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Proto-RNA Synthesis Using Glycerol and Phosphate Anion

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ABSTRACT: Nucleic acids are important molecules for all life, serving as the primary molecules for information storage and reproduction. RNA has long been thought to be the molecule that initiated protein synthesis and soon after, life. However, RNA has recently been thought to be too complex to synthesize prebiotically. Through a 1:1 mole ratio condensation of glycerol and phosphoric acid, I generated glycerol-phosphate oligomers that resemble a primitive form of the carbon-phosphate backbone of RNA. 75 mmol $MgCl_2$ was added to some samples to see if the Mg cation could stabilize the reaction intermediates and further promote longer proto-RNA oligomers. These samples were all heated at 120 °C for 96 hours. Using ^{1}H-NMR and ^{13}C-NMR, the products of this reaction were characterized, and successful phosphate binding to glycerol was observed. Mass spectrometry was used to determine the length of the oligomers that were formed. I observed successful production of monomers, dimers, trimers, tetramers, and pentamers, with significantly less production in samples containing Mg^{2+}. This indicates the potentiality of glycerol-phosphate as an ancient ancestor to modern RNA. Additionally, the products were observed under a light microscope to see the formation of any physical structures. Vesicle-like structures were observed in most samples, ranging from 10-30 microns in size, except those containing Mg^{2+}. This indicates the potential ability of glycerol-phosphate oligomers to spontaneously arrange into vesicles according to its intramolecular hydrophobicity.

INTRODUCTION

Nucleic acids are an extremely important aspect to all life, carrying genetic information essential for reproduction. However, with such complex roles in life, their structure is equally complex, making them inherently difficult to synthesize. RNA, the single stranded molecule thought to be the precursor to the double stranded DNA, has long since been thought to be the basis for the origin of life, evolving and catalyzing reactions that gave way to DNA and protein synthesis.[1] However, recently RNA has been the subject of controversy, with many believing it to be too complex itself to have arisen prebiotically.[1] Instead, other primitive molecules are being investigated that resemble RNA. Such molecules are called proto-RNA.

In this experiment, I focused on the synthesis of one such molecule: an oligomer consisting of glycerol and phosphate to mimic the carbon-phosphate backbone of RNA. Through condensation of glycerol and phosphoric acid, both molecules being present prebiotically in the environment, successful formation of longer oligomers would open the possibility of glycerol-phosphate as a precursor to RNA. In addition, based on the present role of Mg^{2+} in promoting RNA polymerization, the potential role of Mg^{2+} in proto-RNA polymerization was investigated. A second major goal of the study was to explore whether any reaction products would result in amphiphilic molecules that could self-assemble into vesicles which have a lipid bilayer similar to a model protocell membrane.

MATERIALS AND METHODS

Materials

Glycerol, 85% phosphoric acid, $MgCl_2$ were purchased from Sigma-Aldrich. The concentration of glycerol was 92.09 g/mol, the concentration of 85% phosphoric acid was 97.99 g/mol, and the concentration of $MgCl_2$ was 95.21 g/mol.

Calculation of Reactant Concentrations

To run this reaction, a 1:1 mol ratio was decided on as the concentration between glycerol and phosphoric acid.[2] Due to the viscosity of glycerol and its pipetting difficulty, it was measured by weight. A mass of 5g of glycerol equivalent to 0.054 mol was used and then the required amount of 85% phosphoric acid in water was calculated to equal 0.054 mol. This resulted in 3.69 mL of phosphoric acid, and 3.97 mL (equivalent to 5g) of glycerol. In a separate trial, I used 75 mM magnesium chloride ($MgCl_2$), in addition to the same concentrations of glycerol and phosphoric acid as above.

Experimental Design

In three separate 10 mL test tubes, 5 g of glycerol (3.69 mL) and 3.97 mL of phosphoric acid were thoroughly mixed. Glycerol was measured by weight due its viscosity and difficulty pipetting. This reaction was run in triplicate two times. The first trial's samples were labeled 1A, 1B, and 1C, the second trial 2A, 2B, and 2C. Another experiment was run, also in triplicate, in which 0.055g of $MgCl_2$ (75 mmol) was added to the 1:1 glycerol:phosphoric acid mixture. These samples were labelled 3A, 3B, and 3C. Each sample was tested with pH paper before heating, with all of them showing around a pH of 3. All samples were heated for 96 hours in a vacuum oven (provide make and model) at 120 °C.[2] The reacted samples were then refrigerated for storage until further analysis.

Compound Identification

Several techniques were employed to deduce the structure and identity of the product, including ^{1}H-NMR, ^{13}C-NMR (Varian NMRS 500-01, Varian, Palo Alto, CA, USA) and electron spray ionization mass spectrometry (ESI MS). ^{1}H-NMR allows for structural identification based on the chemically different protons present in the molecule. ^{13}C-NMR was also run to attempt to eliminate some of the noise seen the ^{1}H-NMR spectrum from unreacted phosphoric acid still present in the sample. For each NMR technique, a small portion of the sample was diluted in pure deuterium oxide (D_2O), as this minimizes resonance from the solvent. Mass spectrometry was employed to determine the presence of oligomers, as chains of different lengths had unique masses that could be differentiated with this technique.

Microscope Observation

Samples 1A through 1C, as well as sample 3B from the Mg trials, were observed under an inverted fluorescence light microscope (Olympus IX51, Olympus America Inc., Melville, NY, USA) to observe the formation of any physical structures. Small amounts of sample were removed and placed on a glass slide, then covered by a cover slip. These were observed at a size of 100 microns to determine the presence of any physical structures.

RESULTS

Reaction

After 96 hours of heating in a vacuum oven at 120 °C, a gradient of color was observed in each trial (Figures 1, 2 and 3). The darkest sample was noticeably more viscous whereas the clearest samples were positioned towards the center of the oven. This result suggests a greater extent of reaction for the samples closer to the walls, and it is speculated that a much higher temperature exists towards the walls of the oven than the center of the oven.

