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IN-15682

d/s SA 939211

**Oligoglyceric Acid Synthesis by
Autocondensation of Glyceroyl Thioester**

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(NASA-CR-177130)	OLIGOGLYCERIC ACID	N86-28128
SYNTHESIS BY AUTOCONDENSATION OF GLYCEROYL		
THIOESTER (Salk Institute for Biological		
Studies)	22 p HC A02/MF A01	CSCS 07A
		Unclas
		G3/23 43507

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Summary: The autocondensation of the glyceroyl thioester, S-glyceroyl-ethanethiol, yielded oligoglyceric acid. The rates of autocondensation and hydrolysis of the thioester increased from pH 6.5 to pH 7.5 in 2,6-lutidine and imidazole buffers. Autocondensation and hydrolysis were much more rapid in imidazole buffers as compared to 2,6-lutidine and phosphate buffers. The efficiency of ester bond synthesis was about 20% for 40 mM S-glyceroyl-ethanethiol in 2,6-lutidine and imidazole buffers near neutral pH. The size and yield of the oligoglyceric acid products increased when the concentration of the thioester was increased. The relationship of these results to prebiotic polymer synthesis is discussed.

Key words: Glyceroyl thioester - Polymerization - Polyester - Oligoglyceric acid - Thioester - Prebiotic chemistry - Molecular evolution.

Abbreviations: Glc, glyceric acid; $(\text{Glc})_n$, glyceric acid oligomers where n = chain length; Glc-SEt, S-glyceroyl-ethanethiol; $(\text{Glc})_2$ -SEt, S-glyceroyl-glyceroyl-ethanethiol; $(\text{Glc})_3$ -SEt, S-Glyceroylglyceroylglyceroyl-ethanethiol; Glc-Hydrox, glyceric acid hydroxamate; Glc-Im, N-glyceroyl-imidazole; Im, imidazole; DMF, N,N-dimethylformamide.

Introduction

In an effort to understand how energy was produced for the origin of life, we have studied chemical reactions that resemble the initial energy-yielding reaction of the glycolysis. Since this glycolytic reaction involves the oxidation of glyceraldehyde-3-phosphate to give an 'energy-rich' glyceroyl thioester which is used to drive the synthesis of ATP, we have studied the nonenzymatic formation of thioesters from glyceraldehyde and a thiol, and have examined thioester-driven phosphoanhydride synthesis. We showed that glyceraldehyde and a thiol could be converted to lactoyl thioester under anaerobic conditions and glyceroyl thioester in the presence of oxygen (Weber 1984a, b). We also obtained evidence of alanyl thioester synthesis in similar anaerobic reactions in the presence of ammonium ion (Weber 1985). Our studies of thioester-driven phosphoanhydride synthesis demonstrated that thioesters can act as an energy source for the synthesis of pyrophosphate, tripolyphosphate, and phosphorylimidazole (Weber 1981, 1982).

Glyceraldehyde's role in prebiotic chemistry may not have been limited to its being an energy source for phosphoanhydride synthesis. Our earlier studies indicate that glyceraldehyde could act as a source of both energy and monomers for the synthesis of prebiotic macromolecules, since lactoyl, glyceroyl, and alanyl thioesters derived from glyceraldehyde are in fact 'activated' monomers which have the energy needed for polymerization to polyesters or polyamides. Amino acid thioesters have previously been shown to condense to give peptides (Weber and Orgel, 1979). We now report the autocondensation of an hydroxy acid thioester, Glc-SEt, that yields oligoglyceric acid.

The synthesis of glyceraldehyde on the primitive Earth most likely occurred by the oligomerization of formaldehyde (Gabel and Ponnamperna, 1967; Reid and Orgel 1967; Mizuno and Weiss 1974). Formaldehyde has been synthesized

under a variety of prebiotic conditions (Garrison et al. 1951; Miller 1957; Getoff et al. 1960; Hubbard et al. 1971; Bar-Nun and Hartman 1978; Miller and Schlesinger 1984), and models of the Earth's primitive atmosphere show photochemical synthesis of formaldehyde in the early atmosphere and its transport to the Earth's surface by rain-out (Pinto et al. 1980; Canuto et al. 1983; Kasting and Pollack 1984).

