Department of Chemistry and Biochemistry

Analysis of East Texas landfowl and waterfowl eggshells

Crystal Davis[1]; Dr. Alyx Frantzen, PhD[1]; Dr. Franta Majs, PhD[2]
[1]Stephen F. Austin State University, [2]University of Florida

STEPHEN F. AUSTIN STATE UNIVERSITY
COLLEGE OF SCIENCES AND MATHEMATICS

Abstract

Calcium carbonate naturally occurs and is a key component in animalia structural physiology, land egg laying amniotes, fossil deposits, pharmaceuticals, organic deposits in soil, and is the main chemical component in limestone. Calcium carbonate is theoretically found to make up ~94% of the chemical composition in chicken eggs with variations among different avian species, which translates to an approximate 40% Ca composition in eggshells in commercial chickens. Eggshell formation is dependent on carbonic anhydrase in the shell glands of egg-laying species and can be affected by environmental dietary factors. If the diet of the avian species contains contaminants, such as strontium or other heavy metals, the calcium can be displaced by the contaminant and cause malformations in eggshell structure. The contaminant can further leach into the albumen and yolk of the egg. This is harmful to any organism consuming the egg or the embryo developing in the egg. The eggshells of various East Texas landfowl and waterfowl are being analyzed to determine calcium, strontium, carbonate, carbon, nitrogen, and chloride composition of the shells. The shells are divided into the sharp and dull ends to determine if there is any difference in chemical composition. Analysis is done using ICP-OES, ICP-MS, LECO carbon nitrogen detection, STA, IR, and IC.

Methods and Materials

Sample Preparation

- Collected eggs and washed with a mild detergent
- Carefully scored to divide eggs into sharp and dull ends then cleaned and removed inner membranes.
- Ground eggshells using a McCrone Micronizing mill and 200 proof EtOH for 3 minutes each to form an eggshell slurry, Greater Rhea egg was ground approximately 30 minutes
- EtOH was evaporated from eggshell slurry and dried at 105ºC oven then homogenous to a powder using an agate mortar and pestle
- 0.1000 g of each sample was digested using modified EPA Method 3050B for Sediments, Sludges, and Soils[6]
- Powdered samples used for STA
- Preparation of 0.1 M HCl and 0.01 M NaOH
- Standardization of HCl and NaOH using KHP

Instrumentation

- Perkin Elmer STA 6000 coupled with Perkin Elmer Spectrum One FT-IR in nitrogen and air backgrounds
- Perkin Emler ELAN DRC II ICP-MS
- Thermo Fisher iCAP 7400 ICP-OES
- LECO CN 628

Figure 1. Cleaned and dried halves of eggshells before grinding. SFA White, Greater Rhea, SFA Brown, Bantam Seabright, Dorking, Araucana Cross, White Leghorn, Duck, and Barred Rock(Top left to right descending).

Introduction

Eggs are a major component of the life cycle of avian species and the environment. The composition of the egg reflects the diet consumed by the animal and can be used as an indicator of the environment it lives in[1]. Proper calcium intake is imperative for the success of offspring and the production of eggs. Improper calcium intake or presence of heavy metals, like strontium, can disrupt the hatching success of the eggs[2].

Calcium carbonate is the main chemical component of eggshells and makes up about 94% of the eggshells in poultry chicken eggshells and about 92% in duck eggshells[3,4]. Other trace metals like Sr, Ba, Mn, As, Cd, Cu, Pb, Hg, Se, V, and Zn could also possibly be found in the chemical composition of the eggshells[2,5]. The presence of some of these heavy metals can lead to egg malformation, embryo death, transfer of the metal to the yolk and consumption by other species.

The $CaCO_3$ is monitored using STA-IR, following the decomposition of the carbonate to its respective oxide and CO_2. The combustion of any organic material results in H_2O and CO_2 peaks would be seen in the IR. ICP-OES, ICP-MS, and a LECO CN analyzer will be used to detect the percents of other possible chemical components of the eggshells.

Results

Table 1. Percent $CaCO_3$, %CO_3^{2-} , and %C by mass of various eggshells from avian species in East Texas using STA-IR in air and nitrogen backgrounds, back titration, and LECO C N detection.