^{1}H-NMR

Using ^{1}H-NMR, a large peak was observed at ~4.7 ppm, with a group of smaller peaks ranging from 3.4-3.8 ppm (Figures 4, 5 and 6). These were compared to a standard spectrum for glycerol-3-phosphate obtained from the Human Metabolome Database.[3] Noticeable similarities were observed between the spectra, suggesting the presence of glycerol-3-phosphate, the desired monomer in the samples. However, there are many more small peaks compared to the standard spectrum likely due to the formation of side products during the reactions. Using the normalized intensity from the H-NMR spectra that appeared to contain to the fewest side reactions (fewest unrelated peaks), I obtained an estimate of the reaction conversion rate by integrating the area under the peaks (Figure 7). Glycerol and phosphate were observed to react with ~60% efficiency when heated for 96 hours at 120 °C. However, in the presence of Mg^{2+}, the reaction efficiency drops to 14%. The proposed mechanism of Mg^{2+} stabilizing the reaction intermediates had the opposite effect, nearly inhibiting the reaction altogether.

Additionally, I was able to conclude that the phosphorylation of glycerol was selective for the primary alcohol, with phosphate binding to the central alcohol less frequently. This can be observed by the sharp peaks at a chemical shift of ~3.5 ppm (Figures 4A and 4B). This corresponds to the standard glycerol-3-phosphate spectrum.[3] This is likely due to less steric hinderance between the phosphoric acid and glycerol molecules at the C2 position. The slight shifts between the different samples and the standard spectrum are speculated to be a result of the differing pH's between the samples.

^{13}C-NMR

^{13}C-NMR was used to minimize noise in the spectra from unreacted phosphoric acid. However, most of these spectra were extremely complex due to the presence many different products, including unreacted glycerol, being present. The sample with the least observed side reactions, Sample 1B, was analyzed, with the observed peaks indicating the potential presence of both glycerol-2-phosphate and glycerol-3-phosphate due to the peaks present at ~70-70.5 ppm(Figure 8). Due to the similarity of the two products spectra and the complexity of the readings, it is impossible to definitively state whether one was produced exclusively over the other. These results are consistent with the data from the ^{1}H-NMR and reinforces the presence of side products during the reaction.

ESI-Mass Spectrometry

Linear oligomers as long as pentamers were identified in Samples 1A (Figure 9) and 3B (Figure 12) and

oligomers as long as tetramers in 1B (Figure 10). This proves the proposed reaction mechanism of glycerol and phosphate condensing in a series of reactions to form a chain. Oligomer yield decreased significantly with length greater than dimer. Length of oligomer and concentration were observed in an inverse relationship, indicating longer oligomers take more time to form. However, sample 1C (Figure 11) showed no production of any oligomers longer than a dimer. This result indicates that darker, more viscous samples (Samples 1A, 1B, and 3B) contain oligomers of longer length compared to lighter colored samples (1C, 2B, and 2C). Given more time, it is not unreasonable to assume longer chains more typical of nucleic acids could form. Also, the sample containing Mg2+ did show oligomers as long as pentamers, but in very small yields, and these oligomers were not identified by ^{1}H or ^{13}C-NMR. This, combined with the reduced peak intensity from NMR, indicates Mg2+ inhibits the polymerization reaction, as well as the phosphorylation of glycerol.

Microscopic Observations

All samples analyzed with mass spectrometry were subsequently observed under a compound light microscope (Figure 13). In samples 1A, 1B, and 1C, vesicle-like structures were observed in a variety of sizes ranging from 10 microns to ~30 microns . These structures were also observed to be moving throughout the solution, with the smallest ones moving more quickly. These findings suggest the spontaneous formation of vesicles my glycerol-phosphate polymers. This could be due to the difference in hydrophobicity between the phosphate group and glycerol molecule; however, this would require further investigation. No vesicle-like structures were observed in the blank sample, consisting of unreacted glycerol and phosphoric acid.

CONCLUSIONS

Glycerol and phosphoric acid were observed to undergo successful condensation reactions to form oligomers of varying length at ~60% efficiency. The presence of Mg^{2+} substantially inhibits this reaction, as well as the binding of phosphate to glycerol. Many side products are also produced during these reactions, likely due to the lower efficiency. The concentration of these oligomers decreases as they get longer, indicating they take longer to form as they get longer. Given enough time, it is possible for chains longer than pentamers to form. When observed under a microscope, vesicle-like structures can be seen ranging from 10 μM to ~30 μM. These could form due to the different polarities of the phosphate and glycerol molecules. These vesicles are model protocells. Ultimately, glycerol and phosphoric acid have shown the capability to form long chains that resemble very primitive forms of RNA carbon-phosphate backbones and excitingly, also show formation of model protocell membrane-like vesicles.

Moving forward, I would re-run these reactions with more careful control of the temperatures to ensure longer chains are formed. Additionally, more samples need to be run through ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR to verify these results. With respect to the microscope results, the cause of the vesicle formation should be investigated, determining the relationship between structure and the special arrangement of molecules.

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3 Wishart, D; Knox, C; Guo, A; Eisner, R; Young, N; Gautam, B; Hau, D; Psychogios, N; Dong, E; Bouatra, S; Mandal, R; Sinelnikov, I; Xia, J; Jia, L; Cruz, J; Lim, E; Sobsey, C; Shrivastava, S; Huang, P; Liu, P; Fang, L; Peng, J; Fradette, R; Cheng, D; Tzur, D; Clements, M; Lewis, A; De Souza, A; Zuniga, A; Dawe, M; Xiong, Y; Clive, D; Greiner, R; Nazyrova, A; Shaykhutdinov, R; Li, L; Vogel, H; Forsythe, I. HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* 2009. 37. 603-614.

Appendix I – Figures

Figure 1. Reaction products from Trial 1. Samples 1A (left) through 1C (right) are shown.

Figure 2. Reaction products from Trial 2. Samples 2A (right) through 2C (left) are shown.

Figure 3. Reaction products from Trial 2. Samples 3A (right) through 3C (left) are shown.

