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Joseph T. Romero
Augustana College, Rock Island Illinois

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The Effects of Clay On Glycerol/Carboxylic Acid Polymerization: Implications of Mineral Controls In Prebiotic Chemistry

a senior thesis written by

Joseph T. Romero

in partial fulfillment of the graduation requirements
for the major in geology

Augustana College
Rock Island, Illinois 61201

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ABSTRACT

Proto-enzymatic catalysis on early Earth is an important consideration in prebiotic chemistry research as it leads to the understanding of the emergence of the metabolic reaction networks. Contemporary enzymes are comprised of either organic or inorganic factors scaffolded by globular protein structure whereas we consider hyperbranched polyesters as a possible extant scaffold that is easier assembled than the peptide one. The goals of this project are to consider prebiotically plausible starting materials for such structure formation utilizing a subset of conditions for polymer synthesis involving the presence of mineral surfaces as well as temperature and hydration differential. As likely candidates for the formation of hyperbranched polyesters on early Earth, reactions of glycerol with certain carboxylic acids (citric, tartaric, succinic, and fumaric) were studied. Two variants of the clay montmorillonite (KSF and K10) were introduced into the polymerization milieu to observe its effects in the process. To monitor polymer growth, their weights were taken at hourly intervals to ascertain water loss due to the condensation-dehydration reaction of the polyesterification. To characterize the resulting polymers' structures, acid-base titrations were performed to estimate the number of acid groups esterified during the experiment. Size-exclusion chromatography coupled with mass spectrometry was also used to elucidate the polyester structure and degree of branching. The presence of the clays was found not to affect the number of ester bonds formed or the kinetics of the polymerization product. The addition of clay, however, has been shown to favor the formation certain polymer conformations. These preliminary results indicate a potential selective pressure that clay minerals introduce into the chemical evolution of the hyperbranched polyester enzyme scaffolds.

INTRODUCTION AND BACKGROUND

Minerals in the creation of biopolymers on prebiotic Earth have been discussed as having a large role in the advent of life. This study delves into the problem of how the presence of clay minerals, specifically montmorillonite KSF and K10, affects the formation of condensation polymers, both in terms of number of bonds formed as well as final conformation. This in turn will provide insight on the relationship between geology and biochemistry and their importance in early Earth environments. Not only could this study help enlighten the development of life on Earth, but it could also uncover environmental conditions that make extraterrestrial biogenesis a possibility. For example, the recently discovered phyllosilicate clays on Mars (like montmorillonite) may have been able to catalyze polymerization reactions if water was indeed present at one point of its history (Joshi and Aldersley, 2013).

Mineral Catalysis

Some minerals are able to act as catalysts in conditions simulating prebiotic Earth. They are able to convert inorganic molecules into both simple organic molecules like formaldehyde and complex organic molecules like RNA. This process has a high activation energy required to occur, so some sort of catalyst was necessary to diversify biopolymers. Mineral surfaces are able increase the reaction rate of polymerization or promote a specific outcome of a reaction, important aspects when considering a "pool" of monomers being converted into a properly established polymer. According to the surface properties of the mineral, small molecules may be able to adsorb through chemical bonding, electrostatic forces, or dipole-dipole interaction; this provides a type of scaffolding for the biopolymers to start building off of. Because adsorption onto mineral surfaces is selective depending on the

specific mineral, adsorbates receive a type of template that allows for specific architectures, a sense of order in prebiotic conditions (Schoonen et al., 2004).

Clays As Catalysts

Clay minerals are typical weathering products of common silicate minerals like feldspar, mica, amphibole, and olivine; the specific clay formed depends on the environmental conditions. They are characterized by a large surface area due to their layered structure, as well as naturally charged particles that lead to relatively strong electrostatic interactions (Hui Zhou and Keeling, 2013). Montmorillonite is highly likely to have been present on early Earth because of its formation in warmer environments; tropical zones with seasonal contrast favor smectites like montmorillonite. Also, since biomolecules likely formed in an aqueous environment, like near or in early seas, the following sequence of clay minerals from shore to basin supports the use of these specific clays: kaolinite, illite, chlorite, palygorskite, and sepiolite, with montmorillonite present through the entire range (Galan, 2006).

Clays are one type of mineral that are able to promote condensation reactions of biopolymers like nucleic acids and peptides. They do so by allowing for oligomerization of nucleotides, an intermediate length of monomers necessary to reach before achieving polymerization. The basal planes of
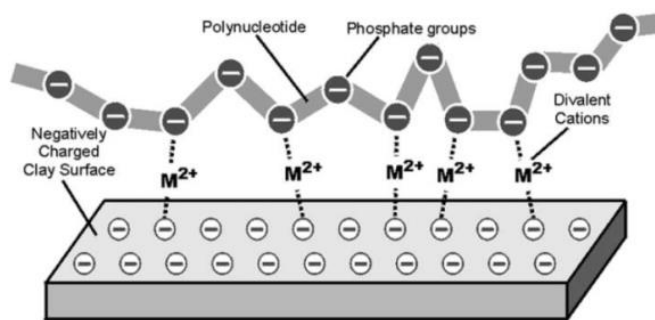

Figure 1. "Cation bridge" formed between the negative surface of the clay and the positive divalent cations of the polynucleotide (Franchi et al., 2002).

clays have a negative charge, while their edges have a positive charge, allowing for differentiation in the polymerization process (Figure 1). Since enzymes and membranes

present in modern day cells didn't exist at the onset of these molecules, clays as well as zeolites are able to act as a primitive selective force to prevent random accumulation of monomers. They can provide compartmentalized microenvironments that allow for polymers to grow in spite of a degrading environment (Schoonen et al., 2004).