Eggshell Sample	%$CaCO_3$ Nitrogen	%$CaCO_3$ Air	%CO_3^{2-} Air	%CO_3^{2-} Nitrogen	%$CaCO_3$ Back titration	% CO_3^{2-} Back titration	%C LECO	% CO_3^{2-} LECO
Barred Rock 1 sharp end (rotten)	94.71	95.94	64.57	63.77	93.87	56.28	10.64	53.16
Barred Rock 1 dull end (rotten)	94.25	94.01	63.70	63.90	94.04	56.38	10.14	50.65
Barred Rock 2 sharp end	93.85	93.71	64.59	64.47	92.96	55.73	10.82	54.04
Barred Rock 2 dull end	93.38	93.66	64.52	64.41	93.35	55.97	11.41	57.03
White Leghorn 1 sharp end	95.62	95.62	63.19	63.20	93.82	56.25	10.26	51.28
White Leghorn 1 dull end	96.99	97.24	62.45	62.38	95.18	57.07	9.65	48.21
White Leghorn 2 sharp end	94.53	94.09	64.33	64.16	93.16	55.85	12.56	62.74
White Leghorn 2 dull end	93.98	93.97	64.63	64.48	88.31	52.95	12.57	62.82
Araucana Cross 1 sharp end	93.30	93.12	64.93	65.07	91.18	54.67	12.53	62.58
Araucana Cross 1 dull end	93.28	93.21	64.59	64.55	91.52	54.87	12.25	61.20
Araucana Cross 2 sharp end	93.05	93.01	64.59	64.50	90.74	54.40	12.22	61.05
Araucana Cross 2 dull end	92.98	92.70	64.54	64.56	92.35	55.37	12.34	61.64
Dorking sharp end	89.50	94.13	64.30	61.46	92.74	55.61	13.20	65.93
Dorking dull end	96.57	96.64	62.61	62.39	96.63	57.93	10.47	52.34
Bantam Sebright sharp end	92.33	92.57	64.98	65.13	90.48	54.25	13.46	67.23
Bantam Sebright dull end	91.68	91.10	65.56	65.39	87.63	52.54	13.78	68.84
Duck 1 sharp end (rotten)	90.92	91.26	66.18	65.94	89.28	53.53	14.19	70.91
Duck 1 dull end (rotten)	91.15	91.06	65.75	65.84	91.71	54.98	14.00	69.96
Duck 2 sharp end	91.80	91.62	65.37	65.62	83.99	50.35	13.96	69.73
Duck 2 dull end	92.85	92.76	64.83	64.89	91.51	54.87	13.67	68.31
Duck 3 sharp end	93.22	92.69	65.18	64.97	91.95	55.13	13.22	66.06
Duck 3 dull end	93.29	92.88	64.55	64.93	91.35	54.77	12.93	64.62
Greater Rhea hole end	93.64	93.44	63.40	63.36	94.12	56.43	10.69	53.40
Greater Rhea full end	94.47	94.49	63.23	63.07	95.84	57.46	11.27	56.29