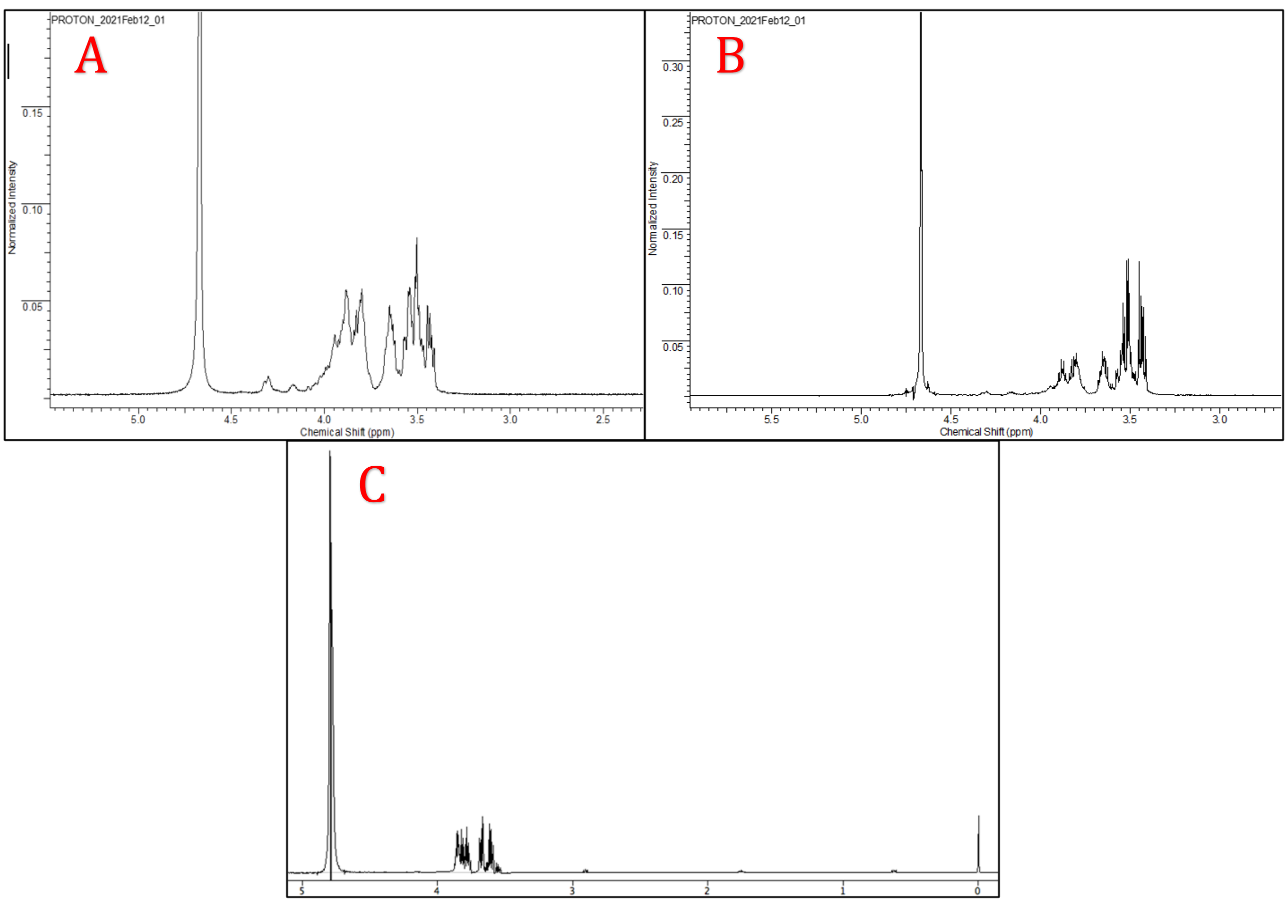

Figure 4. (A) Displays the H-NMR results of Sample 1A. (B) Displays the H-NMR results for Sample 1B. (C) H-NMR results for glycerol-3-phosphate to be used for comparison. Data obtained from the Human Metabolome Database.[3]

