BIOPOLYMER FOR BIOREMEDIATION

A Major Qualifying Project Report:

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

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Date: January 9, 2008

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ACKNOWLEDGEMENTS

I would like to thank Professor Michael A. Buckholt, Professor Jill Rulfs, and Professor Theodore C. Crusberg for their guidance on this project. Their comments, suggestions, and instruction in using laboratory equipment throughout the project have been greatly appreciated.

Additional thanks go to my project partners Christopher Mayo, Gregory Parks, Elisabeth Curlyo, and Mary Gwendolyn for their help in the laboratory and the writing of this paper.

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ABSTRACT

ε-poly-L-lysine (ePL) is a biopolymer that has potential uses in a variety of fields including medicine, bioelectronics, and the food industry (as a food preservative). The goal of this project was to obtained ePL producing bacteria or fungi from soils suspected to be polluted by heavy metals, including chromates. The project concentrated on testing all the soil samples collected for chromium and testing the specificity of the method of Itzhaki (a method to measure concentrations of ePL).

INTRODUCTION

1.1 What is ε-poly-L-lysine?

ε-poly-L-lysine (ePL) is a linear homopolymeric compound created by linkage of lysine monomers at the α-carboxyl and ε-amino groups creating a peptide bond (Shima and Sakai, 1981b). It is naturally secreted by various *Streptomycetaceae* bacteria and some filamentous fungi (Nishikawa and Ogawa 2002; Shima and Sakai, 1981a,b; Skozan et al., 1997; Takehara et al., 1999) and is more commonly found than α-poly-L-lysine. However, in synthetic manufacture of polylysine only the α-poly-L-lysine can be created. Therefore, the production of ePL is currently carried out by organisms that produce it and is then extracted from them.

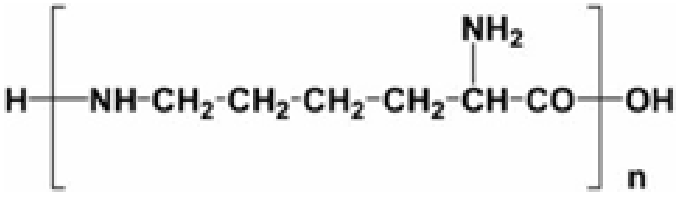

Figure 1. Chemical formula of ε-poly-L-lysine (Chisso Corporation, 2007)

1.2 Uses of ε-poly-L-lysine

The cationic nature of ePL allows it to inhibit the formation of cell membranes of a wide range of microbes, including yeasts, fungi, and both gram-positive and gram negative bacteria (Yoshida and Nagasawa, 2003). Since ePL is non-toxic to humans even at high doses and exhibits antimicrobial activity (Shima and Sakai, 1977; Shima et al., 1982, 1984), the molecule has been utilized as a food preservative in Japan for many years (Hiraki et al., 2003).

Studies have also been performed to investigate the use of ePL as an emulsifying agent (Ho et al., 2000), a dietary agent (Kido et al., 2003), a drug-delivery carrier (Shen and Ryser, 1978, 1979, 1981), a gene delivery carrier (Chiou et al., 1994; Dorudi et al., 1993), a basis for development of hydrogels (Kunioka, 1995; Kunioka and Choi, 1995), and a coating material for biochips and bioelectronics (Ostuni et al. 1999; Cai et al., 2002; Wallace et al., 2000).

1.3 Purpose and goals of the experiment

Heavy metal binding of ePL has not been sufficiently explored and holds a lot of potential. If ePL proves to be useful in removing heavy-metals from water sources, the cheap mass production of the homopolypeptide would be an extraordinary resource for locations where heavy-metal pollution is a problem. The properties of ePL make it an ideal molecule to use in bioremediation. It is highly cationic and has the ability to bind heavy metals, specifically chromates. Additionally, since ePL is a biopolymer it is also biodegradable, which would mean that the method would also be environmentally friendly and safe. Presently, there is a lot of heavy-metal pollution in water and an inexpensive process is needed to reduce heavy metal release from industries, which are allowed to discharge certain amounts of heavy metals into water.

The goal of this project was to collect soil samples from known areas of high pollutants in order to find high yielding ePL producing strains of bacteria or fungi that have been environmentally challenged with chromium. Once the soil samples were collected, a chromium assay was performed in order to determine the amount of chromium present in the soil. In preparation for a larger project, which would involve searching for other ePL producing strains of bacteria or fungi other than *Streptomyces albulus*, the specificity of the method of Itzhaki was

tested. The purpose of that was to determine how useful the method of Itzhaki (Kahar et al., 2001) would be in measuring the amount of ePL in the organisms isolated by that project. Lastly, a positive control stain, *Streptomyces albulus* Routien, was fermented and tested for any ePL production.