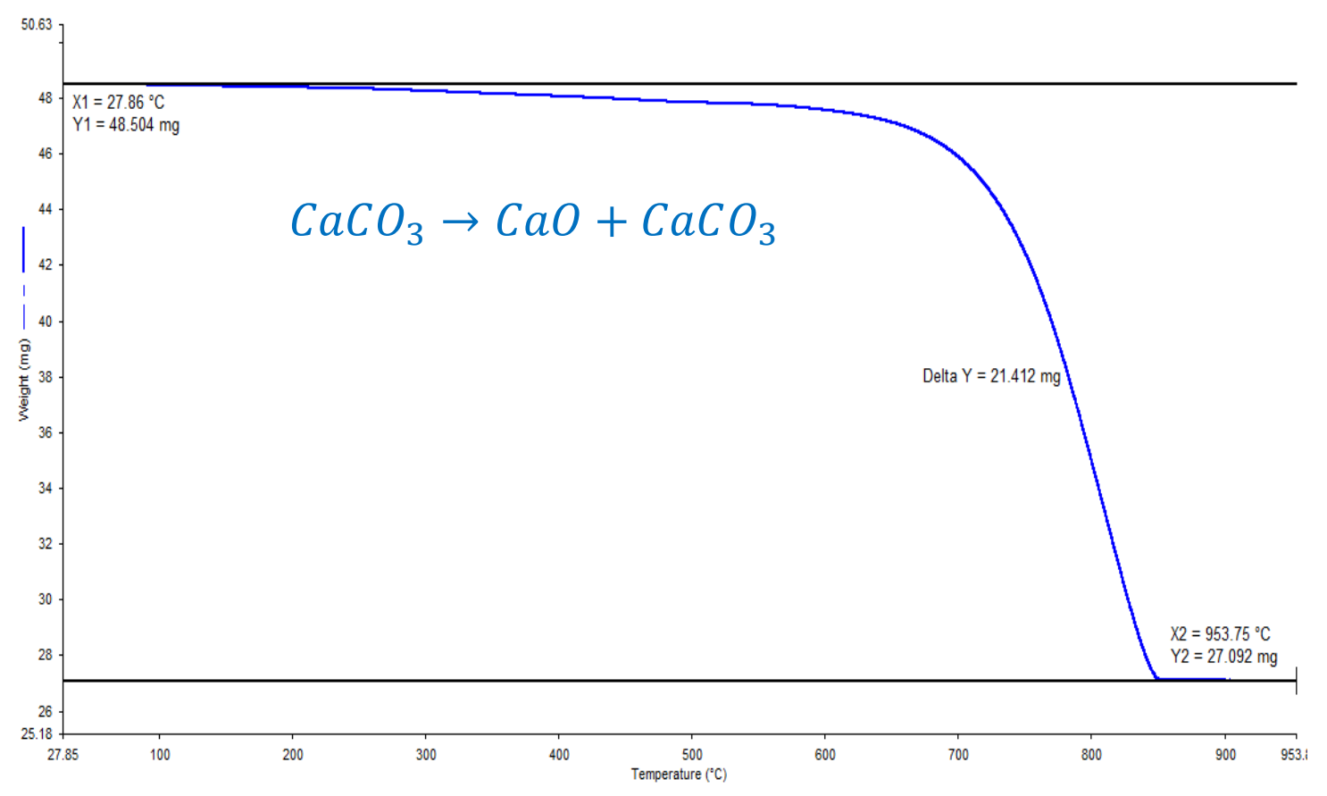


Figure 2. Decomposition of $CaCO_3$ using Thermal Gravimetric Analysis by TGA. $CaCO_3$ decomposes to CaO and CO_2 between 600 °C and 950 °C