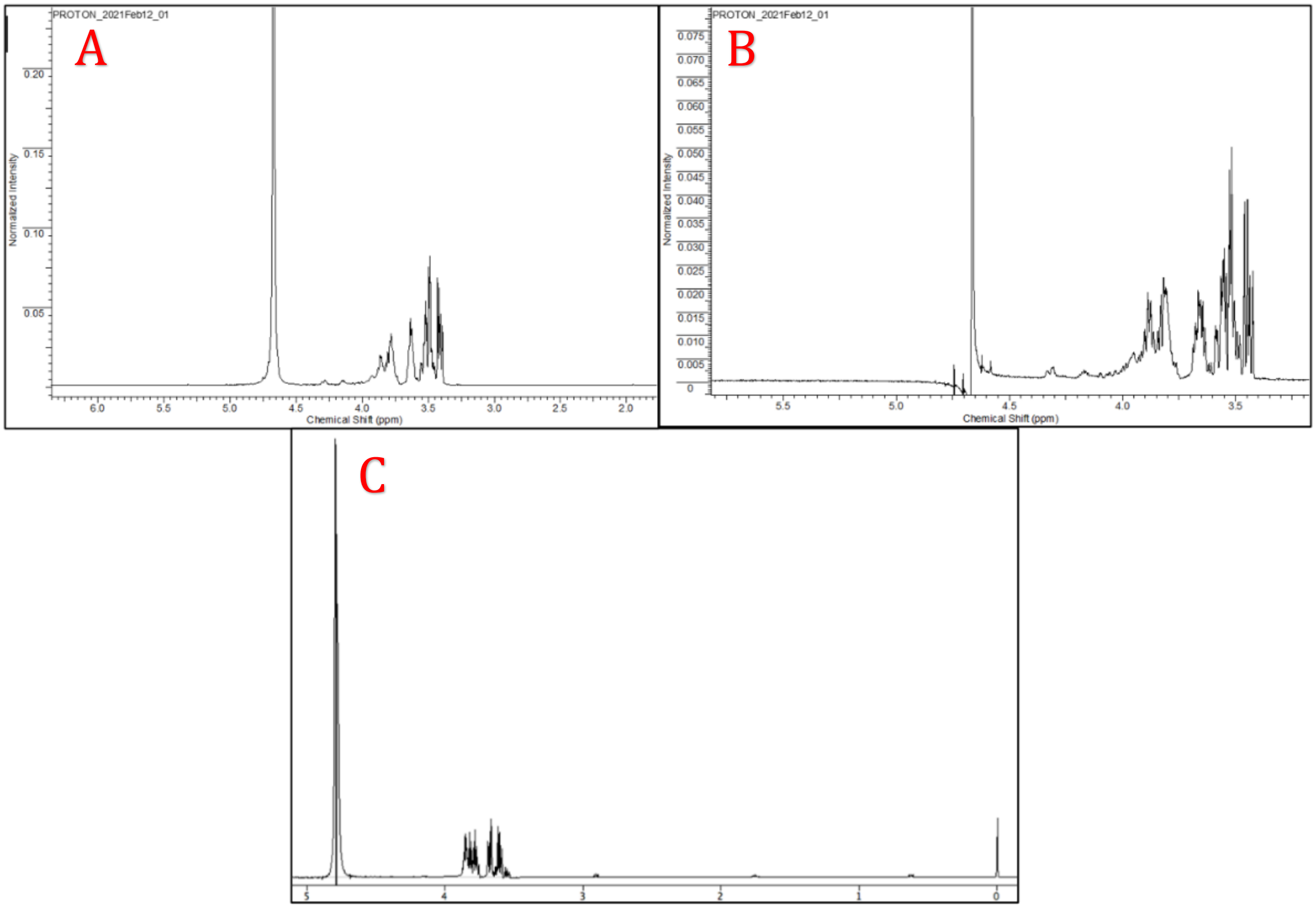

Figure 5. (A) Displays the H-NMR results of Sample 2A. (B) Displays the H-NMR results for Sample 2B. (C) H-NMR results for glycerol-3-phosphate to be used for comparison. Data obtained from the Human Metabolome Database.[3]

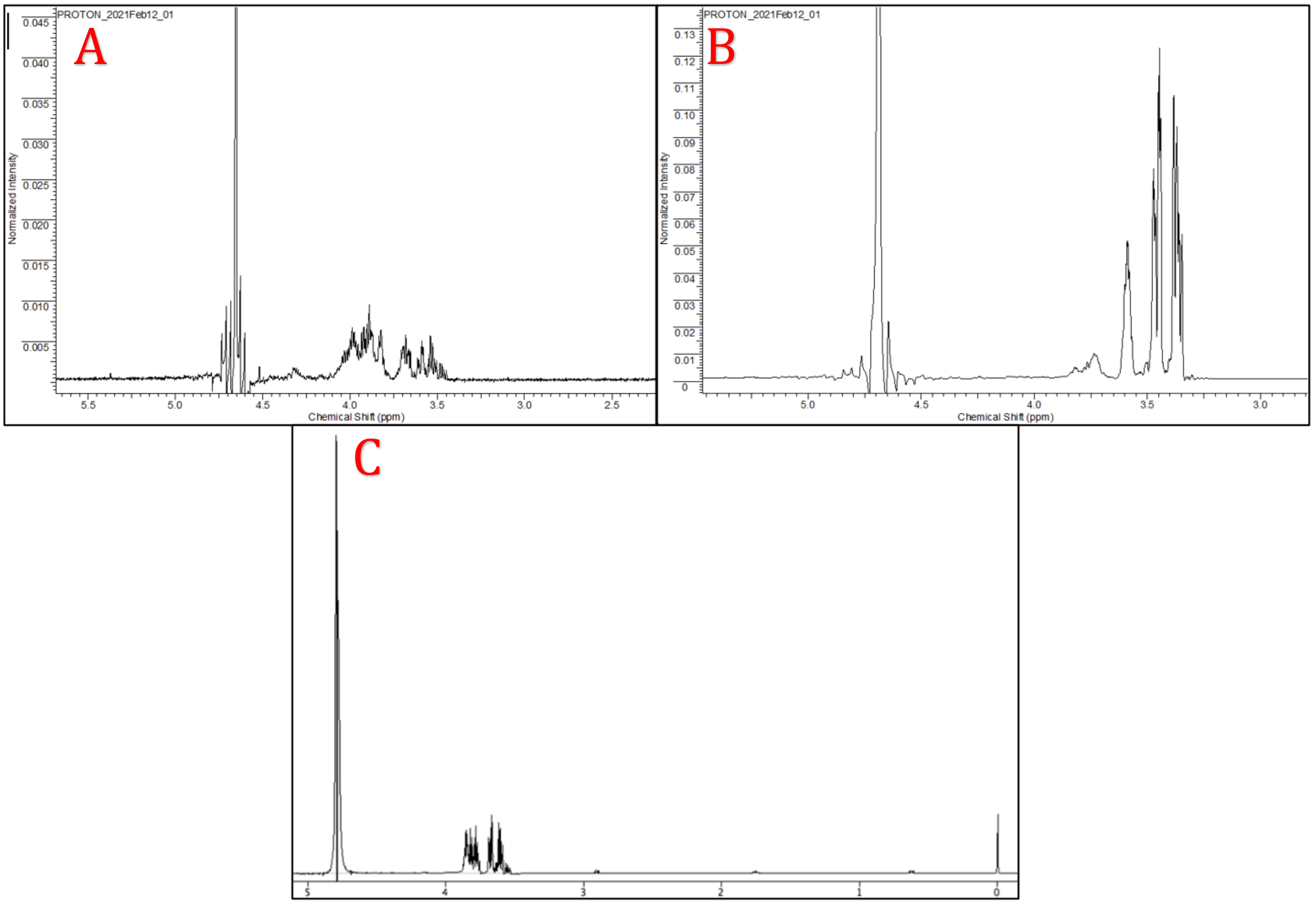

Figure 6. (A) Displays the H-NMR results of Sample 3A. (B) Displays the H-NMR results for Sample 3B. Both samples had 75 mmol Mg^{2+} added to the reaction. (C) H-NMR results for glycerol-3-phosphate to be used for comparison. Data obtained from the Human Metabolome Database.[3]