Experimental

Materials. L-Glyceric acid (hemicalcium salt), Dowex 50W-X4 resin, and 5,5'-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma Chemical Co.; L-[¹⁴C(U)]serine from New England Nuclear; 4-dimethylaminopyridine, ethanethiol, 1,3-dicyclohexylcarbodiimide, and sodium nitrite from Aldrich Chemical Co.; hydroxylamine hydrochloride and methyl red from Matheson, Coleman and Bell; Centrex microfiltration units (nylon filter, 0.2 μm pore size) from Schleicher and Schuell.

Glyceric acid hydroxamate was prepared by the method of Thompson (1951).

Chromatography and Electrophoresis. Paper chromatography was carried out by descending elution on Whatman 3MM in System 1 with n-butanol-formic acid-water (8:1:2, v/v/v), and in System 2 with tert-butyl alcohol-formic acid-water (7:1.5:1.5, v/v/v). High-voltage paper electrophoresis on Whatman 3MM paper used a buffer of 0.03 M potassium phosphate (pH 7.1) in System 3. Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. The products containing [¹⁴C]glyceric acid were located by running the electrophoretograms through a Baird RSC-363 radiochromatographic scanner. The areas of the paper that contained the radioactive products were cut out,

placed in vials that contained 20 ml of scintillator made with Liquifluor (New England Nuclear), and counted in a Beckman scintillation counter. Radioactive products were identified by co-chromatography with commercially available standards, whenever possible. Organic acids were detected by spraying with methyl red. Thioesters were seen as dark spots under ultraviolet light.

← Insert
Table 1

Preparation of L-[¹⁴C]glyceric acid. A modified version of the method of Lok et al. (1976) was used to synthesize L-[¹⁴C]glyceric acid from L-[¹⁴C]-serine. L-Serine (10.3 mg, 0.098 mmole) and 12 μ l of concn HCl were added to a solution of L-[¹⁴C(U)]serine (250 μ Ci, 1.7 μ mole) in 0.60 ml water. The solution was cooled to 2°C and then sodium nitrite (7.0 mg, 0.1 mmole) was added in 1.0 mg portions every 30 min. The reaction solution was allowed to stand 24 h in a cold room at 9°C. Two more 1.0 mg additions of sodium nitrite were made and the reaction solution was allowed to stand at 9°C for 24 h and at ambient temperature for an additional 24 h. Purification of L-[¹⁴C]glyceric acid was achieved by paper chromatography on Whatman 3MM paper [R_f = 0.57; developing solvent: tert-butyl alcohol-formic acid-water, 7:1.5:1.5, v/v/v]. [¹⁴C]glyceric acid was eluted from the paper with water which was removed in vacuo. The residue was redissolved in 1.2 ml of water and the solution filtered through a Centrex microfiltration unit (nylon filter, 0.2 μ m pore size, Schleicher and Schuell). The water was removed in vacuo and the residue dried in a desiccator over P₂O₅ and NaOH pellets for 24 h. The residue was dissolved in 300 μ l DMF. The yield of L-[¹⁴C]glyceric acid was 34% based on radioactivity.

Preparation of S-glyceroyl ethanethiol. The method of Neises and Steglick (1978) was used to synthesize Glc-SEt. Glyceric acid (hemicalcium salt) was first converted to the free acid by passing 1 g of the hemicalcium salt dissolved in about 20 ml of H₂O through a column containing 40 ml of Dowex 50W-X4 resin which had been prewashed with 20 ml of 2 M HCl followed by 225 ml of water. The eluent was dried in vacuo and then in a desiccator over P₂O₅ in vacuo for 24 h. The residue was stored at -80°C until used in order to prevent spontaneous oligomerization that occurs at ambient temperature. The free acid form of glyceric acid (84.8 mg, 0.8 mmole) was dissolved in 340 µl of DMF. Next, 50 µl of a DMF solution of L-[¹⁴C]glyceric acid (14 µCi, 2.5 mCi/mmole), 4-dimethylaminopyridine (4.0 mg, 0.032 mmole), and ethanethiol (118 µl, 99 mg, 16 mmole) were added to this solution. The reaction vial was sealed and cooled to 2°C. 1,3-Dicyclohexylcarbodiimide (107 mg, 5.2 mmole) was added and reacted with stirring for 5 min at 2°C and then 1 h at ambient temperature. Forty microliters of glacial acetic acid and 500 µl of DMF were added, the reaction solution filtered through a Centrex microfilter unit, and the precipitate washed with an additional 750 µl of DMF. The DMF of the filtrate was removed in vacuo, the residue taken up in 700 µl of methanol, and a small amount of precipitate removed by centrifugation. Purification of Glc-SEt was achieved by preparative thin-layer chromatography of this crude preparation on a Silica Gel GF plate (1500 micron layer) from Analtech Inc. [R_f = 0.66; developing solvent: chloroform-methanol-acetic acid, 85:15:5, v/v/v]. The adsorbent layer that contained Glc-SEt, which was visible under ultraviolet light, was scraped off and the thioester eluted with methanol in a Centrex microfiltration unit by centrifugation. The methanol was removed in vacuo, and the residue dissolved in 750 µl of water and filtered through a Centrex microfiltration unit. The water was removed in vacuo. The residue was taken up in a final 500 µl of water, filtered

