

Lake Campus Professional Development Grant

Prepared for
Wright State University Lake Campus
7600 Lake Campus Drive
Celina, OH 45822

Submitted by
Charles, N. Ciampaglio, PhD
Professor, Earth and Environmental Science
Wright State University Lake Campus

Title: “Optimization of the Aqueous Formic Acid Solution Formulation and Dissolution Procedures Used in the Removal of Fossil Vertebrate Material for Carbonate Rocks”

Period of Study:	Spring – Summer 2016
Date Submitted:	February 8, 2016
Principal Investigator:	Dr. Charles N. Ciampaglio
Amount Requested:	\$2,764.23

Narrative

Research Focus

My laboratory addresses fundamental issues in chondrichthyan (sharks, rays, and their relatives) phylogeny, evolution, and distribution, by conducting detailed analyses of Late Paleozoic (Devonian – Permian) exposures throughout the Eastern and Mid-Continental United States. This period of time includes the apparent environmental degradation of habitats world-wide and concluded with the largest biotic catastrophe of all time – the End Permian Mass Extinction. In particular, there are several on-going undergraduate and graduate projects focused on the analysis of species abundance, diversity, and morphological variation of dental and dermal elements contained within lithological units spanning the Late Devonian through the Permian (which encompasses a “lead-up” to the End-Permian Mass Extinction). In order to identify evolutionary patterns, such as the diversity and morphological innovation of the chondrichthyans as a group, we must first isolate the chondrichthyan remains that are contained within carbonate rocks such as limestones and dolostones.

Statement of Relevance

The paleontological literature contains few detailed procedures for acid preparation of macrofossils. Many times workers only identify the acid reagent used without mention of concentrations or procedures employed. Jeppsson reports detailed procedures using acetic acid (1985, 1999) and formic acid (1995), principally for the safe extraction of microscopic conodont elements from carbonates, yet ignores the extraction of macrofossils such as teeth, scales, and other boney remains.

The acetic acid system that Jeppsson advocates is a 10% acetic acid solution that is slightly buffered with trisodium phosphate. While this technique is sound, it is a very slow process that requires large amounts of acid solution with respect to a given amount of sample to be dissolved. Part of the problem lies with the acidity of acetic acid itself, with a pK_a of 4.76. On the positive side, being a fairly weak acid, it usually does not damage even the most delicate of the chondrichthyan remains.

Jeppsson (1995) also advocates a 10% aqueous formic acid solution that is buffered *in situ* using calcium carbonate and trisodium phosphate. While this stronger acid, with a pK_a of 3.77, requires much less volume with regard to mass of sample to be dissolved, and a much quicker dissolution time (typically three times as fast as acetic acid solution), it does have its drawbacks. Buffering the acid raises the pH considerably, and then remains consistently higher compared to an unbuffered solution, thus diminishing its ability to dissolve carbonate material. Using too little acid solution, or allowing evaporation of the aqueous component results in the formation of copious amounts of calcium formate, which coat the remaining carbonate material, effectively quenching the reaction between acid and carbonate matrix. While the cost per liter of 95% formic acid is only slightly more expensive than glacial acetic acid (100%), using a 7% or perhaps a 5% aqueous solution would lower the effective cost per liter of acid considerably.

Proposed Study

Since using an aqueous formic acid solution is faster and much more economical than aqueous acetic acid, optimizing the acid dissolution procedure would allow much higher quantities of carbonate material, at much higher rate of dissolution, to be dissolved at a much lower cost than the methods currently employed. Optimization will consist of four parts: (1) comparing buffered versus unbuffered aqueous formic acid solutions, (2) prevention of calcium formate crystals and increased reaction rate via heating and continual stirring of the acid solution, (3) testing various acid concentrations to determine the

lowest, yet effective aqueous formic solution that can be employed, and (4) determining the effective length of time the acid solution retains the ability to dissolve carbonate matrix.