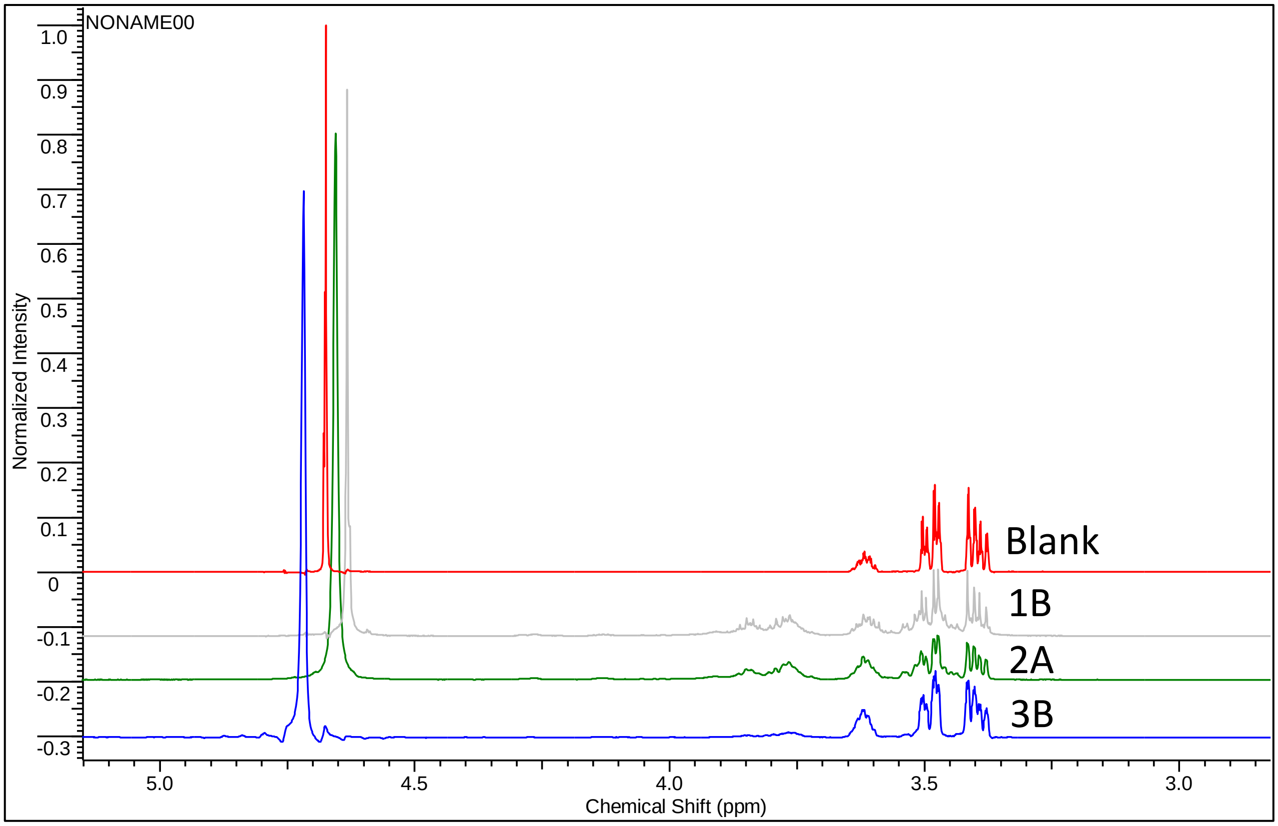

Figure 7. Displays normalized H-NMR results for shown samples. Samples were selected based on clear NMR resolution. H-NMR peaks were used to calculate reaction conversion rate (1B = 5.12H; 2A = 4.75H; 3B = 20.73H).

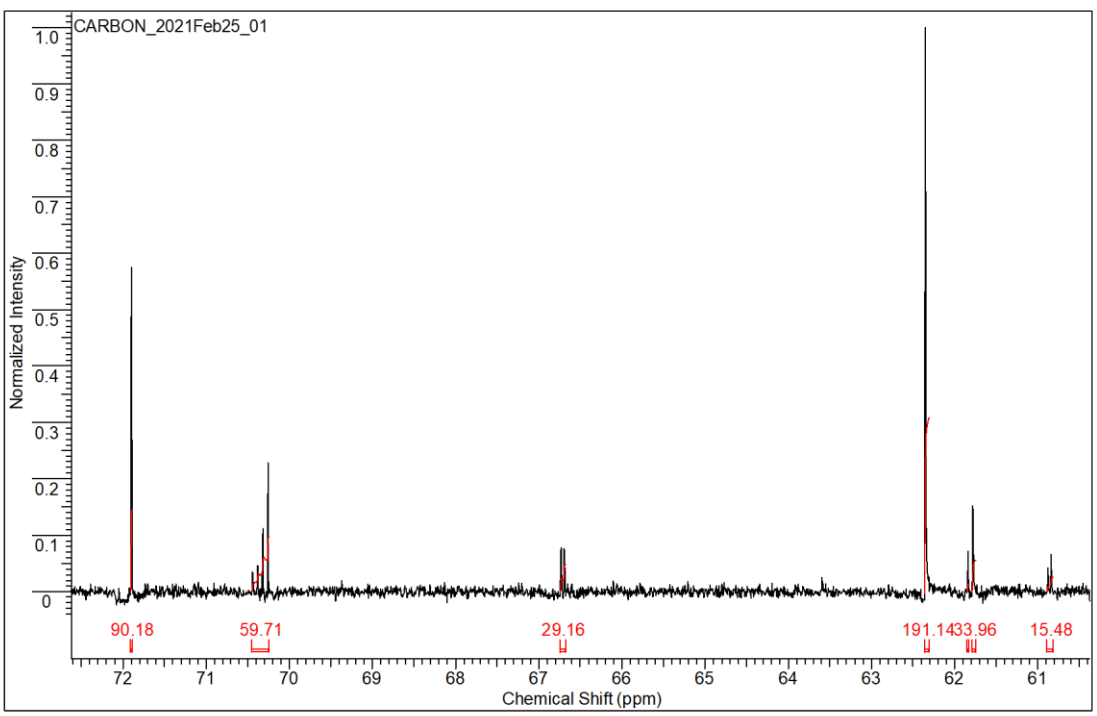

Figure 8. C-NMR results for Sample 1B. Peaks between 70 and 71 ppm indicate presence of glycerol-3-phosphate and/or glycerol-2-phosphate.