through a Centrex microfiltration unit, and stored at -80°C until used in reactions. The yield was 0.121 mmole or 15% based on the amount of glyceric acid used in the preparation.

Glc-SEt was characterized by hydrolysis (pH 12, 30 min) to glyceric acid and a thiol, and by reaction with neutral hydroxylamine to give glyceric acid hydroxamate (Stadtman 1957). The absorption spectrum (200-300 nm) resembled that of other thioesters (Stadtman 1957 and references therein). At the wavelength of maximum absorbance (240 nm) the molar extinction coefficient was found to be 4700 by relating the absorbance at 240 nm of Glc-SEt to the concentration of thiol released by its ammonolysis (Lynen, 1951). Thiol concentration was measured with 5,5'-dithiobis-(2-nitrobenzoic acid) (Zahler and Cleland, 1968).

Autocondensation of Glc-SEt. In a typical solution reaction, 40 μl of buffer, 20 μl of water, and 20 μl of 160 mM L-[^{14}C]Glc-SEt were added to a reaction tube. At various times, 15 μl aliquots were removed for electrophoresis in System 3, which separated glyceric acid oligomers from each other and from Glc-SEt that remained near the origin. The region of the electrophoretogram that contained Glc-SEt was cut out and sewed onto another strip of Whatman 3MM paper for re-chromatography in System 2. The radioactive reaction products on the electrophoretogram and chromatogram were located and measured as described earlier.

The dry state reaction was carried out by heating at 40°C under a nitrogen stream the residues from drying in vacuo 10 μl aliquots of a solution of 160 mM L-[^{14}C]Glc-SEt in 160 mM imidazole (pH 7.0). At various times, a reaction tube was removed from the heating block, 10 μl of 2.5 M formic acid was added, and the resulting solution was subjected to chromatography in System 1. The radioactive products were located and measured as described earlier.

Characterization of Reaction Products. The identity of the oligoglyceric acids is supported by their stability in weak acid and rapid alkaline hydrolysis in 0.2 M NaOH that is complete within 2 min. The chain lengths of (Glc)₂, (Glc)₃, and (Glc)₄ are confirmed by their reaction with alkaline hydroxylamine (Hestrin 1949; Jencks et al. 1960) to give their expected Glc-hydrox/Glc ratio. The structure of (Glc)₂-SEt is supported by its absorption spectrum (200-300 nm) which resembles other thioesters (Stadtman 1957 and references therein), and by its containing about twice the Glc residues per thioester linkage as Glc-SEt. This determination assumes that the extinction coefficients for (Glc)₂-SEt and Glc-SEt are the same. The identity of (Glc)₃-SEt is supported by the presence of radioactive Glc residues in its structure and by its absorbance of ultraviolet light on chromatograms, a behavior that is characteristic of thioesters.

Results

Table 2 shows that the autocondensation of 40 mM Glc-SEt in 2,6-lutidine buffers yields (Glc)₂ and (Glc)₃. In these lutidine buffers the decomposition of Glc-SEt due to hydrolysis and autocondensation is slow with a half-life of about 16 days at pH 7.0. The rate of decomposition increases as the pH increases from 6.5 to 7.5. The efficiency of ester bond synthesis is about 14% at pH 6.5 and increases to about 22% at pH 7.0. From pH 7.0 to 7.5 the efficiency remains constant. Increasing the lutidine buffer concentration from 0.4 M to 0.8 M results in a small increase in the rate of Glc-SEt decomposition.