Carboxylic Acids

The four carboxylic acids used in our experiments (citric, tartaric, succinic, and fumaric acid) are multifunctional; in other words, they have more than one hydroxyl group within the molecule to create an ester bond when mixed with glycerol (Figure 2). This quality allows for greater degrees of branching and exponenti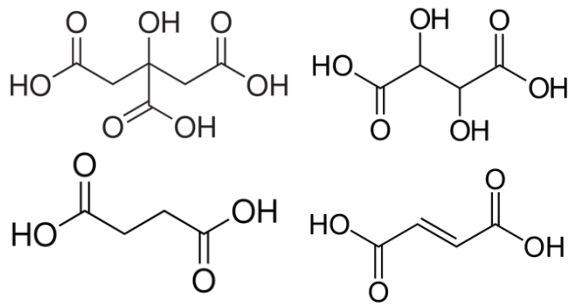ally greater numbers of possible polymer conformations. Additionally, we chose to use prebiotically plausible carboxylic acids to simulate an early Earth environment. All have been found in meteorites or detected in interstellar dust (Cooper et al., 2011). Citric acid is of particular interest because of its role in the citric acid cycle and other metabolic

Figure 2. Carboxylic acids. Citric (upper left), tartaric (upper right), succinic (lower left), and fumaric acid (lower right).

processes that are vital in biological systems today (Holser, 2011). Because the polymerization of these carboxylic acids with glycerol forms polyesters, it is important to consider that the ester bonds formed have a ΔG of -1 kcal/mol, whereas peptide bonds found in modern day enzymes have a ΔG of +2-4 kcal/mol. In other words, polyesters have a negative free energy of formation and are involved in exergonic reactions, possible having a role as a polypeptide precursor (Mamajanov, 2014).

Glycerol

Like certain carboxylic acids, glycerol is considered a prebiotically plausible molecule. It is sugar alcohol that is small enough to form relatively easily and therefore does not require extremely complex systems (Figure 3). Glycerol has been found in
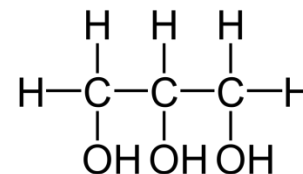

Figure 3. Structure of a gylcerol molecule.

meteorites as well as laboratory-produced organic residues produced by UV irradiation of interstellar/pre-cometary ice analogs (Nuevo et al., 2011). In other words, it is possible to form glycerol abiotically, and therefore it may have been present on prebiotic Earth. Additionally, glycerol is a precursor for the synthesis of triacylglycerols, which is a type of lipid. Lipids in modern day biological systems are used as energy storage for cellular metabolism, so this gives glycerol an additional facet of potential importance in the evolution of complex biochemical systems (Nuevo et al., 2011).

Importance of Cations in Polymerization

The role of cations in the formation of condensation polymers is important as well. Monovalent cations like lithium, sodium, and potassium facilitate the elongation of oligmoers into polymers. As such, the addition of inorganic salts like sodium chloride promotes the polymerization process; this is called salt-induced peptide formation (SIPF) (Rode and Suwannachot, 1999). At the same time, divelent cations like copper, calcium, or magnesium are more efficient mediating monomers' adsorption onto mineral surfaces. In fact, adsorption onto clays occurs much more efficiently when both cations and monomeric building blocks were present. The cations don't only facilitate adsorption, but that they are also directly associated in between nucleic acids and mineral surfaces. It is believed that they

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act as a bridge between a negative surface of the mineral and phosphate groups of genetic polymers (Franchi et al., 2002).

Mineral salts could be a possible source of these ions in prebiotic conditions. Sodium chloride, especially, is a big contender because of its presence in ocean waters—the likely milieu of prebiotic reactions. When catalyzing RNA molecules, it was found that maximum catalytic activity was reached at NaCl concentrations between 0.8 and 1.2 M. Because this resembles the NaCl levels in ancient oceans (0.9-1.2 M), NaCl likely played a large part of polymerization reactions. Also, the presence of NaCl decreased the yield of cyclic dimers during polymerization; in other words, longer chains of monomers were able to form by preventing the formation of cyclic structures (Joshi and Aldersley, 2013).
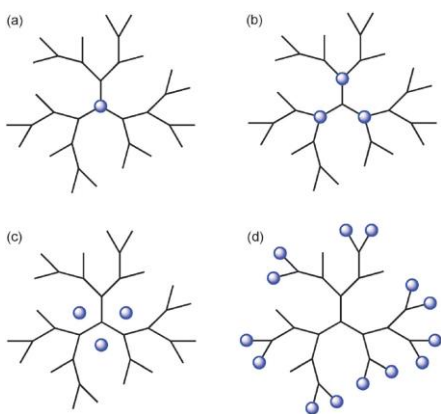
Hyperbranched Polymers

Figure 4. Hyperbranched polymers with catalytic groups (a) at the core, (b) covalently bound to the interior, (c) encapsulated within the interior, and (d) at the periphery (Kirkorian et al., 2012).