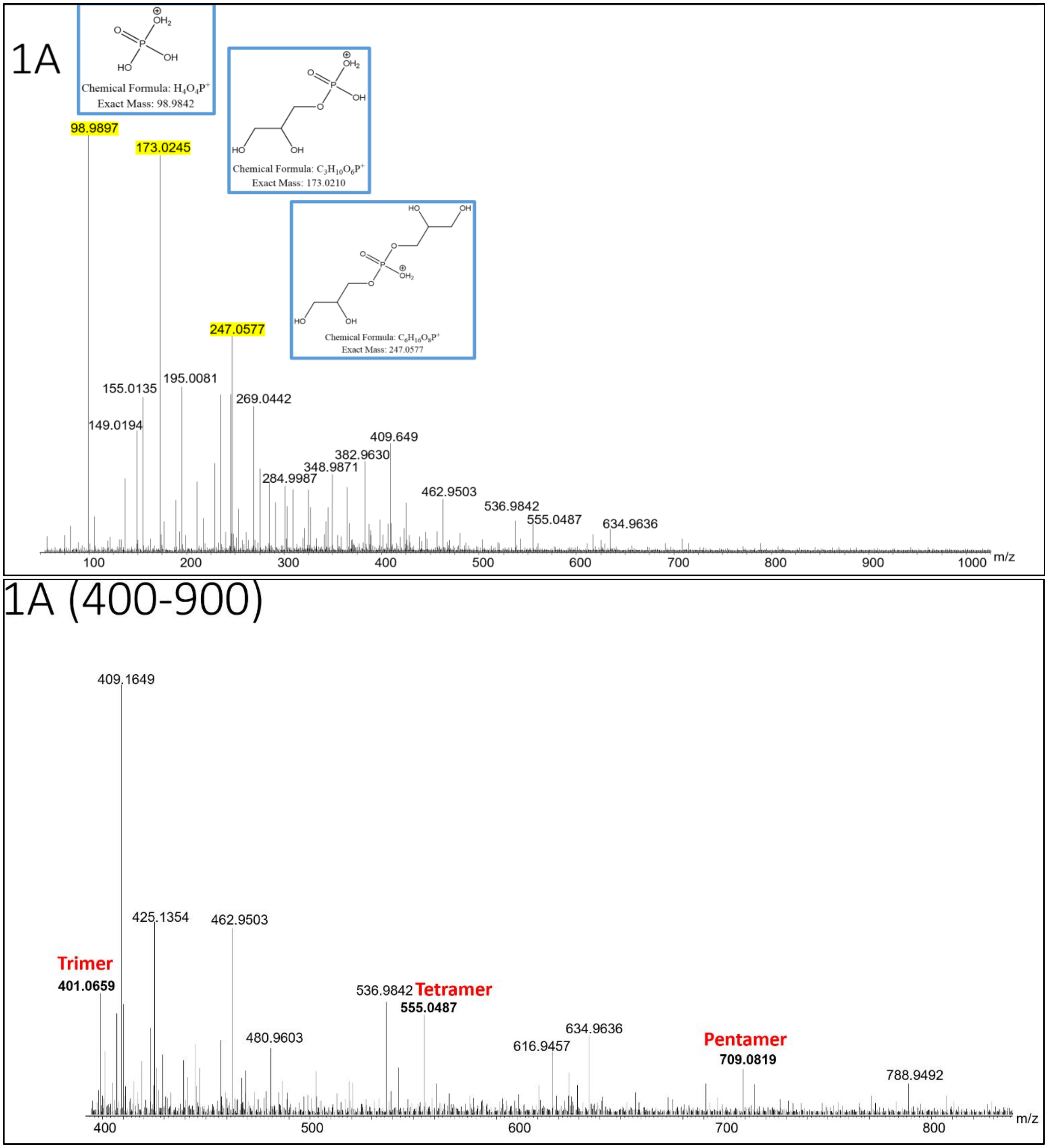

Figure 9. Mass spectrometry results for Sample 1A with significant peaks highlighted. Indicates successful formation of longer oligomers. Mass 100-1000 products (top panel) and focusing on mass 400-900 products (bottom panel) are shown.

