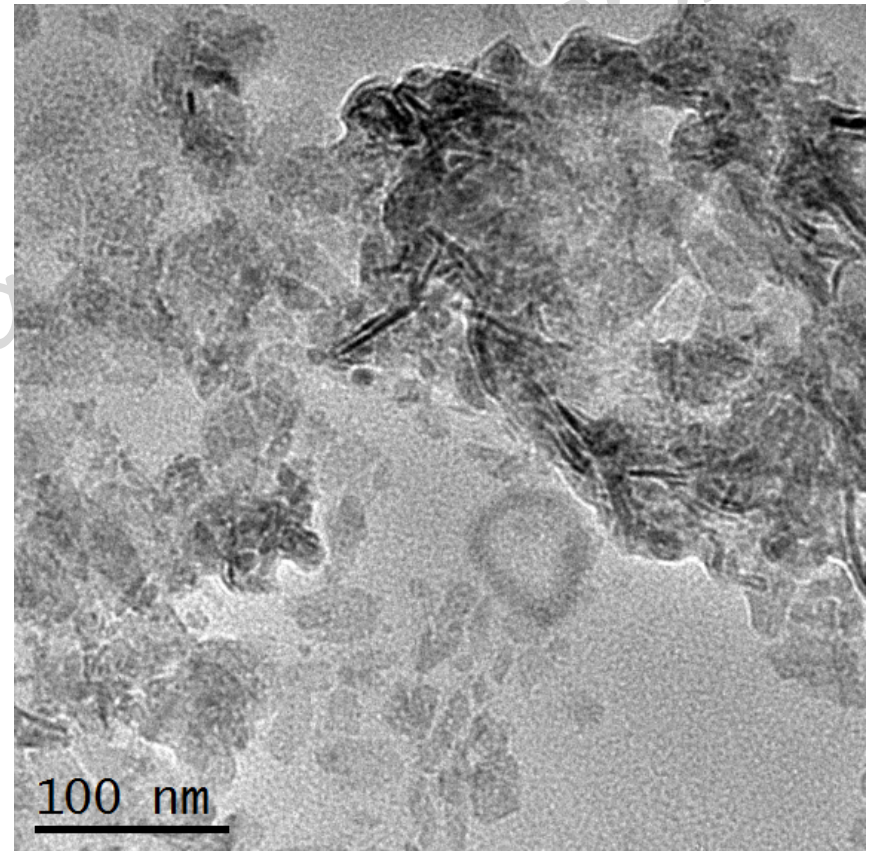
## Ca, P, and O containing particles

- Average particle diameter:  
 $20 \pm 5$  nm
- 57 particles counted with  
100% of particles with one  
dimension less than 100nm



## Ca, P, and O containing particles

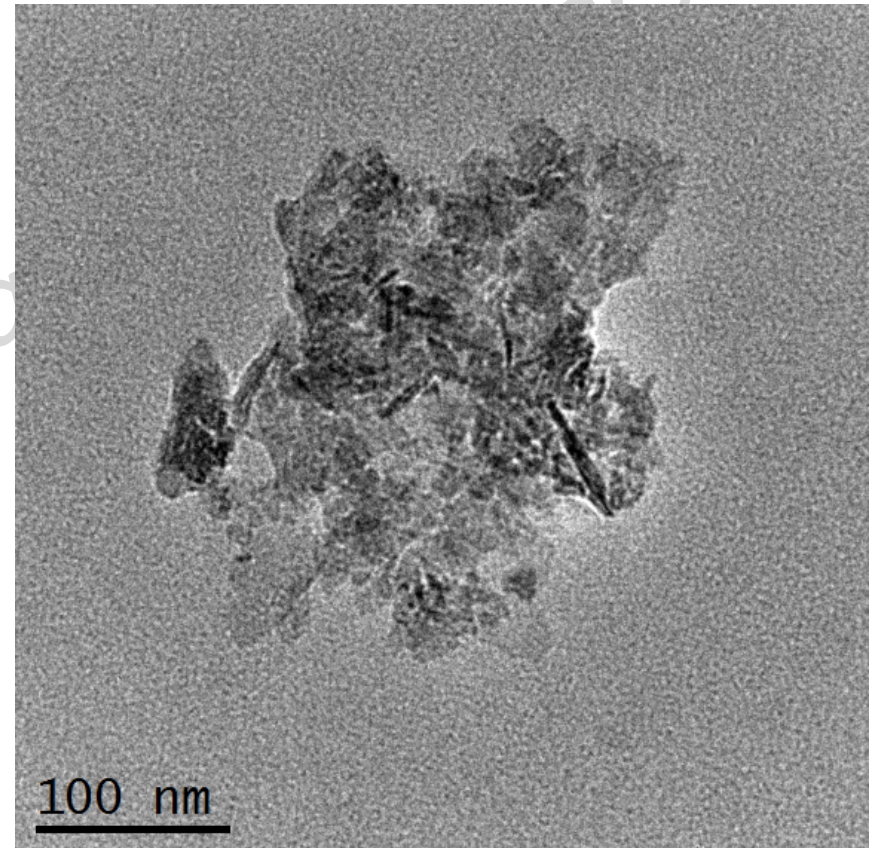
- Average particle diameter:  
 $20 \pm 5$  nm