← Insert
Table 2

Table 3 shows the autocondensation of 40 mM Glc-SEt in imidazole and phosphate buffers. The rate of reaction of Glc-SEt is much more rapid in the imidazole buffers compared to either lutidine or phosphate buffers. In imidazole

buffer (pH 7.0) the decomposition half-life of Glc-SEt is about 1.2 days; whereas, in lutidine or phosphate buffers (pH 7.0) it is about 16 days. Decomposition of (Glc)₂ and (Glc)₃ also occurs in imidazole buffers at all pH's studied. Although the efficiency of ester bond synthesis is about the same in the imidazole and lutidine buffers it is substantially less in phosphate buffers.

← Insert Table 3

Table 4 shows the effect of metal ions on Glc-SEt autocondensation. Zn²⁺ is the only divalent ion that influences the reaction. In the imidazole buffer, Zn²⁺ slows the rate of Glc-SEt decomposition. However, in the lutidine buffer Zn²⁺ accelerates the rates of Glc-SEt decomposition and (Glc)₂ synthesis. Precipitated solids are present in the Zn²⁺ reaction in the lutidine buffer, but not in the imidazole buffer. Presumably, these precipitates are insoluble Zn²⁺ salts.

← Insert Table 4

Table 5 shows the autocondensation of 400 mM Glc-SEt, and Glc-SEt in the dry state. Condensation of 400 mM Glc-SEt yields oligomers as large as the pentamer, (Glc)₅. Under dry conditions oligomers larger than (Glc)₅ are produced from Glc-SEt. These results together with our studies at 40 mM Glc-SEt show that increasing the concentration of Glc-SEt results in the synthesis of larger oligomers. This result is attributed to the increase in the ratio of the autocondensation rate to the hydrolysis rate of Glc-SEt that occurs when the concentration of Glc-SEt is increased. The appreciable yield of oligomers larger than (Glc)₂ indicates that cyclization of (Glc)₂-SEt to the cyclic diester is not a major reaction pathway, since rapid cyclization of (Glc)₂-SEt would block chain elongation beyond the dimer to give mostly (Glc)₂ from hydrolysis of the cyclic diester.

← Insert Table 5

with thioesters to give reactive N-acyl-imidazoles (Stadtman 1954) which are known to react with hydroxyl groups to give esters (Gerstein and Jencks 1964; Weber and Fox 1973; Profy and Usher 1984).

Oligoglyceric acid synthesis from Glc-SEt has several characteristics that distinguish it from related autocondensation reactions of amino acid esters that yield peptides. First, Glc-SEt oligomerization does not appear to be blocked by efficient intramolecular cyclization of (Glc)₂-SEt to the cyclic diester; whereas, oligomerization of amino acid esters is terminated by the analogous intramolecular cyclization of dipeptidyl esters to the cyclic dipeptide-(diketopiperazine) (Weber and Orgel, 1978, 1979, 1980). Second, polyesters such as oligoglyceric acid are susceptible to hydrolysis under mild alkaline conditions (Euranto, 1969; Braud 1985) where polypeptides are relatively stable. Furthermore, oligoglyceric acid can be branched, since each residue has the potential to form α - and β - ester linkages. However, transesterification reactions (Wilfong 1961; Koskikallio 1969; Sridharan and Mathai 1974) are expected to lead to a dominance of β -linkages in oligoglyceric acid, since glyceroyl esters of the C2-hydroxyl group (α -linkage) are expected to be more unstable than esters of the C3-hydroxyl group (β -linkage). This difference in stabilities is attributed to the strong inductive effect of the ester group adjacent to the C2-hydroxyl group which causes it to be more acidic and its esters less stable (Gerstein and Jencks 1964; Dean 1979; Rochester 1971).

Prebiotic Significance

Glc-SEt autocondensation is considered a model of condensation reactions that may have produced polyglyceric acid on the prebiotic Earth. This autocondensation reaction together with 1) the synthesis of glyceroyl thioester by oxidation of glyceraldehyde in the presence of a thiol (Weber 1984 a, b), and

2) the synthesis of glyceraldehyde from formaldehyde (Gabel and Ponnamparuma 1967; Reid and Orgel 1967; Mizuno and Weiss 1974) constitute a model pathway for the prebiotic synthesis of polyglyceric acid from formaldehyde. It seems very likely that formaldehyde was available on the primitive earth, since it can be produced under a variety of prebiotic conditions (Garrison et al. 1951; Miller 1957; Getoff et al. 1960; Hubbard et al. 1971; Bar-Nun and Hartman 1978; Miller and Schlesinger 1984), and model studies of the early Earth show its photochemical synthesis in the primitive atmosphere and its rain-out to the Earth's surface (Pinto et al. 1980; Canuto et al. 1983; Kasting and Pollack 1984). If we assume that the oxidant for glyceroyl thioester synthesis is a second molecule of triose (glyceraldehyde or dihydroxyacetone), the standard free energy (ΔG°)¹ of the overall pathway that converts formaldehyde(6) into a glyceroyl residue(1) of polyglyceric acid and glycerol(1) is calculated to be a very favorable -32.4 kcal/mol (convention III, Jencks 1976).