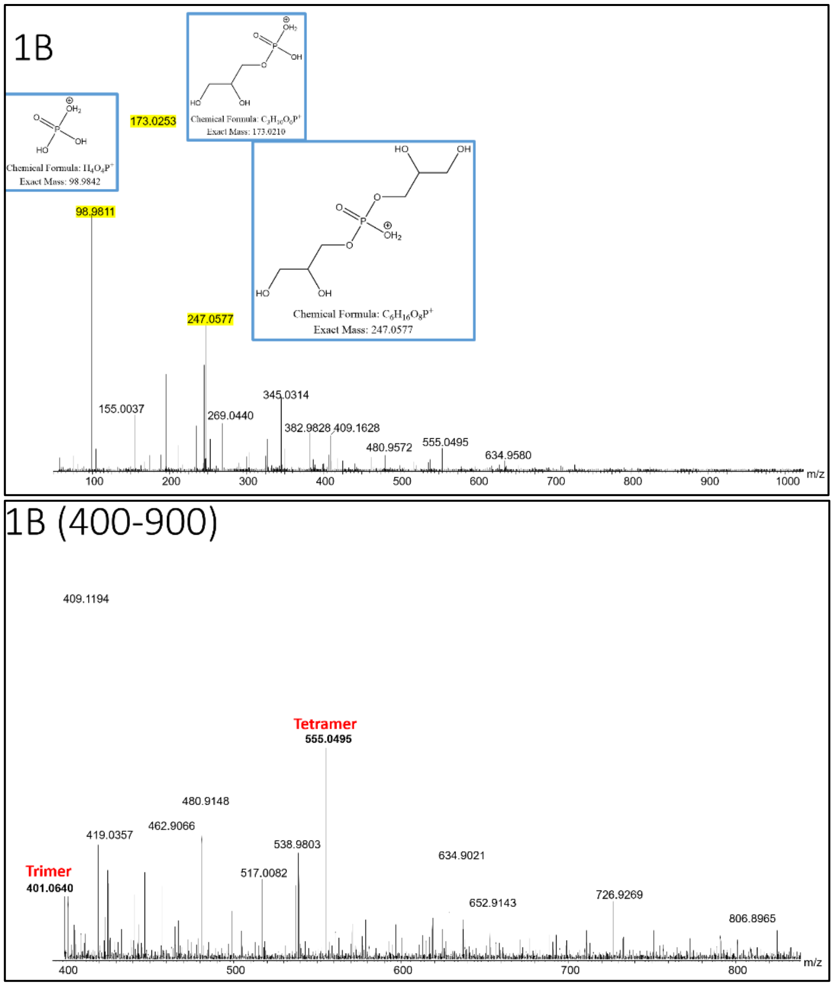

Figure 10. Mass spectrometry results for Sample 1B with significant peaks highlighted. Indicates successful formation of longer oligomers. Mass 100-1000 products (top panel) and focusing on mass 400-900 products (bottom panel) are shown.

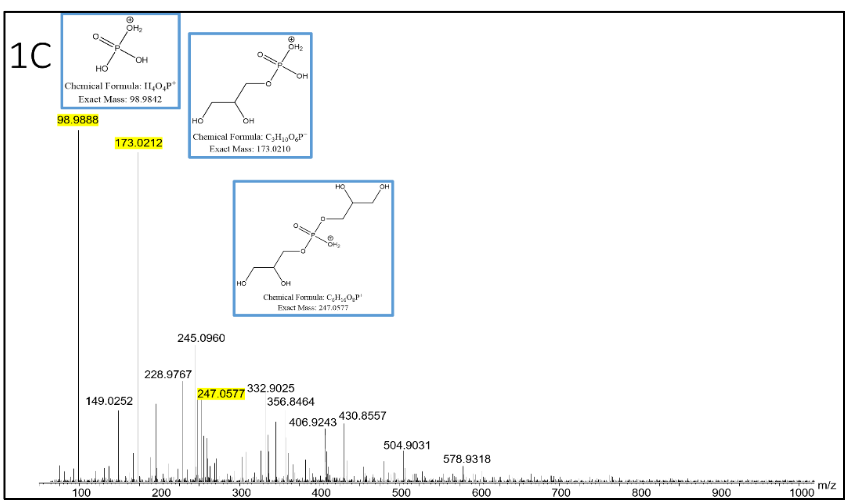

Figure 11. Mass spectrometry results for Sample 1C with significant peaks highlighted. No oligomer of higher length than a dimer was detected. Mass 100-1000 products (top panel) and focusing on mass 400-900 products (bottom panel) are shown.

