Chemical Analysis of the Organic Binding Materials in Paint using Silylation Dervatization Techniques and GC-MS

Alexis Gonzales, James Karnezis, and Michelle Shulman, Ph. D

School of Science, Chemistry Department, Saint Mary's College of California, 1928 Saint Mary's Road, Moraga, CA 94556, United States

Abstract: For an undergraduate chemical instrumentation course, organic components used to create a supposed 12th century Russian icon painting were analyzed by GC-MS after derivatizing both the fatty acid and amino acid components in samples taken from the painting, using a MTBSTFA silylation reagent. The original icon was expected to contain egg tempera as the pigment binder and traditional animal glue - calcium mixture to create the gesso. Amino acid and fatty acid standards were prepared and used in combination with a NIST library to identify the main amino and fatty acids present in the painting. The amino acid hydroxyproline, distinct to gesso, was used to verify the presence of animal glue in the ground layer.

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Background:

Traditional Russian icon painting is a classical art form that has been standardized for centuries. Icons consecrated in early times were usually prepared with many thin layers of gesso applied directly to a piece of wood. This ground layer was usually composed of a mixture of chalk, commonly gypsum, and glue made from animal hide. This combination was used to smooth the surface and improve binding of the pigment to the wood foundation. Characteristically, animal hide is largely composed of collagen with hydroxyproline as a unique identifiable amino acid that is not found in egg yolk. Most typically, mineral pigment was combined with egg temperas as a binder and laid directly onto the gesso surface. Egg yolks have a large fatty acid and protein component. Once the painting was finished and dried, a varnish was applied as a protectant.

A Russian icon of unknown provenance and composition will be analyzed for verification of these two classes of components. The primary objective will be to create paint models and profile standards to compare against actual paint samples using GC-MS. Standards are created by mixing known amino acids and fatty acids in uniform amounts so that an organic component profile can be made as a reference source. Silylation of the amino and fatty acids has been shown to be a successful method to derivatization, allowing these organic components to be both amenable to and identifiable by GC-MS analysis, despite their polarity and small sample size[1, 2, 3]. Complementary techniques can be used in combination to give a complete compositional analysis of the painting and identify potential anomalies within the icon painting.

This experiment was a continuation of a final Capstone project for an undergraduate upper division chemical instrumentation course.

Methods:

Amino Acid and Fatty Acid Standards:

Select amino acid standards were used to compare retention times and verify matches found using the NIST library. Standards were run individually and then combined to create a profile (see figure 1). Amino acids, Proline, Hydroxyproline, glycine, alanine, aspartic acid, glutamine, and leucine all obtained from Sigma Aldrich were weighed to 1 mg on an analytical balance, combined with 1 mL of HPLC-grade H_20 and vortexed until the mixture was homogenous. A 10 μL portion was transferred to a glass conical microvial and dried under nitrogen to ensure a 0.01mg of sample residue without any water inside the vial. Caution was taken to ensure that the vial was completely dry since the silylation reagent was highly reactive with water.

Derivatization

Approximately 25 μL of MTBSTFA + 1% TSA (Thermo) and 50 μL of acetonitrile was added directly into the microvial and vortexed until homogenous. The vial was left to react at room temperature for approximately 20 minutes and left to react overnight at 20 °C. A 1 μL sample from each vial was run on a Varian 450-GC 240-MS instrument for analysis using the method conditions listed below.

GC-MS conditions on a Zebron ZB-5MSi column

GC-MS method conditions were adapted from Schilling[2]:

The linear velocity of the helium carrier was set to 45 cm/sec; splitless injector set to 300 °C; GC oven temperature program: 80 °C for one minute; 75 °C/min to 180 °C; 10 °C/min to 270 °C; isothermal for two minutes; solvent delay for 2 minutes. Figure 1 displays the GC-MS profile resulting from a mixture of amino acid and fatty acid standards.

Mock Painting Preparations

Gesso Preparation

A traditional recipe containing rabbit skin glue and chalk was used to mimic how gesso would have historically been prepared. Natural Pigments high bloom strength rabbit skin glue was added to water (5:1 v/v). The mixture was heated over a steam bath and small additions of chalk were slowly added until a solution mixture thickened and was removed from the heat. The cooled mixture was painted onto a cardboard swatch in multiple thin layers until a smooth thick layer was obtained. A dried gesso layer may be sanded down, if thick enough, to obtain a smooth surface.