In regards to the polymers themselves, hyperbranched polymers may be a potential product in the condensation reactions. Hyperbranched polymers (HBPs) are able to mimic an enzyme's structure by incorporating binding or catalytic sites within their configuration (Figure 4). Since enzymes were not available on prebiotic Earth, this has huge implications for the creation of more complex biomolecules. The terminal function groups of the HBPs act as reactive centers for other monomer to bind, further promoting polymerization. Also, they are able to partake in both homogeneous catalysis as well as heterogeneous catalysis; this allows for benefits of both to be utilized, such as a higher loading of catalytic sites and easy reagent

access for more polymerization activity. HBPs have a high chemical and thermal stability, an important trait in developing in the extreme environments of early Earth. Like the minerals they adsorb to, HBPs provide microenvironments thanks to the catalytic sites' variable locations within the molecule, providing protection for polymerization to occur (Kirkorian et
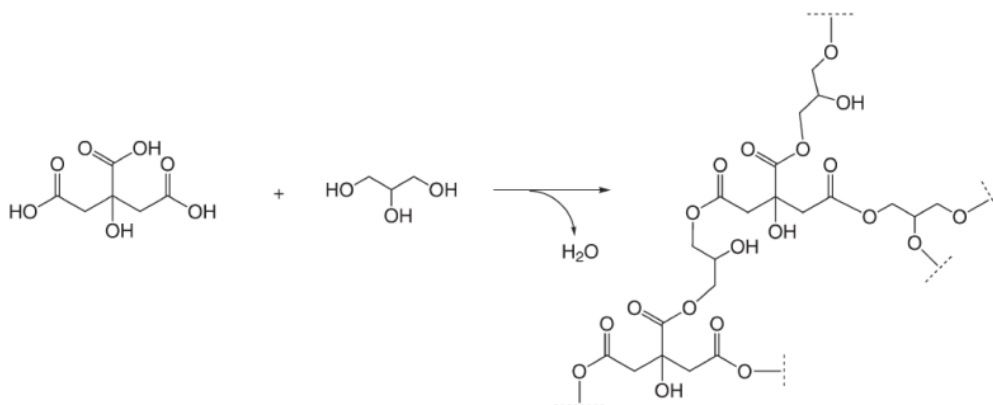

Figure 5. Condensation reaction synthesizing branched polyester from glycerol and citric acid (Halpern et al., 2013).

al., 2012). Figure 5 describes the process of HBP formation between glycerol and citric acid.

Wet-Dry Cycles

A potential origin for both HBPs and other polymers in prebiotic conditions is the presence of wet-dry cycles. Repeated and cyclic changes in hydration and temperature can mimic day
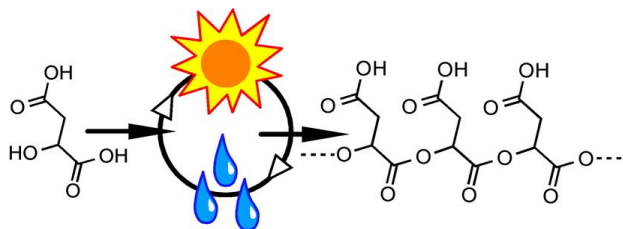

Figure 6. Ester polymerization induced by wet-dry cycles (Mamajanov et al., 2014).

and night, seasonal changes, or changes in tides analogous to early Earth environments. Heating during the dry phase promotes the formation of ester bonds, in turn driving the polymerization of polyesters. During the wet phase when hydrolysis is possible, lower temperatures are favorable in polymerization by inhibiting the hydrolyzing process. A polymerization ratchet where both oligomers and monomers coexist and cycle is an

important step in creating more complexity in the system (Figure 6). Oligoesters that are soluble in the aqueous milieu of the wet phases are labile, making them eligible to be repeatedly cycled as building blocks for different polymeric states (Mamajanov et al., 2014).