All experiments will be conducted using 2-gallon, HDPE plastic buckets and modified lids (the lids modified for housing stirring motors, pH sensors, temperature probes, and if necessary, carbon dioxide sensors, and compressed air to purge the gasses the system evolves). The chemical system will be composed of 4-liters of aqueous formic acid solution (10 % solution unless otherwise noted) and 400 grams of limestone (broken into roughly 2.5 cm cubes) from the Mississippian-Age Burlington/ Keokuk Formational boundary located in the Nelson Brothers Quarry, Biggsville, IL. This is a fairly pure limestone that contains abundant vertebrate remains, with small amounts of silica, phosphate, and clay grains. Stirring will be performed using 12-volt DC, 60 rpm geared motors with a stainless steel shafts and plastic blades attached to the spindles via a coupler. For reaction systems that are stirred, stirring will occur for 60 seconds at one hour intervals. For systems that are heated, heating will be continuous in order to maintain a 30°C temperature, using submersible, temperature controlled, 50W Aqueon aquarium heaters. pH and temperature measurements will be determined using Vernier PH-BTA and TMP-BTA sensors, respectively. Carbon dioxide concentration will be measured using a Vernier CO@-BTA sensor housed within a modified 2-gallon bucket and lid vessel. Purging the vessel of residual carbon dioxide will be accomplished using compressed air via a microcomputer controlled solenoid system built “in-house”. The systems will be monitored using the Vernier Lab Quest2 data collection unit. Residual material (vertebrate remains and undissolved matrix) will be collected via a set of stacked US Sieves (with mesh opening sizes of 4.76 mm, 2.00 mm, and 0.25 mm, respectively).

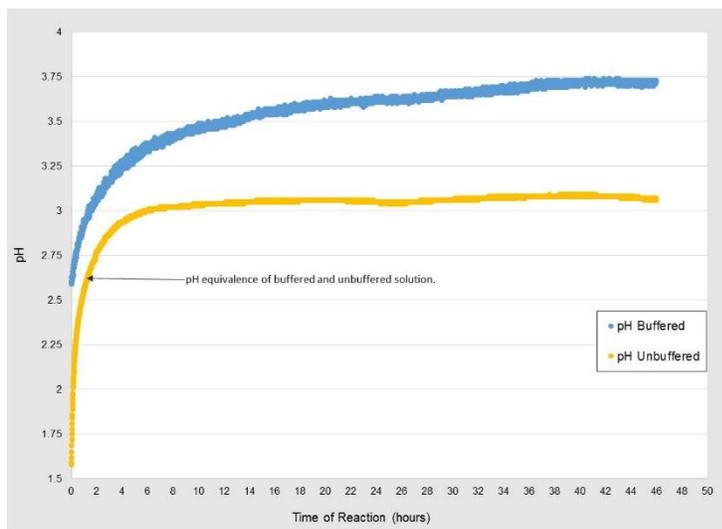


Figure 1: pH of buffered and unbuffered 10% formic acid solution reacted with 400 grams of vertebrate bearing limestone, from the Burlington/Keokuk Formational Boundary.

System	Rock Unit	Starting Amt.	End Amt.	Amt. Residual
Stir and Heat	Brush Creek LS	687.00 g	0.00 g	1.58 g
Control	Brush Creek LS	687.00 g	31.00 g	4.17 g

Table 1: Preliminary study stirring and heating versus control, 10% formic acid solution.

Preliminary results show that the pH of a non-buffered solution of 10% formic acid reaches the initial pH of a buffered solution within an hour (Figure1), but remains lower throughout a 72 hour period. Furthermore, vertebrate material collected from the non-buffered solutions appears to be undamaged. In order to fully test safety of using the unbuffered systems (consistently lower pH than buffered systems), a series of trials of buffered versus non-buffered solutions will be run on identical carbonate samples that contain “spikes” (delicate, highly ornamented conodont specimens that do not exist in the carbonates undergoing dissolution). After 72 hours the solution and residual vertebrate material will be sieved through fine screens, the spikes collected, and their condition (degree of ornamentation and completeness) compared.

A set of stirred and heated versus unstirred and unheated formic acid

dissolution trials will be conducted. The mass of matrix before and after, as well as the mass of residual material before and after will be measured. The presence, and amount of formate crystal formation will also be noted. Preliminary results show that the mass of matrix dissolved is much greater for the heated and stirred solution, yet the amount of recovered material is less (see Table 1). At first this may seem counterintuitive, but upon analysis it was obvious that the material recovered from the stirred and heated reaction vessel was nearly pure vertebrate material with small amounts of silica present. This contrasts

with the material recovered from the control vessel, where the recovered material contained copious amounts of lithic fragments (undissolved pieces of matrix) and clay. Preliminary results also show that the stirred and heated system reached and consistently remained at a higher pH than the control (see Figure 2). This is an indication of increased reaction rate and efficiency.