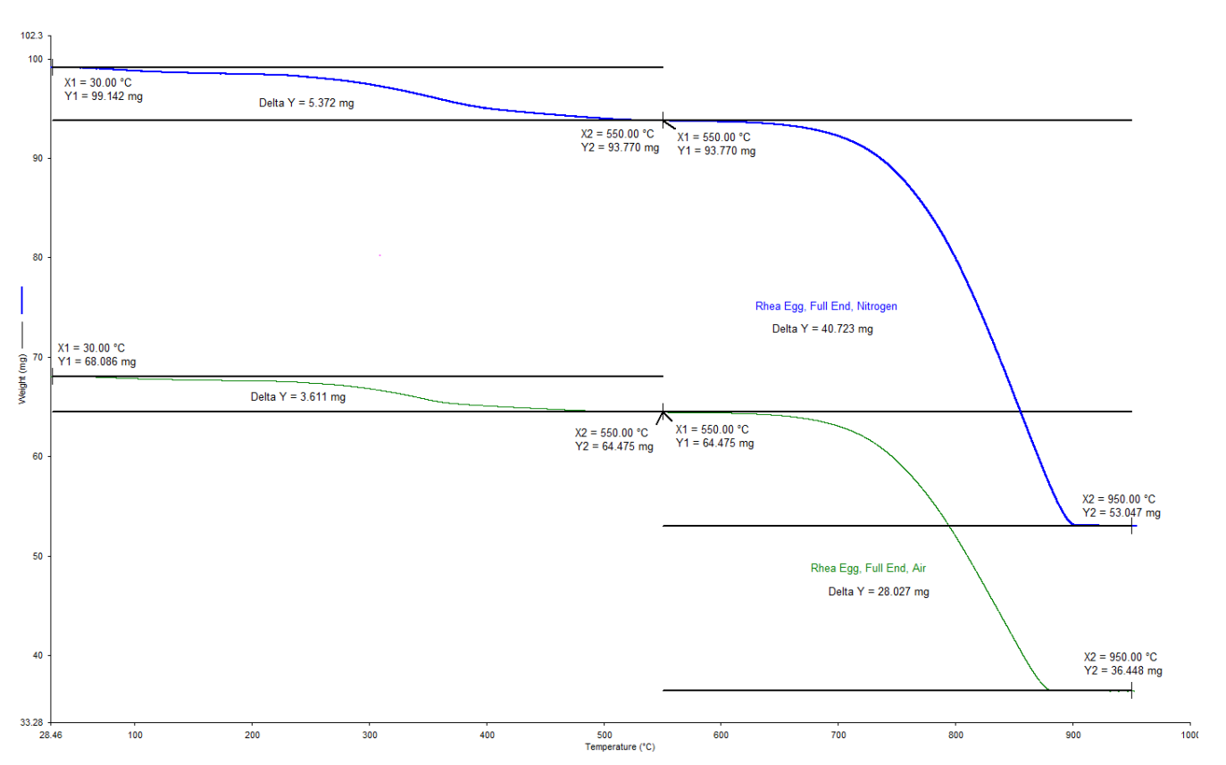


Figure 3. STA plot of the thermal gravimetric mass loss of the Greater Rhea full end in nitrogen(blue) and air(green) backgrounds.