Paint Preparation

Various mineral pigments obtained from Rublev Colours Natural Pigments and Sinopia were combined with egg yolks and mixed until homogenous to create egg tempera paint. More or less pigment was added to the egg yolk depending on the viscosity of paint desired. Mineral pigments were selected based on what was thought to be used in ancient Russian icon painting during the 12th century. The pigments used included vermillion, titanium white, cadmium red, malachite, and chromium oxide green. Facemasks and lab goggles must be worn when working with mineral pigments, and this portion of the experiment must be performed inside of a hood. The egg tempera paints were individually painted onto cardboard swatches directly or on top of a gesso layer. A paint containing a mixture of all pigments was also painted onto a cardboard swatch, both directly and onto a gesso layer, as a mock standard for any mixed pigments in the painting. A sample size comparable to a 12-point font period was removed from the edge

of the swatch under a dissecting microscope, placed into a dry glass conical microvial, and left at room temperature until ready for use.

Ammonia Extraction:

To remove possible interfering matrices, such as calcium components in the gesso layer based on solubility as described by *Gautier et al*., samples were dissolved in 200 μL of ammonia (2.5 M NH_3) in 1.5 mL eppendorf tubes. The prepared samples were placed in a sonicator for two hours. Another 200 μL portion of ammonia was added directly into the tube and sonicated for another two hours. Samples were centrifuged for 20 seconds and the liquid was decanted into clean glass conical microvials. The vials were then dried under nitrogen to ensure that the cleaned samples were completely dry.

Hydrolysis:

Dried samples were combined with 100 μL of 6 M HCl, vortexed, and placed in a 105 °C oven for 20-24 hours. Once removed, the vials were allowed to cool to room temperature and the HCl was evaporated under nitrogen flow while on a heating block. Samples were reconstituted in 40 μL of HPLC-grade H2O, centrifuged on a tabletop centrifuge for three minutes, and dried under nitrogen on a heating block. Samples were reconstituted in 40 μL of absolute ethanol, centrifuged for three minutes, and dried under nitrogen on a heating block. Caution must be taken then heating the samples to enure that it does not degrade with excess heating. Vials must also be completely dry prior to adding the silylating reagent, which is highly reactive with water, and may lead to extraneous results in the GC-MS analysis.

Derivatization:

The MTBSTFA + 1% TBDMCS siylation reagent was added directly to the dried vial, followed by 50 μL of Acetonitrile. Samples were vortexed until homogenous and were left out at room temperature for approximately twenty minutes before placing them into a 20 °C fridge to react overnight.

Painting Sample

Samples approximately the size of a 12-point font period were taken from a damaged end of the painting using a scalpel under a dissecting microscope. The previously mentioned ammonia extraction, hydrolysis, and silylating derivatization were used to analyze the painting sample.

GC-MS

All samples, from both the mock standards and the painting, were subjected to the same method conditions used for the amino acid and fatty acid standards.

A “blank injection”, meaning nothing was injected into the GC-MS, was run in between each sample injection to ensure that any residues on the column after each run were burned off to prevent any cross contamination between samples, which may lead to erroneous peaks on chromatograms.

Results/Discussion:

Amino acid standards were run individually to obtain both corresponding retention times, used for comparison to times obtained for the painting sample, and mass spectral data, used to complement the mass spectral information obtained for the painting (Figure 1: amino profile). Siloxane compounds resulting from side reactions of the MTBSFTA silylation reagent, were reduced by using the minimum amount of reagent, using glassware for all liquid transfers and storage, and increasing the time of exposure of the reagent to the amino acid. These parameters can be adjusted accordingly to improve detection. Some amino acids were more successful and easier to derivatize than others, which was indicated

by relative peak areas within the same chromatogram. Others such as proline appeared at multiple retention times if the amino acid was only partially silylated.