A series of trials will be conducted where upon the concentration of the aqueous formic acid solution will be varied. Currently, the standard employed is a 10% formic acid solution. The standard will be used as the control and nine, seven, and five percent solutions will be compared to the control system. Comparison will consist of the mass of material dissolved, and mass, and quality

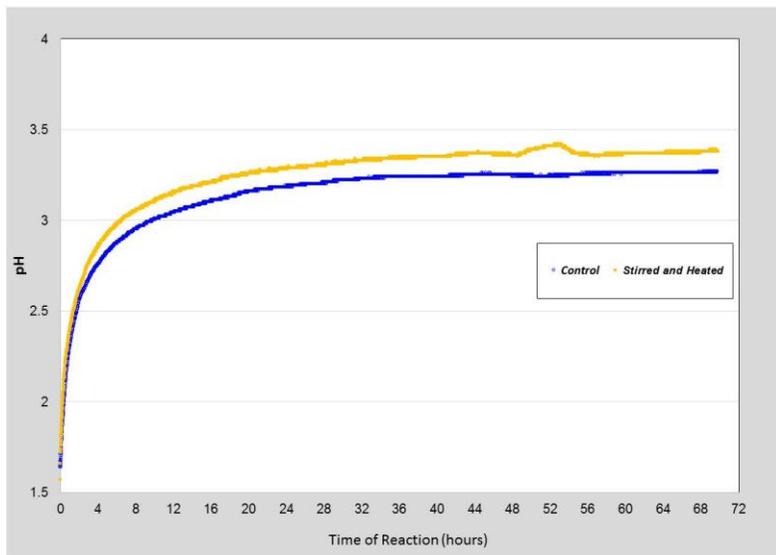
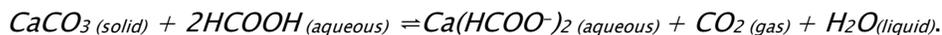


Figure 2: pH of stirred and heated system versus control. Both systems employed a 10% formic acid solution which was reacted with 687.00 grams of Brush Creek Limestone, Athens Co., OH.

of the material obtained after dissolution.

To determining the effective length of time the acid solution retains the ability to dissolve carbonate matrix the amount of CO₂ generated will be measured in one hour time intervals. The amount of carbon dioxide evolved by the system is an excellent indication of reaction rate:



Measuring the amount of carbon dioxide present must be performed with care. Since carbon dioxide is a heavier than air gas its concentration will increase (via accumulation in the vessel) over time. In order to negate this effect, a five second burst of compressed air will purge the system. After one minute has passed the level of carbon dioxide will be measured. This process will be repeated at one hour intervals for 72, 96, 120, and 144 hour trials in both stirred and heated, and control systems. The point at which the amount of carbon dioxide levels-off, and or becomes negligible will be the indication that the effectiveness of the acid has ceased.

References

- Jeppsson, L., Fredholm, D. and Mattiasson, B., 1985, "Acetic Acid and Phosphatic Fossils - A Warning", *J. of Paleontology*, 59(4), pp. 952-956.
- Jeppsson, L. and Anehus, R. 1995, "A Buffered Formic Acid Technique For Conodont Extraction", *J. of Paleontology*, 69(4), pp. 790-794.
- Jeppsson, L., Anehus, R. and Fredholm, D., 1999, "The Optimal Acetate Buffered Acetic Acid Technique For Extracting Phosphatic Fossils", *J. of Paleontology*, 73(5), pp. 964-972.

<u>Timeline</u>	
Preliminary Research	Completed
Ordering Equipment and Consumables	March 2016
Comparison of Buffered vs. Non-Buffered Systems Studies	April 2016
Increasing the Effective Rate of Reaction Studies	May 2016
Determination of Acid Effectiveness vs. Acid Concentration Studies	June 2016
Length of Acid Effectiveness Studies (includes building specialized apparatus)	July – August 2016
Presentation at the National GSA Meeting to be held in Denver, CO.	September 22 – 25, 2016

Principal Investigator Qualifications:

Charles N. Ciampaglio, Ph.D.
Professor of Earth and Environmental Science
Wright State University, Lake Campus
230 H Faculty/Staff Suites
7600 Lake Campus Drive
Celina, OH 45885
Date of appointment: September 1, 2003

EDUCATION

DUKE UNIVERSITY, Durham, North Carolina
Ph.D. September 2002
Major: Paleontology
Minor: Geology

DUKE UNIVERSITY, Durham, North Carolina
Master of Arts: February 2000
Major: Zoology
Minor: Geology

TOWSON UNIVERSITY, Towson, Maryland, 1991-1997
Degree: B.S. (*summa cum laude*)
Major: Chemistry Minor: Mathematics
Major: Physics Concentration: Biology