Size Exclusion Chromatography

Size exclusion chromatography is a widely used method of separating molecules based on their size. This is performed by running a liquid sample through a porous column in a chromatographer; the smallest molecules are temporarily "trapped" within these pores while the larger ones sink through the milieu faster. In this way, the largest molecules in the sample reach a sensor at the bottom of the column first and are separated temporally from smaller molecules that fall more slowly (Figure 7).
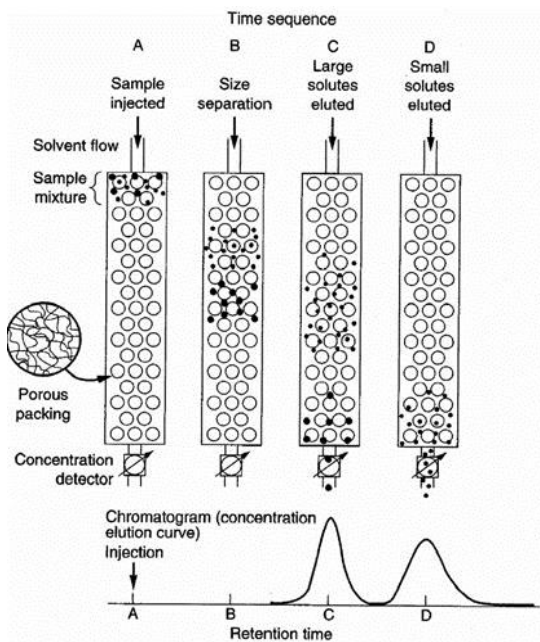
As is the case with this experiment, size exclusion chromatographers are usually paired with mass spectrometers as a means of determining the molecular weights of the different molecules separated by the column based on peaks formed when the molecules reach the bottom. This experiment adds another facet to the complexity of the chromatographers due to the branching nature of the HBPs formed from glycerol and carboxylic acids. Rather than spherical molecules falling through the columns, molecules with protruding branches get caught in the pores and fall more slowly to the bottom.

Figure 7. Diagram of the size exclusion chromatography process (Adeli 2013).

METHODS

Sample Drying and Weighing

Dehydrated samples of glycerol, citric acid, and tartaric acid were dissolved in deionized water to create 0.01 M solutions of each. These solutions were then mixed together at different molar ratios both with and without montmorillonite KSF and K10 present. The different combinations used were as follows: 1glycerol:1 citric acid, 2 glycerol:1 citric acid, 1 glycerol:1 succinic acid, 1 glycerol:1 citric acid + 0.5g KSF, 1 glycerol:1 citric acid + 0.5g K10, 2 glycerol:1 citric acid + 0.5g KSF, 2 glycerol:1 citric acid+ 0.5g K10, 1 glycerol:1 succinic acid + 0.5g KSF, 1 glycerol:1 succinic acid + 0.5g K10, and as controls, 2 H_2O + 0.5 g KSF as well as 2 H_2O + 0.5 g K10.

The samples were heated constantly for 24 hours at 85ºC and weighed at 24 hour increments (Figure 8). We discovered that the weight had already stabilized between the measuring periods, so we chose instead to recreate the samples and measure them at an hourly basis. In this way, we attempted to determine, based on changes in weight (via water loss due to the condensation reactions occurring), whether introduction of the clays allowed for a greater or lesser degree of polymerization within the solutions.
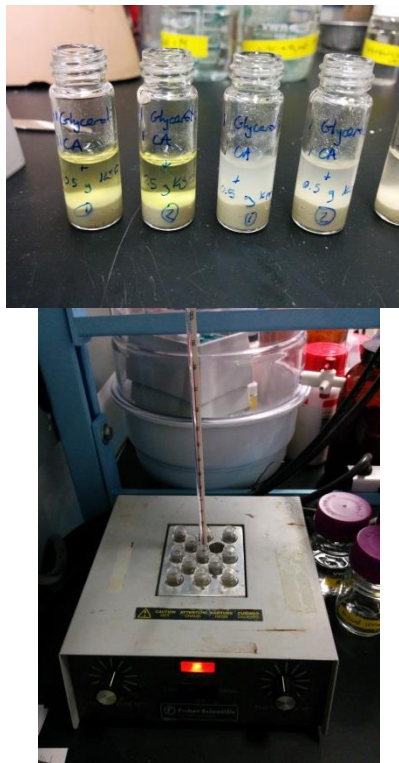

Figure 8. Example of sample vials with glycerol/carboxylic acid solutions with clay (top) and the oven used to heat vials (bottom).

Acid-Base Titrations

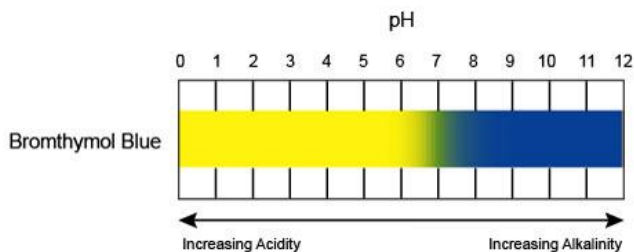4 mL solutions of 0.01 M citric acid and tartaric acid were titrated using 0.01 M NaOH. The indicator bromothymol blue was used to determine when the titration was complete (Figure 9). Additionally, samples of 2 glycerol:1 citric acid polymer and 1 glycerol:1 tartaric acid polymer dissolved in 4 mL H_2O were dialyzed (for contaminant removal) and then titrated using 0.01 M NaOH.

Figure 9. Graphic of the pH sensitivity of bromothymol blue.

Complete titration determined when all of the reactable acid groups within the carboxylic acids have bound to the glycerol.

Divalent Salt Addition to Polyesters

Solutions of 250 mM $CaCl_2$, $CoCl_2$, $CuCl_2$, and $MgCl_2$ were mixed with solutions of 2 glycerol:1 citric acid, 2 glycerol:1 citric acid, 2 glycerol:1 citric acid + 0.5 g KSF, and 2 glycerol:1 citric acid + 0.5 g K10. The samples were then heated at 85º C for 48 hours to allow for complete desiccation. To remove the resulting polymers from the clay samples after drying, the remaining clay + polymer solid was washed with deionized water. The supernatant was then removed and dried once more. These final polymers along with those formed without the clay addition were then dissolved in 2 mL of deionized water and dialyzed.