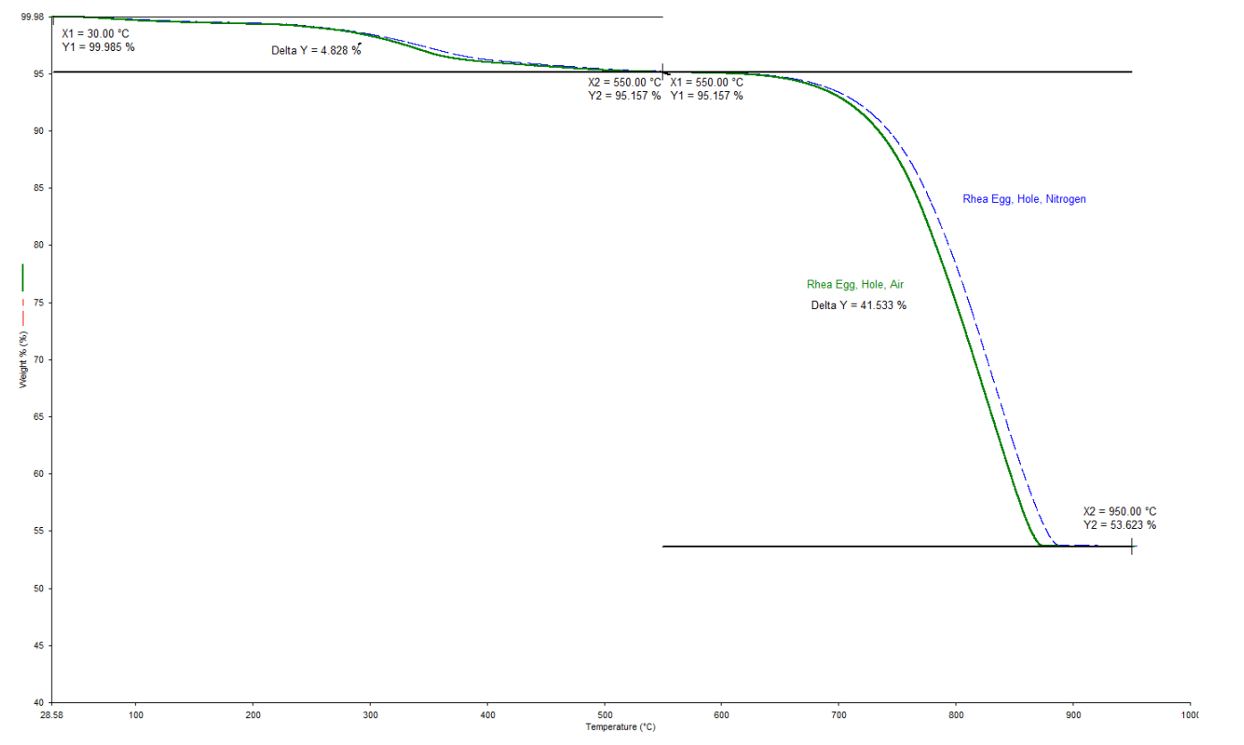


Figure 4. Weight percent of mass loss from the thermal event of the Greater Rhea full end in nitrogen(blue) and air(green) backgrounds. The percent mass loss in both nitrogen and air were