The following was accomplished in this project:

1. Collecting soil samples from areas suspected and/or known to contain high concentrations of heavy metals (specifically chromium).
2. Testing of all soil samples for the presence of chromium.
3. Testing the specificity of the method of Itzhaki in measuring ePL.
4. Fermenting of *Streptomyces albulus* Routien (ATCC, Manassas, VA).
5. Determining if *Streptomyces albulus* Routien produces ePL and whether it can serve as a positive control for comparison to other organisms grown from the soil samples.

MATERIALS AND METHODS

2.1 Soil sample collection

Soil samples were obtained from areas that were either known or suspected to be polluted with heavy metals, including chromates. All the samples were taken at about 6 inches below the surface of soil along the water's edge. Multiple samples (different places in the same area) were taken from each location. These locations include Singing Dam (Sutton, MA), West Hill Dam (Uxbridge, MA), West River (Uxbridge, MA), Wilson Pond (Easthampton, MA), and Paint Shop Pond (Wellesley, MA). The Singing Dam in Sutton, MA was reported to be the area of highest heavy metal deposit concentration in the Blackstone River (Hamrick et al, 2002). The soil samples were taken out of the ground using a can, which was driven into the ground. This method allowed the collection of several layers of soil, which was then transferred temporarily into a zip lock bag. At the laboratory the collected soil samples were transferred into glass containers stored in a 4°C refrigerator until further use.

The collected samples were named according to the location from which it came from, followed by a number that indicates the location at that specific site. Table 1, lists all the locations of the collected samples, as well as, their corresponding abbreviations used in the experiments.

Figure 2. Soil sample collection at Singing Dam site (Sutton, MA)

Figure 3. Soil sample collection at West River site (Uxbridge, MA)

Soil Sample Location	Soil Sample Abbreviation
Singing Dam (Site 1)	SD1
Singing Dam (Site 2)	SD2
Singing Dam (Site 3)	SD3
Singing Dam (Site 4)	SD4
Paint Shop Pond (Site 1)	PSP1
Paint Shop Pond (Site 2)	PSP2
Paint Shop Pond (Site 3)	PSP3
Paint Shop Pond (Site 4)	PSP4
Wilson Pond (Site 1)	WP1
Wilson Pond (Site 2)	WP2
Wilson Pond (Site 3)	WP3
West River (Site 1)	WR1
West River (Site 2)	WR2
West River (Site 3)	WR3
West Hill Dam (Site 1)	WHD1
West Hill Dam (Site 2)	WHD2
West Hill Dam (Site 3)	WHD3
West Hill Dam (Site 4)	WHD4
West Hill Dam (Site 5)	WHD5
West Hill Dam (Site 6)	WHD6

Table 1. A list of all soil sample locations and the sample sites

2.2 Preparation of soil samples for the chromium assay

Concentrations of chromium in each of the soil samples were determined using the diphenylcarbazide method from *Standard Methods for the Examination of Water and Wastewater* (Prepared and published jointly by the American Public Health Association, American Water Works Association [and] Water Pollution Control Federation, 13th edition, 1971). The reaction between hexavalent chromium and diphenylcarbazide produces a red-violet product which is measured spectrophotometrically at a wavelength of 540nm.

Briefly, portions of each the collected samples were placed in individual tubes and then left in a drying oven uncapped at 65°C for three days. One gram dry weight of each of the samples was mixed with 10mL of 1M HCl and placed on shakers for at least 24 hours at room

temperature. The collected samples were centrifuged at 2,500 rpm for 5 minutes to remove any particles in the mixture and the supernatant removed for assay.

2.3 Preparation of chromium solution

Analytical grade $K_2Cr_2O_7$ (ACROS Organics, St. Louis, MO) was used to make all chromium standard solutions used in the experiments. A stock solution of 1 g/L was prepared by dissolving the powder in reagent grade water. The stock solution was diluted in water to make the standards for the assay, which ranged from 0.50 mg/L to 5.00 mg/L of $K_2Cr_2O_7$.

The molecular weight of $K_2Cr_2O_7$ is 294.18 g/mol, of which 35.35% of the powder is actually chromium. Therefore, in 1000.00 mg/L of stock solution there is 353.50 mg/L of chromium. Similarly, the prepared standard solutions represent concentrations of chromium that range from 0.18 mg/L to 1.77 mg/L.

2.4 Spectrophotometric determination of chromium in soil samples

The concentrations of the collected samples were determined by mixing 0.5 mL of sample with 1.0 mL of 0.2 N H_2SO_4 and 200 µL of 0.5% diphenylcarbazide (in acetone). This was diluted up to 10.0 mL with reagent grade water in a 15.0 mL conical tube and left at room temperature 2-3 minutes before measuring the optical density at 540 nm.

Note: For the standard solutions reagent grade water was used as a blank. However, for each of the collected samples, 0.5 mL of sample with 9.5 mL of reagent grade water was used.