Hydrolysis of a dipeptide standard proved just as successful in the hydrolysis of individual amino acids in the mock standards and painting samples, resulting in distinct retention times for the isolated and derivatized amino acids present in the organic binding materials.

Prepared swatches of gesso and egg were analyzed and compared to the amino acid and fatty acid standards to verify that the sample had been successfully hydrolyzed and derivatized, as well as confirm any identification made by the mass spectral component of the instrument. The egg standard, as expected, lacked a hydroxyproline peak, but did contain other key amino and fatty acids not found in standards. Any amino acids that could not be analyzed using individual standards were identified through mass spectral information in combination with a NIST library match (~80%). The expected mass spectral information and molecular ion atomic masses were calculated for these non-standard components, and used to validate any library matches. The gesso layer was determined to be present in the painting sample due to the presence of a hydroxyproline peak in the corresponding chromatogram.

The ammonia extraction was implemented to increase the level of detection of the amino acids in the gesso due to the competing matrix effects in the complex mixture of chalk, water, and rabbit glue. The calcium components in gesso are largely insoluble in ammonia leaving only the amino and fatty acids in solution, increasing the likelihood they will be derivatized and minimizing any possible effects the calcium may have when allowed to react with the silylating reagent.

Samples taken from the supposed icon painting contained a peak identified as a silylated hydroxyproline compound, which indicated the use of gesso as the ground layer for painting. A sample was carefully taken from the icon painting so that no gesso was sampled, and was used to identify the egg tempera as the organic binding component of the paint (Figure 2). Relative amounts in either the egg tempera paint or the gesso could not be determined since no quantitative measurements were made during this experiment.

Conclusion:

It was determined that the proposed Russian icon was composed of the primary organic components expected in a typical icon painting of that time period. Destructive techniques used to determine possible organics in the icon proved to be successful. Silyation of the amino and fatty acids in the binding media and gesso layer were successful at identifying the key amino acid that differs between the two layers. The presence of hydroxyproline indicates that a traditional gesso serves as the ground layer for the icon. Presence of fatty acid and amino acid components, as well as the absence of a hydryproline peak in the derivatized sample of only egg tempera, indicates the presence egg as the pigment binding material. These results are consistent with the known composition of the painting. Overall there were no conclusive aberrations among the organic binding materials that could definitely suggest against its claim as a traditional icon painting.