the same. The weight percent loss in the first thermal event was 4.828%. The weight percent mass loss in the second thermal event was 41.533%.

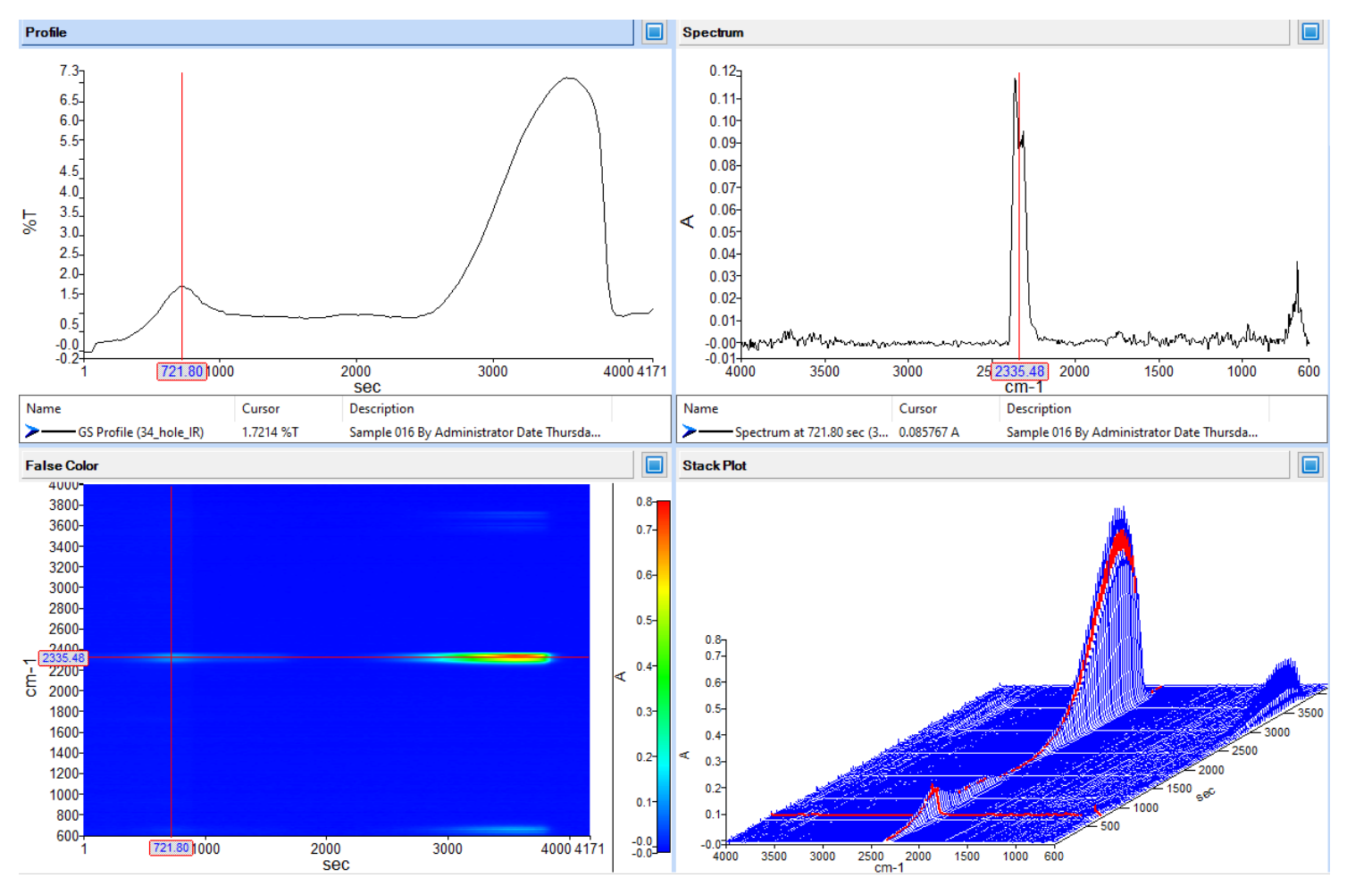
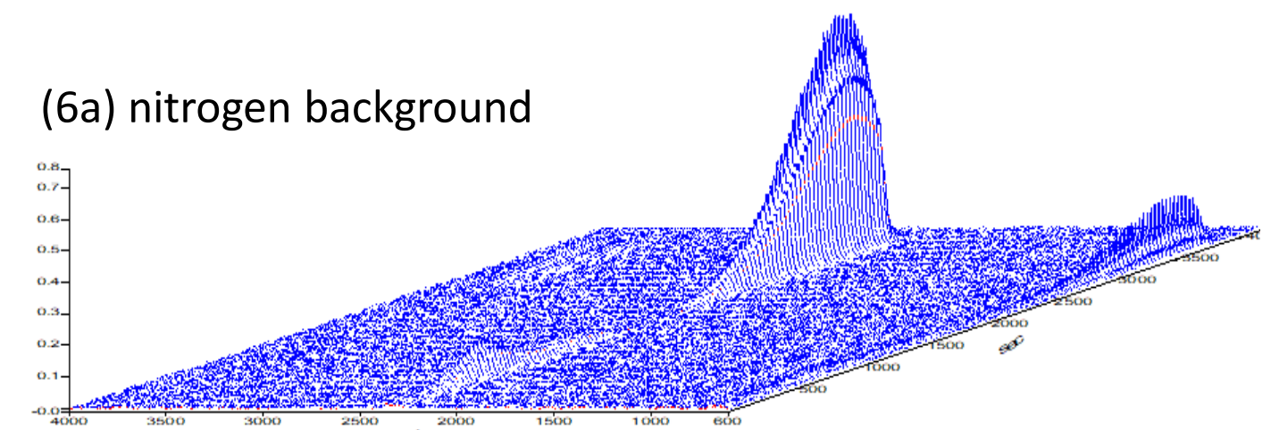