This pathway from formaldehyde to polyglyceric acid has several characteristics that make it an attractive model of prebiotic polymer synthesis. First,

¹This ΔG° was calculated from the ΔG° values of Thauer et al. (1977), and an estimated ΔG° of hydrolysis of a β -ester linkage of polyglyceric acid of -7.6 kcal/mol. This ΔG° of hydrolysis was calculated from the ΔG° of hydrolysis of an analogous acetyl ester, and the pKa of glyceric acid. The ΔG° of hydrolysis of the acetyl ester was calculated (Gerstein and Jencks 1964) from the pKa of the C3-hydroxyl group of polyglyceric acid that had been estimated using Taft polar substituent parameters (Dean 1979; Rochester 1971).

it produces monomer in an activated form, a glyceroyl thioester, which possesses the energy needed for polymer synthesis. This simplifies polymer synthesis by eliminating the requirement for a condensing agent and its bimolecular reaction with monomer. Furthermore, hydrolysis of some of the activated monomer liberates acid (glyceric acid, $pK_a = 3.55$) which could protect polyglyceric from alkaline hydrolysis. Second, the model unites the origin of metabolism and the origin of polymer synthesis into a single process. This unification takes place because the oxidation of glyceraldehyde to glyceroyl thioester can function as the initial energy-yielding reaction of early glycolysis, and as the source of activated monomer for early polymer synthesis. Finally, the resemblance between the oxidative conversion of glyceraldehyde to glyceroyl thioester and the early part of glycolysis gives this model pathway the potential to develop into glycolysis in a straightforward manner. However, this further development probably depends on polyglyceric acid acting as a catalyst which has a rudimentary hereditary capability.

Acknowledgments

This investigation was supported by the National Aeronautics and Space Administration (Grant NSG-7627). I thank Esther Varon for technical assistance in this study.

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Table 1: Chromatographic and electrophoretic mobilities^a

Substance	Relative Mobility		
	System 1	System 2	System 3
Glc	1.00	1.00	1.00
(Glc) ₂	0.74	0.84	0.77
(Glc) ₃	0.50	0.65	0.62
(Glc) ₄	0.32	0.47	0.52
(Glc) ₅	0.21	0.33	0.46
(Glc) _{>5}	0-0.21	0-0.33	0-0.40
Glc-SEt	2.12	-	-
(Glc) ₂ -SEt	1.93	-	-
(Glc) ₃ -SEt	1.57	-	-
Glc-Hydrox	0.55	-	-

^aMobilities are given relative to glyceric acid.

Table 2. Formation of oligoglyceric acid by autocondensation of 40 mM Glc-SET in 2,6-lutidine buffers at ambient temperature.

Buffer	pH	Time (days)	Percentage of total cpm			
			Glc-SET	Glc	(Glc) ₂	(Glc) ₃
0.4 M 2,6-lutidine	6.5	0	98.0	0.6	0.5	0.9
		1	96.8	2.0	0.6	0.6
		5	92.4	5.6	1.1	0.9
		10	85.4	10.8	2.8	1.0
		20	71.8	21.1	5.8	1.3
	6.8	0	98.3	0.4	0.5	0.8
		1	95.3	3.1	0.8	0.8
		5	85.9	9.6	3.2	1.3
		10	72.7	18.7	7.0	1.6
		20	52.9	33.7	11.5	1.9
	7.0	0	98.7	0.3	0.5	0.5
		2	86.0	7.8	4.7	1.4
		5	74.9	13.4	9.2	2.4
		10	62.4	21.9	13.3	2.6
		20	45.4	36.3	16.1	2.2
	7.2	0	99.0	0.2	0.4	0.4
		2	82.2	9.5	6.3	1.9
		5	68.9	16.7	11.6	2.9
		10	54.7	27.3	15.3	2.6
		20	38.0	43.2	16.8	2.1
7.5	0	98.9	0.2	0.4	0.5	
	2	75.6	12.2	9.7	2.7	
	5	57.1	24.5	15.3	3.0	
	10	42.5	38.5	16.9	2.3	
	20	26.0	57.6	15.2	1.3	
0.8 M 2,6-lutidine	7.0	0	99.1	0.2	0.4	0.3
		2	84.8	8.5	5.2	1.5
		5	70.5	16.3	10.6	2.5
		10	54.4	28.3	14.9	2.4
		20	35.0	47.9	15.6	1.7