Wet-Dry Cycles

Samples were made with the following molar ratios: 2 glycerol:1 citric acid, 2 glycerol:1 citric acid + 0.5 g KSF, 2 glycerol:1 citric acid + 0.5 g K10, 1 glycerol:1 fumaric

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acid, 1 glycerol:1 fumaric acid + 0.5 g KSF, 1 glycerol:1 fumaric acid + 0.5 g K10, 2 glycerol:1 fumaric acid, 2 glycerol:1 fumaric acid + 0.5 g KSF, 2 glycerol:1 fumaric acid + 0.5 g K10, 2 glycerol:1 citric acid buffer, 2 glycerol:1 citric acid buffer + 0.5 g KSF, and 2 glycerol:1 citric acid buffer + 0.5 g K10. They were then heated at 24 hour intervals at 85º C. After each 24 hour period, one vial per variation was removed while the others were refilled with 2 mL of deionized water. These hydration differentials simulated wet-dry cycles for four days in total. The citric acid buffer was used to simulate a saline aqueous environment similar to that of an early ocean.

RESULTS

Sample Drying and Weighing

The hourly weighing process of the samples with 1 glycerol:1 citric acid is shown in Figure 10. Similar results were found for the samples containing succinic acid. After normalizing for the weight of the clays added in some vials, we found that there was nearly no change in final weight of the samples after allowing them to dry completely. This signifies that regardless of whether the
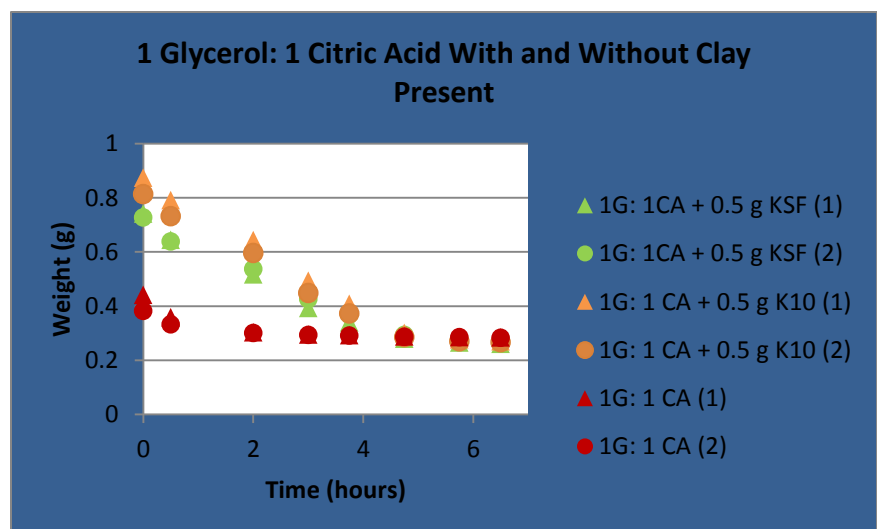

Figure 10. Graphic representation of the hourly change in weight of 1 glycerol:1 citric acid samples with and without clays present.

clays are present or not during the polymerization process, there is no significant change in

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the number of ester bonds formed between the glycerol and the carboxylic acids. The fact that it took longer for the samples with clay to reach a stable dry weight demonstrates its ability to retain water, perhaps allowing the substrates to interact in an aqueous milieu and thus polymerizing to a further degree. This may account for the small weight difference between the samples with and without clay; less weight signifies more water loss which in turn signifies more ester bonds formed via condensation reactions.

Acid-Base Titrations

When the solutions of 4 mL 0.01 M citric acid were titrated, it took between 12-13 mL of 0.01 M NaOH to change the bromothymol blue from yellow (pH < 6.0) to blue (pH > 7.6). This indicates that all three acid groups of the citric acid molecules were neutralized. When the solutions of 4 mL samples of 0.01 M tartaric acid were titrated, it took around 8 mL of 0.01 M NaOH to change the solution from yellow to blue. Since tartaric acid has two acid groups, it is reasonable to conclude that it takes around 4 mL of base to neutralize each acid group.

Comparing these results to those when titrating the polymers, we were able to determine the degree of polymerization of the substrates. Approximately 2.6 of the 3 water equivalents in the 2 glycerol:1 citric acid polymer were lost; in other words, 82% of the possible ester bonds available were formed because not all three acid groups of all citric acid molecules were bound to glycerol. With regards to the 1 glycerol:1 tartaric acid polymer, 2 of the 2 water equivalents were lost, so 100% of the potential ester bonds formed.