SELECTED REFERENCES AND ABSTRACTS

- Ciampaglio, C. N., Cicimurri, D. J., Ebersol, J. A., and Runyon, K. E., 2013, "A Note on Late Cretaceous Fish Taxa Recovered from Stream Gravels at Site AGr-43 in Greene County, Alabama", *Alabama Museum of Natural History Bulletin* 31, V. 1, 84-97.
- Ciampaglio, C. N., and Cicimurri, D. J., Deuter, L. H., and Taylor, M. A., 2013, "A Review of the Chondrichthyans from the Mississippian System of Northern Alabama, USA", *Alabama Museum of Natural History Bulletin* 28, V. 1, 67-80.
- Ciampaglio, C. N., Wray, G. A., and Corliss, B. H., 2005, "A Toothy Tale of Evolution: Convergence in Tooth Morphology among Marine Mesozoic – Cenozoic Sharks, Reptiles, and Mammals", *The Sedimentary Record*, v.3, n.4, pp. 4 – 8.
- Jacquemin, S. J., Cicimurri, D. J., Ebersol, J. A., Ciampaglio, C. N., Jones, M., and Whetstone, Z., 2016, "Quantifying heterodonty in the late Devonian (upper Famennian) sharks *Cladoselache* and *Ctenacanthus* from the Ohio Shale", *PalArch's Journal of Vertebrate Palaeontology* 13:1.
- Jacquemin, S. J., Ebersol, J. A., Dickinson, W. C., and Ciampaglio, C. N., 2015, "Late Pleistocene fishes of the Tennessee River Basin: an analysis of a late Pleistocene freshwater fish fauna from Bell Cave (site A Cb-2) in Colbert County, Alabama", USA, *Peer J* 4:e1648; DOI 10.7717/peerj.1648.
- Cicimurri, D. J., Ciampaglio, C. N., and Runyon, K. E., 2014, "Late Cretaceous Elasmobranchs from the Eutaw Formation at Luxapalila Creek, Lowndes County, Mississippi", *PalArch's Journal of Vertebrate Palaeontology*, 11, 2, pp. 1-36
- Nyberg, K., Ciampaglio, C. N., and Wray, G. A., 2006, "Tracing the Ancestry of the Great White Shark Using Morphometric Analyses of Fossil Teeth", *Journal of Vertebrate Paleontology* 26(4), 806-814.

Current Funding

None

Itemized Budget

<u>Item</u>	<u>Company</u>	<u>Quantity</u>	<u>Price</u>
LabQuest® 2 (LABQ2)	Vernier	2	\$329 ea. (total = \$658)
pH Sensor (PH-BTA)	Vernier	4	\$79 ea. (total = \$316)
Stainless Steel Temperature Probe (TMP-BTA)			
	Vernier	4	\$29 ea. (total = \$116)
CO ₂ Gas Sensor (CO2-BTA)	Vernier	2	\$259 ea. (total = \$518)
2-Gallon Bucket (#3048)	US Plastics	10	\$3.78 ea. (total = \$37.80)
2-Gallon Lid (#3049)	US Plastics	10	\$0.86 ea. (total = \$8.60)
95% Formic Acid (15-Gallon Dru, 151# lb)			
	DudaDiesel	2	\$245 ea. (total = \$490)
			w/ shipping total = \$674.24
Aqueon Submersible Aquarium Heaters (50-watt)			
	Amazon.com	5	\$33.04 ea. (total = \$165.20)
DC Motor with Gearhead 12VDC 74Ma (#253500)			
	Jameco Electronics	8	\$15.95 ea. (total = \$127.60)
6mm Precision Shafting (150mm length; #634294)			
	ServoCity.com	8	\$2.09 ea. (total = \$16.72)
Actobotics Set Screw Shaft Coupler (1/4" to 6mm; RB-Sct-397)			
	RobotShop.com	8	\$4.99 ea. (total = \$39.92)
Small Plastic Push-On Fan Blade, 3 1/2" Dia., CW, 3/16" Bore, 3/4" Blad Depth, 4 Blade (#WBB879747)			
	GolbalIndustrial.com	8	\$3.28 ea. (total = \$26.24)
6mm Prop Adapter & Collet (Sku: GH-34)			
	GraysonHobby.com	8	\$5.25 ea. (total = \$42.00)
5/16" x 0.875" OD Grade 316 Stainless Steel Large OD General Purpose Flat Washer (#78016)			
	Fastenal	80	\$0.1883 ea. (total = \$15.07)
#6-18 x 3/4" Phillips Drive Pan Head Grade 18-8 Type A Point Stainless Steel Sheet Metal Screw (#1172906)			
	Fastenal	40	\$0.071 ea. (total = \$2.84)

ESTIMATED TOTAL: \$2,764.23