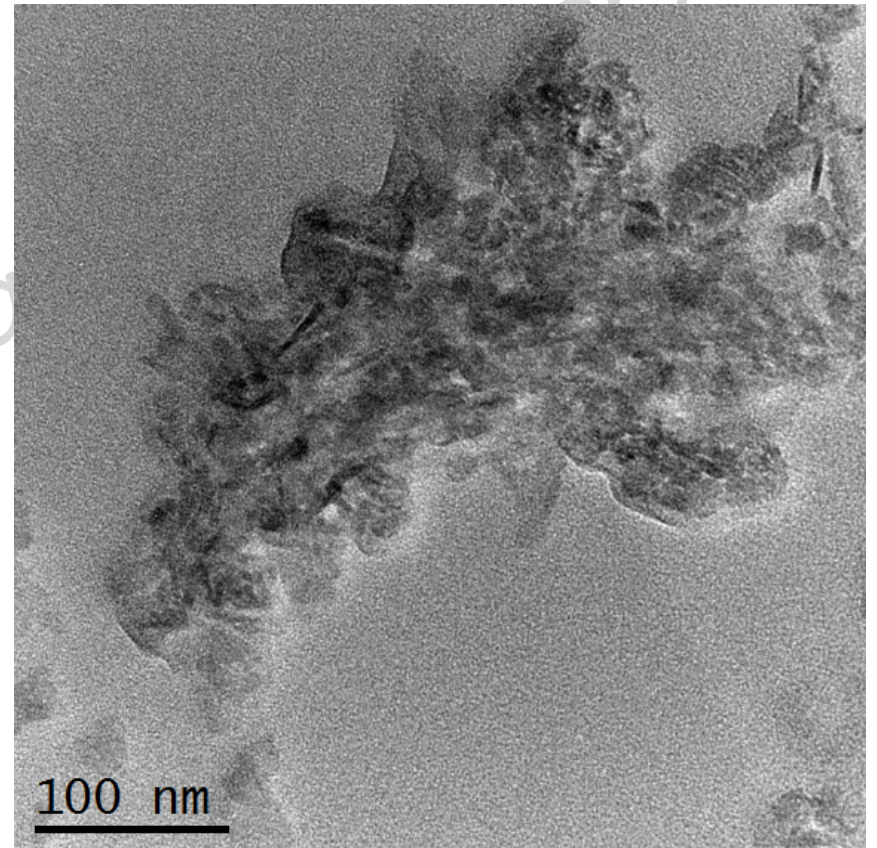
## Ca, P, and O containing particles

- Average particle diameter:  
 $20 \pm 5$  nm
- Majority is not needle-like,  
but some needle-like HA  
exists