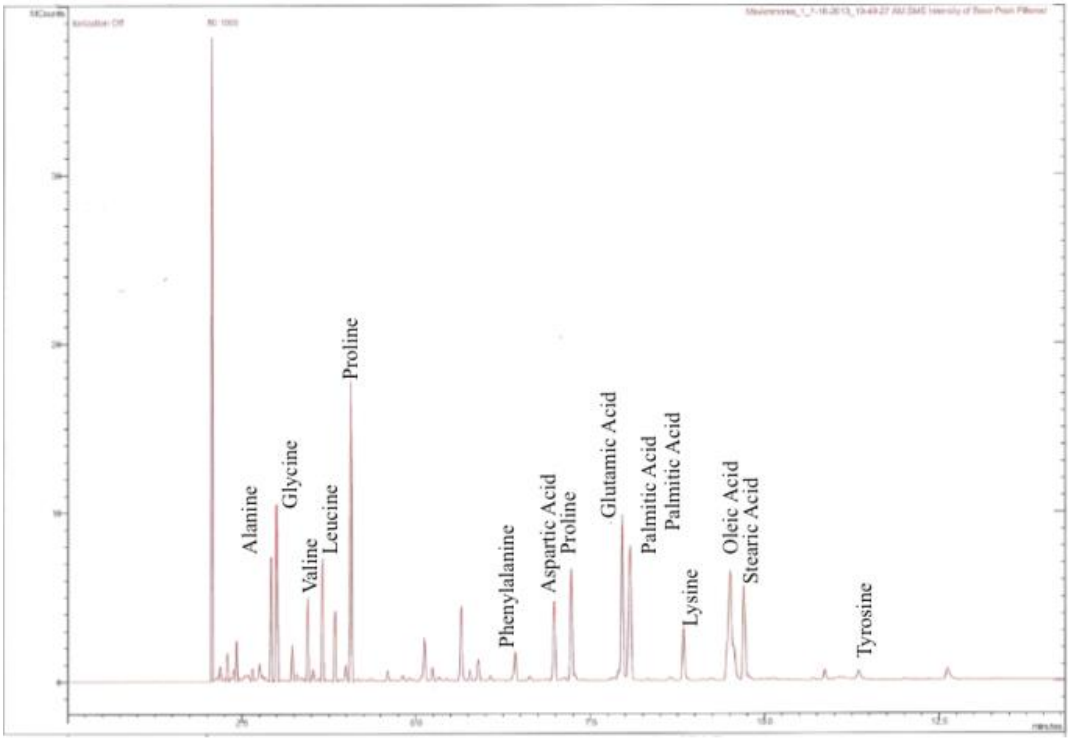

Figure 1. A chromatogram of a mixture of standard pigments, egg tempera, and gesso ground material. A sample from the mock painting mixture was subjected to the ammonia extraction, silyation derivatization, and GC-MS analysis. Unique amino acid and fatty acid peaks were identified according to a NIST library match >80%.

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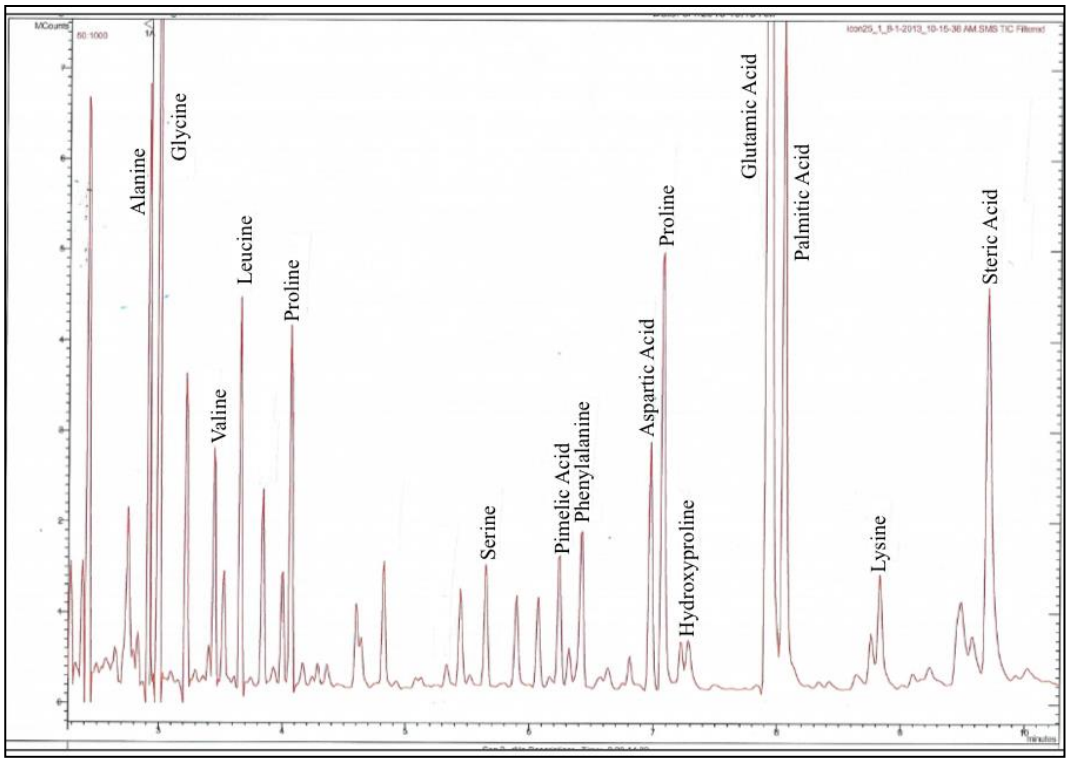

Figure 2. Chromatogram of a painting sample from the damaged edge of the mock 12th century Russian icon painting. The sample was subjected to the ammonia extraction, hydrolysis, and silylation derivatization techniques and analyzed using GC-MS. A key peak is the hydroxyproline peak, indicating the unique presence of gesso as the ground material.

References:

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