Figure 5. FT-IR four plot screen of the spectra of Greater Rhea hole end captured during STA analysis. Time Profile showing %transmittance over time length of spectra taken (top left). Spectrum of sample collected at one frame(top right). False color shows intensity of peak over time taken(bottom left). Stack plot is the 3D accumulation of spectra collected over time(bottom right).

(6a) nitrogen background



(6b) air background

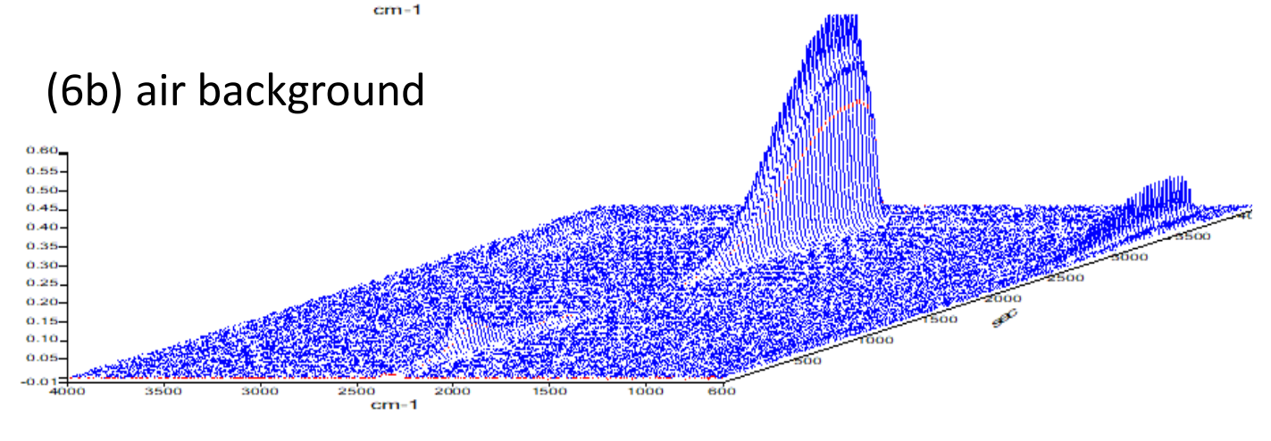


Figure 6. Stack plot of the accumulated FT-IR spectra of Greater Rhea hole end during STA analysis in nitrogen(6a) and air(6b) background collected over time. CO_2 peaks were seen during the first thermal event and more intensely during $CaCO_3$ decomposition, second thermal event. No H_2O peaks were seen during the run in air.

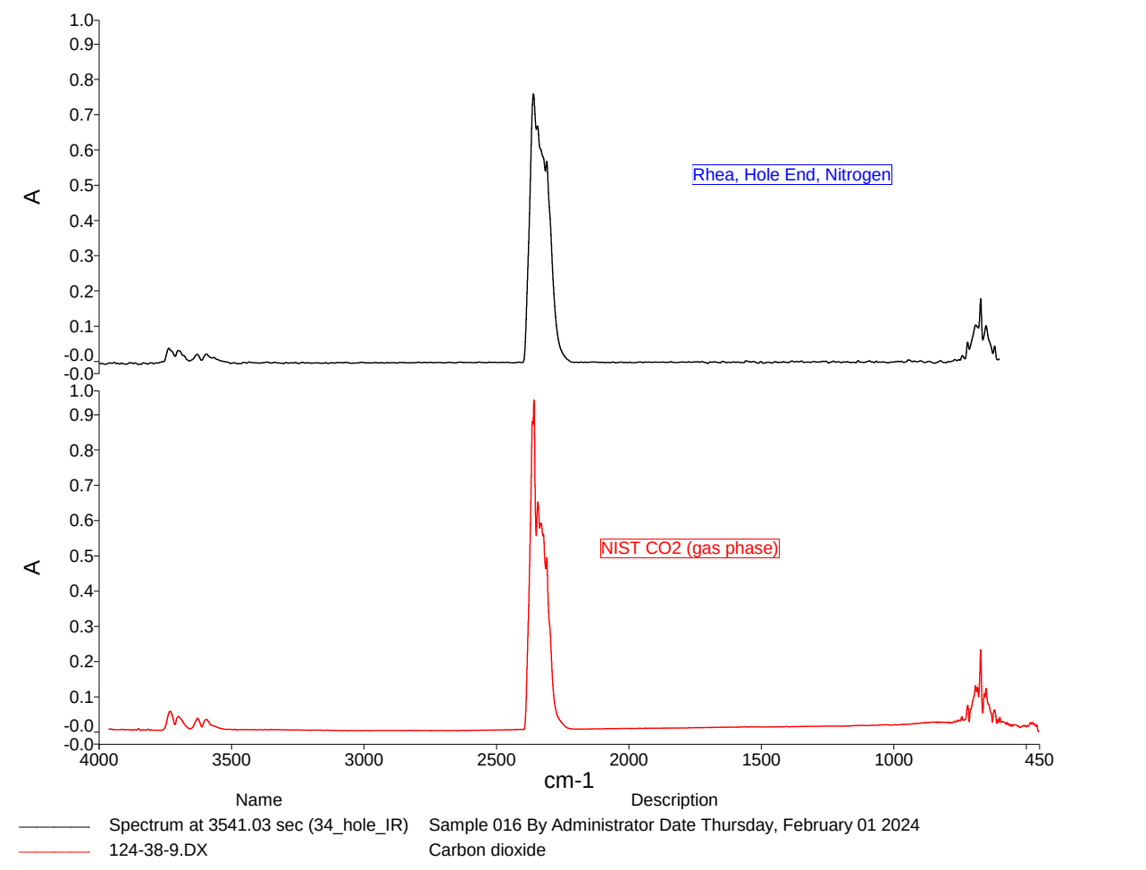


Figure 7. FT-IR spectra of the Greater Rhea hole end during STA analysis in nitrogen background(top spectra) at max absorption time of 3541.03 sec, during second thermal event, compared to NIST CO_2 gas phase reference spectra (bottom spectra) at a 96% match hit.