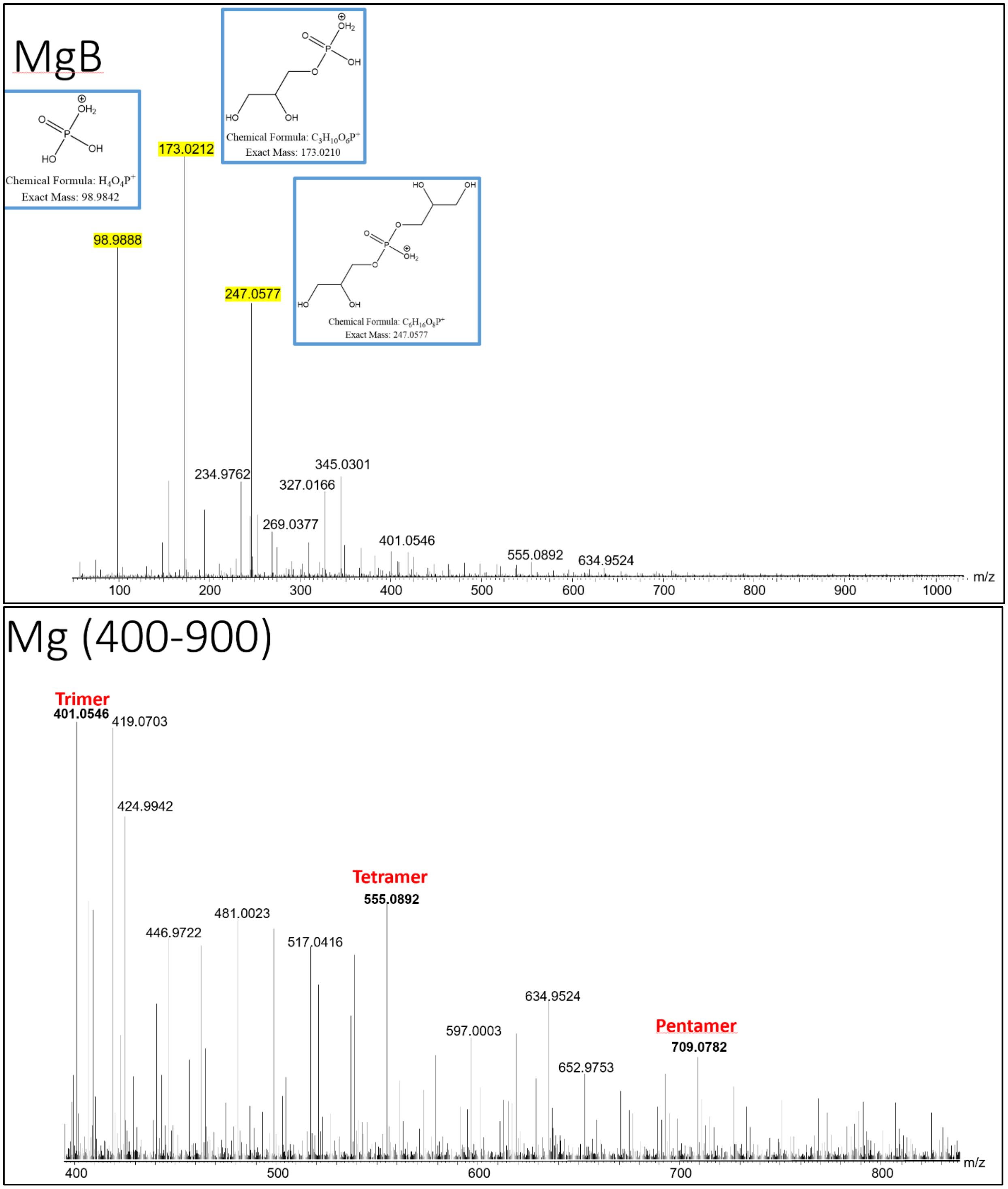

Figure 12. Mass spectrometry results for Sample 3B with significant peaks highlighted. Indicates successful formation of longer oligomers. Mass 100-1000 products (top panel) and focusing on mass 400-900 products (bottom panel) are shown.

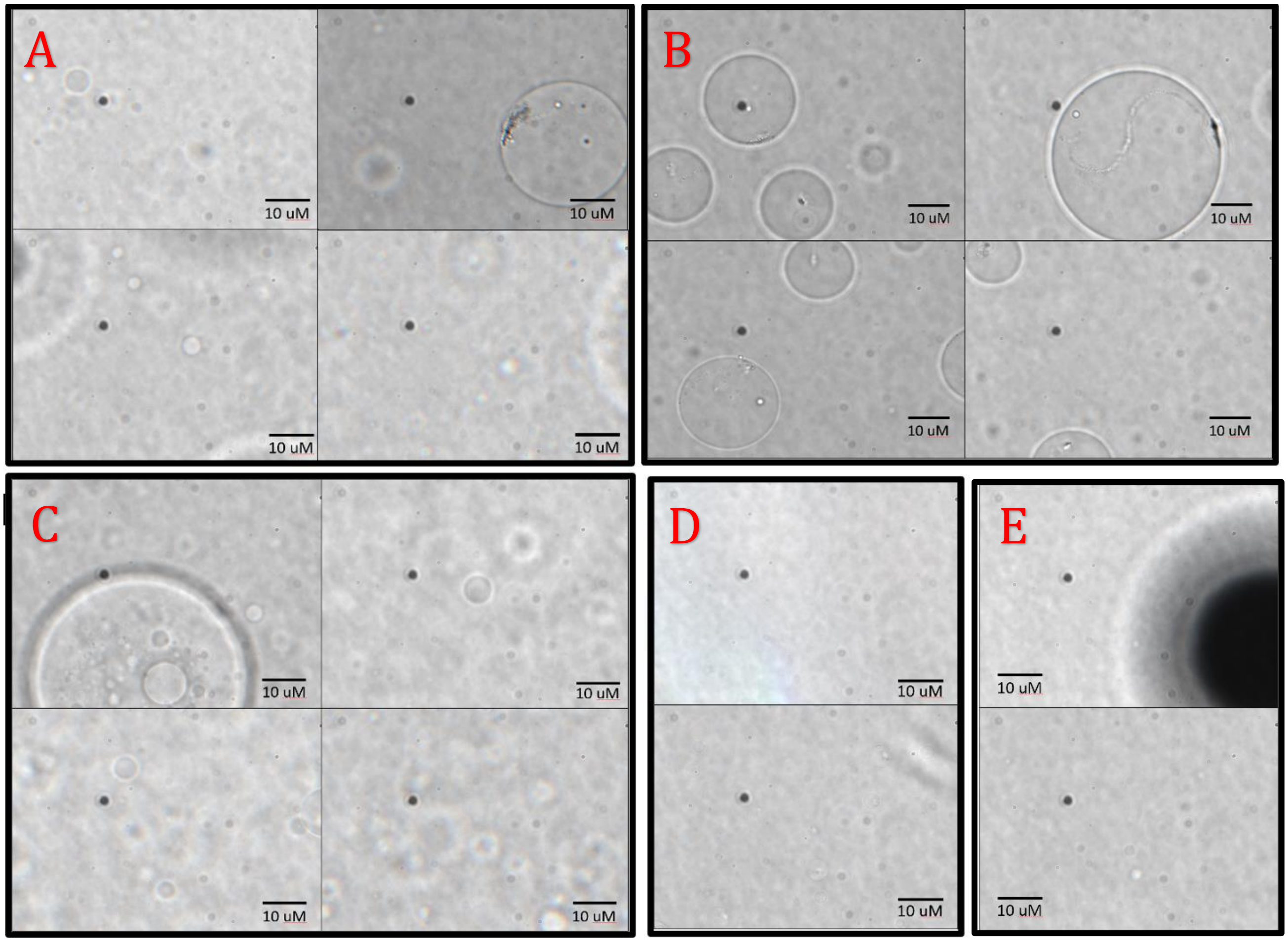

Figure 13. Displays the microscopic analysis of samples 1A through 1C (A through C respectively) as well as sample 3B (D). Sample blank included for reference (E), depicting an air bubble.

Appendix II – Sample Calculations

Calculation of Phosphoric Acid Amount

5 g Glycerol x $\frac{1\ mol}{92.09\ g}$ = 0.054 mol

0.054 mol x $\frac{97.99\ g}{1\ mol}$ x $\frac{1\ mL}{1.685\ g}$ x 1.85 (85% phosphoric acid in water) = 3.69 mL H_3PO_4

Calculation of 75 mmol $MgCl_2$

7.67 mL (total volume) x $\frac{0.075\ mol}{1000\ mL}$ x $\frac{95.21\ g}{1\ mol}$ = 0.055 g $MgCl_2$

Determination of Reaction Efficiency

5.12H – 3H (from phosphate product) = 2.12H (from glycerol SM)

2.12H (glycerol SM) / 5 (glycerol theo.) = 42% remaining

=58% conversion