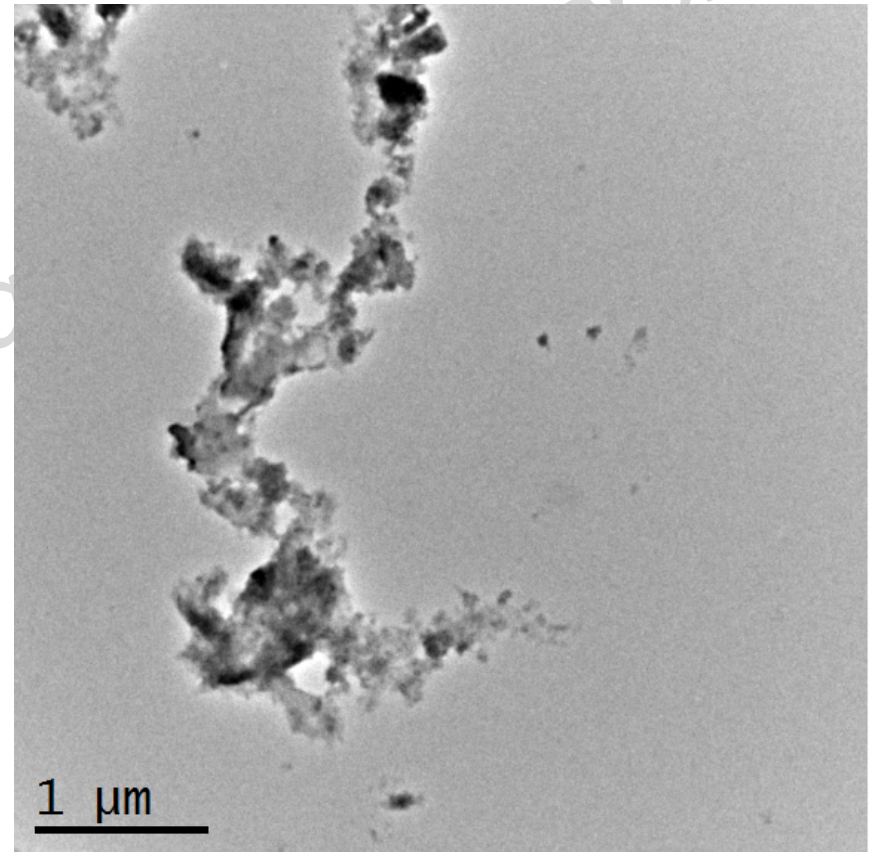
## Ca, P, and O containing particles

- Average particle diameter:  
 $20 \pm 5$  nm



## Ca, P, and O containing particles

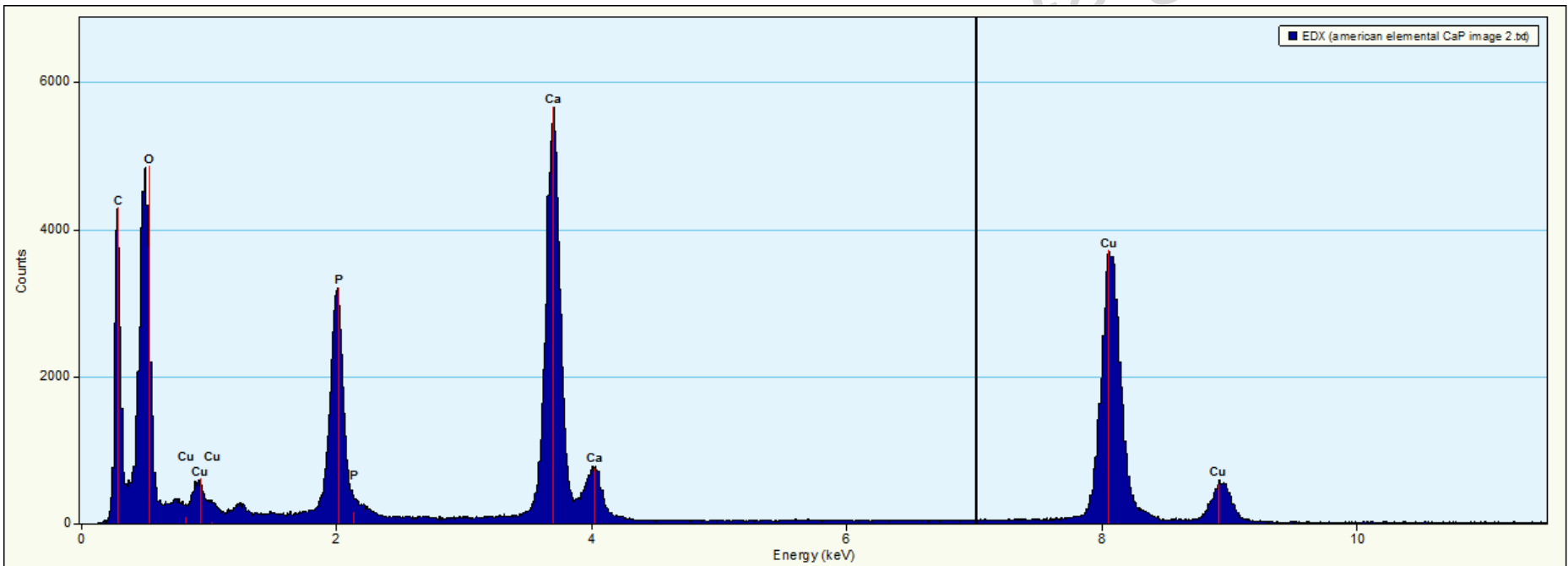
- Average particle diameter:  
 $20 \pm 5$  nm





# EDX of Food-Grade HA (Product 2)

- C and Cu from TEM grid



## Summary of Reference Food Grade Hydroxyapatite

- Two food grade hydroxyapatite (HA) samples were analyzed by TEM and EDX
- One sample was composed solely of needle-like HA (Product 1) while the other sample had majority spherical-shaped HA (Product 2)
- Sample preparation process does not solely form nano HA needle-like structures