Discussion/Conclusion

- The % $CaCO_3$ found in the majority of eggshells was ~ 94%, which was to be expected based on literature[3].
- The % $CaCO_3$ found in duck eggshells (rotten) was ~ 91% which with approximately near the expect literature range of the % $CaCO_3$ of duck eggshells[4].
- There was significant mass loss event present in the eggshells to exhibit a thermal event between 200 °C and 600 °C in all the eggshells, but cannot be identified through STA-IR.
- During STA-IR analysis in air, there was no detectable H_2O peak between 4000-3500 and 2000-1200 cm^{-1} in IR.
- The first thermal event is probably a combination of $MgCO_3$ and protein decomposition. Both processes produce CO_2, with protein decomposition producing additional water or ammonia. If present, both are below the detection limit of the IR.
- Inconsistencies were found between LECO and STA data for several eggshells due to drift in the LECO columns. These will be rerun.

Future Work

- Analysis of digested eggshell samples using ICP-MS and ICP-OES instrumentation to determine the concentration of any trace heavy metals and contributing chemical components to the composition of the shell.
- Confirm % $CaCO_3$ from the STA-IR using standardized carbonate determining procedures: XRF and XRD.
- Confirm presence of $MgCO_3$ using XRF and XRD.

Contact

Crystal Davis
Stephen F. Austin State University
davisc13@jacks.sfasu.edu
Dr. Alyx Frantzen
Stephen F. Austin State University
afrantzen@sfasu.edu

References

(1) Butcher, G.; Miles, R. *Concepts of Eggshell Quality*. edis.ifas.ufl.edu. https://edis.ifas.ufl.edu/publication/VM013.
(2) Mora, M. A. Heavy Metals and Metalloids in Egg Contents and Eggshells of Passerine Birds from Arizona. *Environmental Pollution* **2003**, *125* (3), 393–400. https://doi.org/10.1016/s0269-7491(03)00108-8.
(3) Murakami, F.; Rodrigues, P.; Teixeira De Campos, C.; Antônio, M.; Silva, S. Physicochemical Study of CaCO 3 from Egg Shells Estudo Físico-Químico Do Carbonato de Cálcio Obtido a Partir Da Casca de Ovo. *Ciênc. Tecnol. Aliment* **2007**, *27* (3), 658–662.
(4) Malau, N. D.; Adinugraha, F. *Synthesis of hydrokxyapatite based duck egg shells using precipitation method*. IOPscience. doi:10.1088/1742-6596/1563/1/012020.
(5) Daniels-Abdulahad, M. *NSUWorks NSUWorks All HCAS Student Capstones, Theses, and Dissertations HCAS Student Theses and Dissertations Heavy Metal Content in Eggs from Wild and Managed-Care Heavy Metal Content in Eggs from Wild and Managed-Care Humboldt Penguins Humboldt Penguins*; 2023. https://nsuworks.nova.edu/cgi/viewcontent.cgi?article=1143&context=hcas_etd_all/.
(6) United States Environmental Protection Agency. (1996, December). Method 3050B: Acid Digestion of Sediments, Sludges, and Soils.

Acknowledgements

- This research was supported by the College of Sciences and Mathematics as part of the Summer Undergraduate Research Experience at Stephen F. Austin State University.
- Department of Chemistry and Biochemistry
- Robert A. Welch Foundation Grant AN-0008
- Robert A. Welch Summer Research Experience Grant AN-0008
- Stephen F. Austin Soil, Plant, and Water Laboratory
- SFA Poultry Farm
- East Texas Hobby Farm egg donor