Table 3. Formation of oligoglyceric acid by autocondensation of 40 mM Glc-SET in imidazole and phosphate buffers at ambient temperature.

Buffer	pH	Time (days)	Percentage of total cpm			
			Glc-SET	Glc	(Glc) ₂	(Glc) ₃
0.4 M Imidazole	6.5	0	99.0	0.3	0.4	0.3
		2	64.8	27.3	6.1	1.7
		5	36.4	49.3	11.8	2.5
		10	14.2	70.0	13.9	2.0
		20	1.9	85.5	11.9	0.8
	7.0	0	98.1	0.3	0.7	0.8
		2	32.7	49.0	14.4	3.8
		5	4.7	75.2	17.4	2.7
		10	0.4	85.1	13.6	0.9
		20	0.2	92.3	7.3	0.2
	7.5	0	94.4	0.5	2.6	2.4
		2	12.9	63.8	19.2	4.1
		5	0.9	81.4	16.2	1.4
		10	0.6	89.6	9.5	0.4
		20	0.9	95.9	3.0	0.1
0.8 M Imidazole	7.0	0	93.7	0.8	3.1	2.3
		2	16.0	66.0	15.1	2.7
		5	0.6	85.5	13.0	0.9
		10	0.6	91.0	8.2	0.3
		20	0.4	96.7	2.8	0.2
0.4 M Sodium Phosphate	6.5	0	99.8	0.2	0.0	- ^a
		2	95.4	4.1	0.4	-
		5	88.5	10.8	0.7	-
		10	77.4	20.5	2.1	-
		20	60.7	37.2	2.1	-
	7.0	0	99.7	0.2	0.1	-
		2	93.1	6.5	0.4	-
		5	83.5	14.7	1.8	-
		10	66.0	30.3	3.7	-
		20	44.9	53.0	2.2	-
	7.5	0	99.6	0.4	0.1	-
		2	86.1	13.3	0.7	-
		5	67.7	30.6	1.7	-
		10	41.7	53.0	5.3	-
		20	23.0	76.5	0.6	-

^a The dash indicates that this product was not detected in any radioscan of the reaction; therefore, it was not measured by scintillation counting.

Table 4. Effect of metal ions on the autocondensation of 40 mM Glc-SEt at pH 7.0 and ambient temperature.

Buffer	Salt	Time (days)	Percentage of total cpm			
			Glc-SEt	Glc	(Glc) ₂	(Glc) ₃
0.4 M Imidazole	NaCl	0	97.9	0.5	0.8	0.8
		5	7.4	69.2	19.6	3.6
		10	0.8	81.3	16.4	1.5
	MgCl ₂	0	98.2	0.4	0.6	0.9
		5	7.5	70.9	18.4	3.2
		10	0.5	84.4	14.0	1.2
	CaCl ₂	0	98.3	0.6	0.4	0.7
		5	7.1	72.2	18.0	2.8
		10	0.4	85.9	12.6	1.1
	ZnCl ₂	0	97.9	0.6	0.7	0.8
		5	12.6	68.1	16.5	2.7
		10	1.7	82.7	14.5	1.2
0.4 M 2,6-Lutidine	NaCl	0	98.3	0.7	0.5	0.6
		5	82.6	11.1	5.1	1.2
		10	68.5	20.3	9.3	1.7
	MgCl ₂	0	98.2	0.7	0.5	0.5
		5	82.3	11.9	4.7	1.2
		10	67.9	22.2	8.4	1.5
	CaCl ₂	0	98.4	0.7	0.5	0.4
		5	82.1	11.8	4.8	1.2
		10	66.7	22.8	9.1	1.4
	ZnCl ₂	0	98.2	0.8	0.6	0.5
		5	67.1	24.6	7.4	1.1
		10	42.5	45.7	10.8	1.1