Wet-Dry Cycles and Size Exclusion Chromatography

Figures 11, 12, and 13 show chromatogram results of the polymers created from the wet-dry cycles. The 2 glycerol:1 citric acid solutions shown in Figure 11 demonstrate that the

addition of the clays do in fact affect the polyesterification process of the substrates. Rather than affect the degree of branching or the number of ester bonds formed, however, it seems as though the clays put a selective pressure on a certain conformation of polymer. In this case, whereas samples without clay have peaks corresponding to two 2 glycerol/1 citric acid polymers, the main peak of both KSF and K10 corresponds to a dimer of 1 glycerol and one citric acid. Interestingly, it took longer for samples with KSF to reach a point of conformational pressure than samples with K10, perhaps due to its smaller surface area. The results in Figure 12 are less conclusive due to the lack of variation in the chromatogram peaks.

The Figure 13 results are even less conclusive due to the strange chromatograms created. The peaks after 24 hours correspond to the molecular weight of unreacted citric acid, perhaps insinuating that the salinity hindered polymer formation. However, the peaks after several wet-dry cycles do not correspond to any molecular weights involved in the experiment (of citric acid, glycerol, etc.) begging the question of what molecule was detected by the chromatographer.

DISCUSSION

We determined that the addition of montmorillonite KSF and K10 to the reaction milieu of glycerol and carboxylic acids did not promote further esterification based on water loss during condensation reactions. We did, however, determine that the clays affect the polymerization process by favoring certain polymer conformations over others as the polyesters interacted during wet-dry cycles. Surface area of the clays may play a role in how fast this conformational pressure applies itself, with lower surfaces areas requiring more time
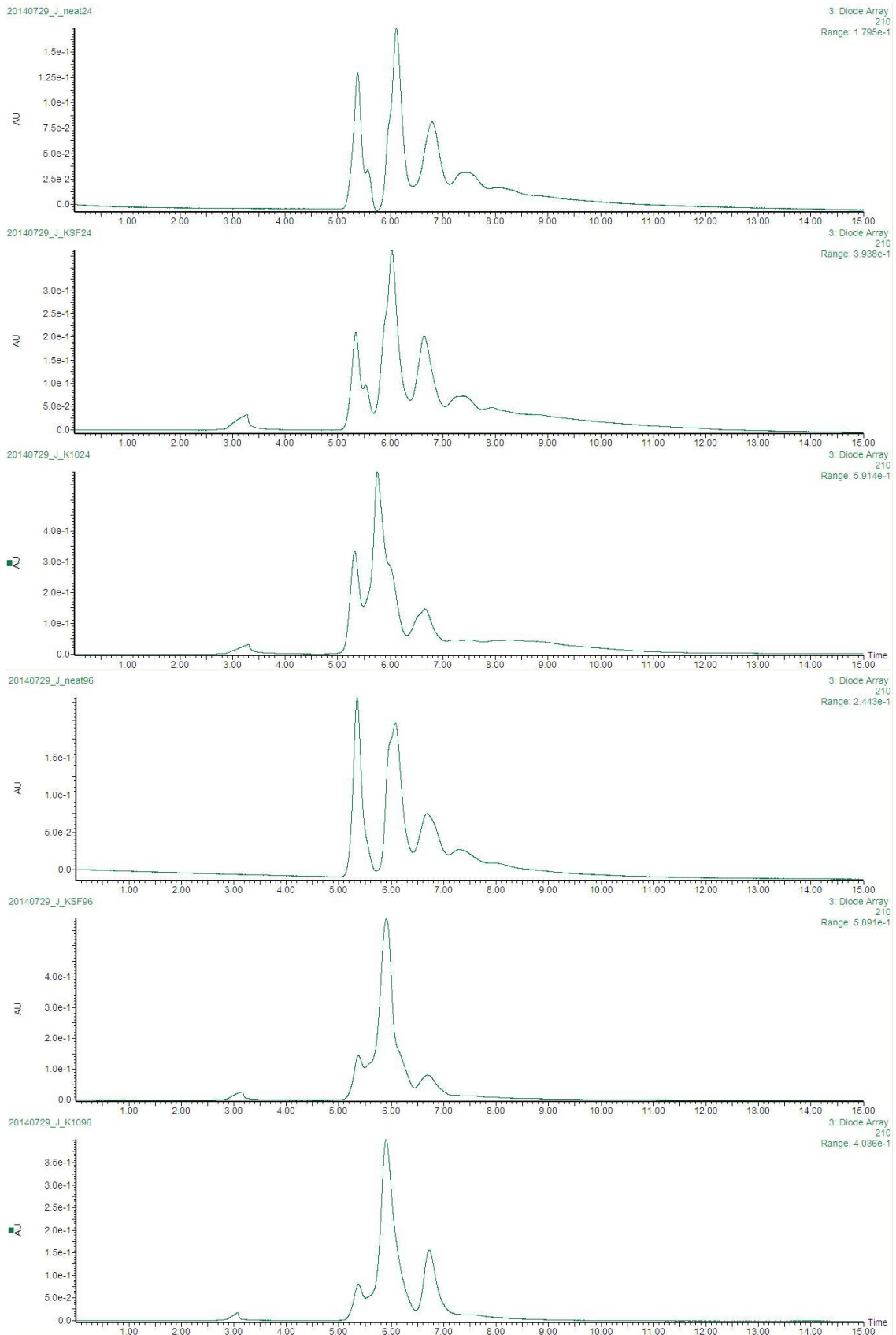
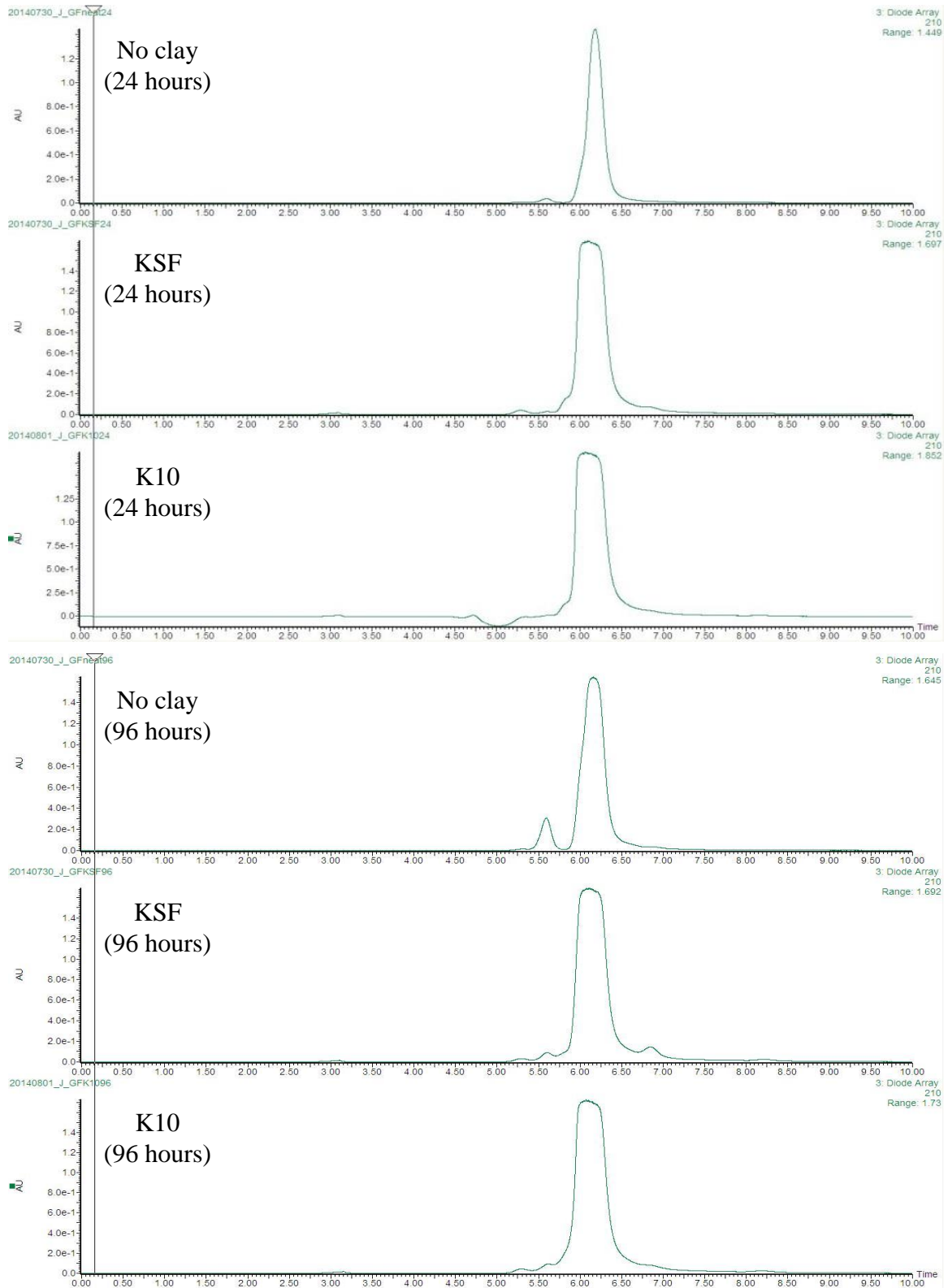**Figure 11. Chromatogram results after the wet-dry cycles of 2 glycerol:1 citric acid.**

Figure 12. Chromatogram results after the wet-dry cycles of 2 glycerol:1 fumaric acid. Results of 1 glycerol:1 fumaric acid were nearly identical.
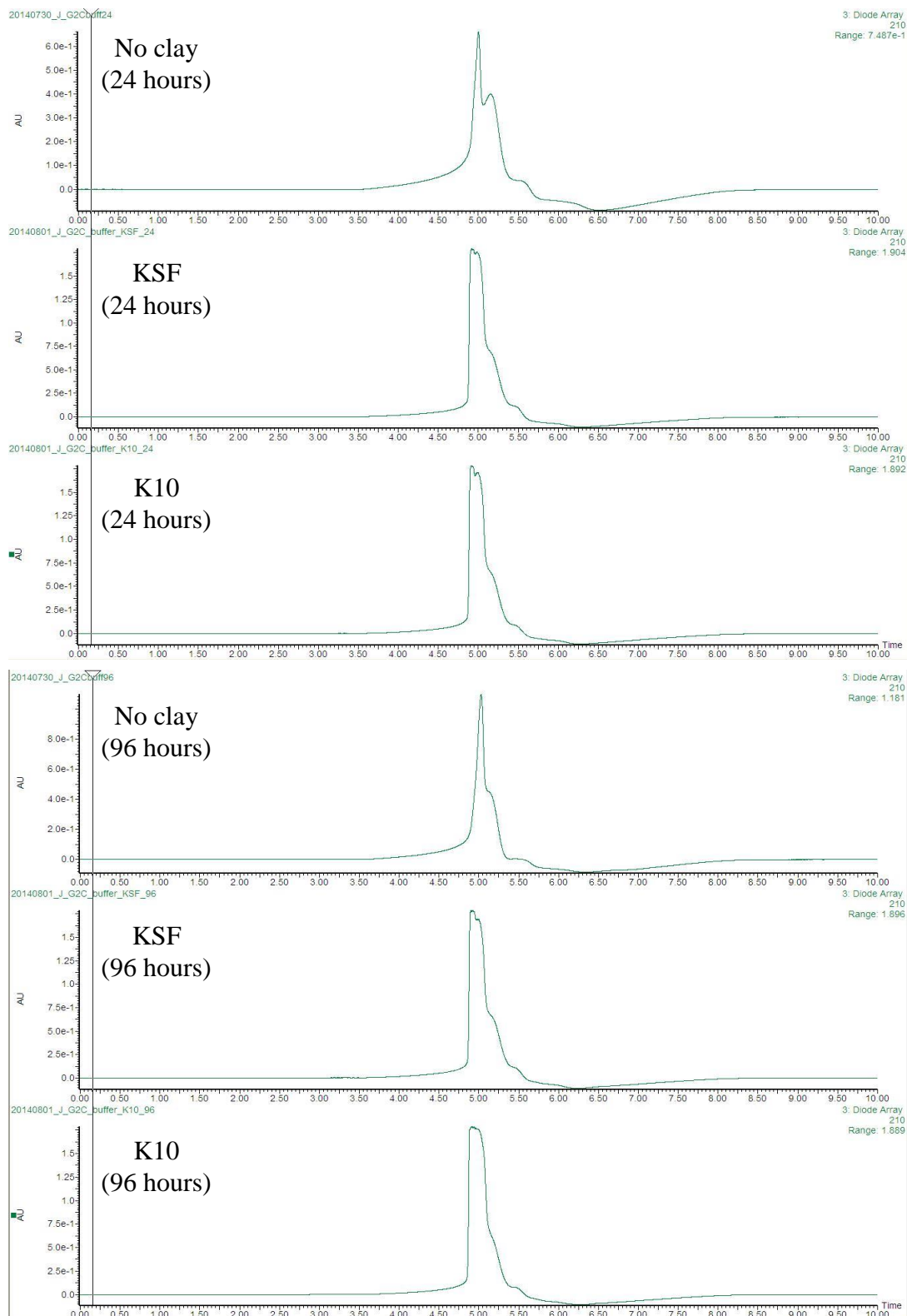
Figure 13. Chromatogram results after the wet-dry cycles of 2 glycerol:1 citric acid buffer.

for polymer adhesion.

Polymers formed from solutions of 1 glycerol:1 tartaric acid had all possible ester bonds formed based on acid-base titration comparison to non-polymerized forms. However, 2 glycerol:1 citric acid polymers only yielded 82% esterification, leaving unreacted acid groups on the citric acid molecules. This could have important implications to the number of conformations possible for glycerol/citric acid polyesters. Because there were open end groups that could have reacted, different isomers of the polyester were possible, which would affect the chemistry and catalysis capability within its environment.

Due to time restraints, this project did not reach the degree of completion required to address the catalytic capabilities of the hyperbranched polymers formed from glycerol and the carboxylic acids. Therefore, introducing the polymers produced from conditions with and without clay to chemical systems susceptible to catalyst interaction (such as primitive metabolic pathways) would help to determine their proto-enzymatic qualities, as well as whether the clays would promote or hinder said catalysis.

Another important route to follow up on is how best to remove the polymers from the clays they were mixed with. While simply washing with deionized water was sufficient for some polymer removal, depending on where they adsorbed to the clay (surfaces vs. in between layers), all potential conformations may not have been detected. This is especially evident when analyzing the samples with divalent salts introduced to the system; the DI water wash removed some polymer, but not enough to titrate or be reliably read by the chromatographer. Because the presence of divalent salts has been shown to promote polymerization, analyzing their effect on the tested systems would add to the complexity of the simulated environments.

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Overall, the results of this project were able to support the idea that minerals (in this case, clays), play a role in the evolution of chemical systems. Likely due to their adsorptive surfaces, chemical substrates capable of polymerizing are influenced to form certain structures over those that would form without the presence of the clays. Depending on which molecules are interacting, the role of minerals in this process has implications on potential hyperbranched conformations; in turn, the resulting polymer could be capable of catalyzing reactions in earl Earth environments. Research in prebiotic geochemistry, then, has potential in facilitating our understanding of how lifeless systems became complex enough to begin the development of life on Earth.

ACKNOWLEDGEMENTS

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