2.5 Method of Itzhaki for analysis and determination of ePL

The purpose of collecting soil samples was to obtain ePL producing bacteria that were challenged environmentally in the presence of chromium. In order to screen for those ePL producing bacteria, the method of Itzhaki would be used to detect and quantify the amount of ePL produced (Kahar et al., 2001).

However, it was important to establish the specificity of the assay before it could be used as a definitive method in verifying an organism's ability to produce ePL. For this, the assay was performed several times using the following: ε-poly-L-lysine (Chisso Corporation, Tokyo, Japan), L-lysine (Sigma-Aldrich, St. Louis, MO), and poly-L-lysine (Sigma-Aldrich, St. Louis, MO).

Solutions were prepared for ePL (powder provided by Chisso Corporation as a 1:1 ratio of ePL to dextrin), L-lysine, and poly-L-lysine starting at a concentration of 1000.00 mg/L. By using a 2-fold dilution, a range of concentrations was made from 1000.00 mg/L to 7.81 mg/L.

For each (ePL, poly-L-lysine, and L-lysine) 0.1 mL of the sample was added to 1.9 mL 0.1mM phosphate buffer (pH 6.6) and 2.0 mL methyl orange solution (0.1 mM methyl orange solution (Sigma-Aldrich, St. Louis, MO)) in a 15.0 mL conical tube. Then, the mixture was placed on a shaker at 30°C for 30 minutes and centrifuged at 2,500 rpm for 5 minutes. The resulting supernatant was then measured in a spectrophotometer at a wavelength of 465 nm using two blanks: a methyl orange blank and a phosphate buffer blank.

2.6 Fermentation of *Streptomyces albulus* Routien

The *Streptomyces albulus* Routien was streaked onto plates containing LB medium and were placed in a 28°C incubator for 5 days. M3G medium (50g glucose, 10 g $(NH_4)_2SO_4$, 5 g

yeast extract, 1.36 g KH_2PO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 0.03 g $FeSO_4 \cdot 7H_2O$, 0.04 g $ZnSO_4 \cdot 7H_2O$) was prepared and autoclaved for 20 minutes at 120°C. Four loops from the *S. albulus* Routien plate were suspended in 2 mL of M3G medium. 0.5 mL of that suspension was placed into a 300 mL baffle flask (sterilized by autoclaving for 20 minutes at 120°C) containing 50 mL of M3G medium. The baffle flask was placed in a shaking water bath at for 96 hours at 26°C and shaking at 250 rpm.

2.7 Measuring production of ePL by *Streptomyces albulus* Routien

The method of Itzhaki was also used to determine whether any ePL was produced by *S. albulus* Routien. At the end of the fermentation, 1 mL of the liquid was placed into a 1.5 mL microfuge tube and centrifuged for 5 minutes at 2,500 rpm. Then 0.1 mL of the resulting supernatant was added to 1.9 mL phosphate buffer and 2.0 mL methyl orange solution into a 15.0 mL conical tube. The mixtures were placed on a shaker at 30°C for 30 minutes and then centrifuged at 2,500 rpm for 5 minutes. The resulting supernatant was measured in a spectrophotometer at a wavelength of 465 nm.

RESULTS

3.1 Chromium assay standard curve

The chromium assay was established using a series of 6 standards ranging from 0.5 mg/L to 5 mg/L of $K_2Cr_2O_7$ (which corresponds to 0.18 mg/L to 1.77 mg/L of chromium).

Concentration of $K_2Cr_2O_7$	Trial 1	Trial 2	Trial 3	Average	Standard Error
5 mg/L	0.069	0.069	0.068	0.069	0.000
4 mg/L	0.054	0.055	0.055	0.055	0.000
3 mg/L	0.039	0.040	0.040	0.040	0.000
2 mg/L	0.027	0.027	0.027	0.027	0.000
1 mg/L	0.015	0.015	0.014	0.015	0.000
0.5 mg/L	0.005	0.006	0.005	0.005	0.000

Table 2. Optical density values of the K2Cr2O7 standard solutions at a wavelength of 540 nm. For each standard solution, the experiment had three replicates and the average and standard error was obtained for those solutions.

The $K_2Cr_2O_7$ standard curve was obtained by using the averages obtained from the triplicate samples (see Table 2 and Figure 4) and has an R-value of 0.998. The assay was then used to determine the amount of chromium present in each soil sample. The samples were prepared as described in the materials and methods section following extraction in 1M HCl and measured using the diphenylcarbazide method (see Table 3). Since it is known that 35.35% of $K_2Cr_2O_7$ consists of chromium, and using the value determined from the $K_2Cr_2O_7$ standard curve, the amount of chromium for each soil sample was calculated accordingly (see Table 4).

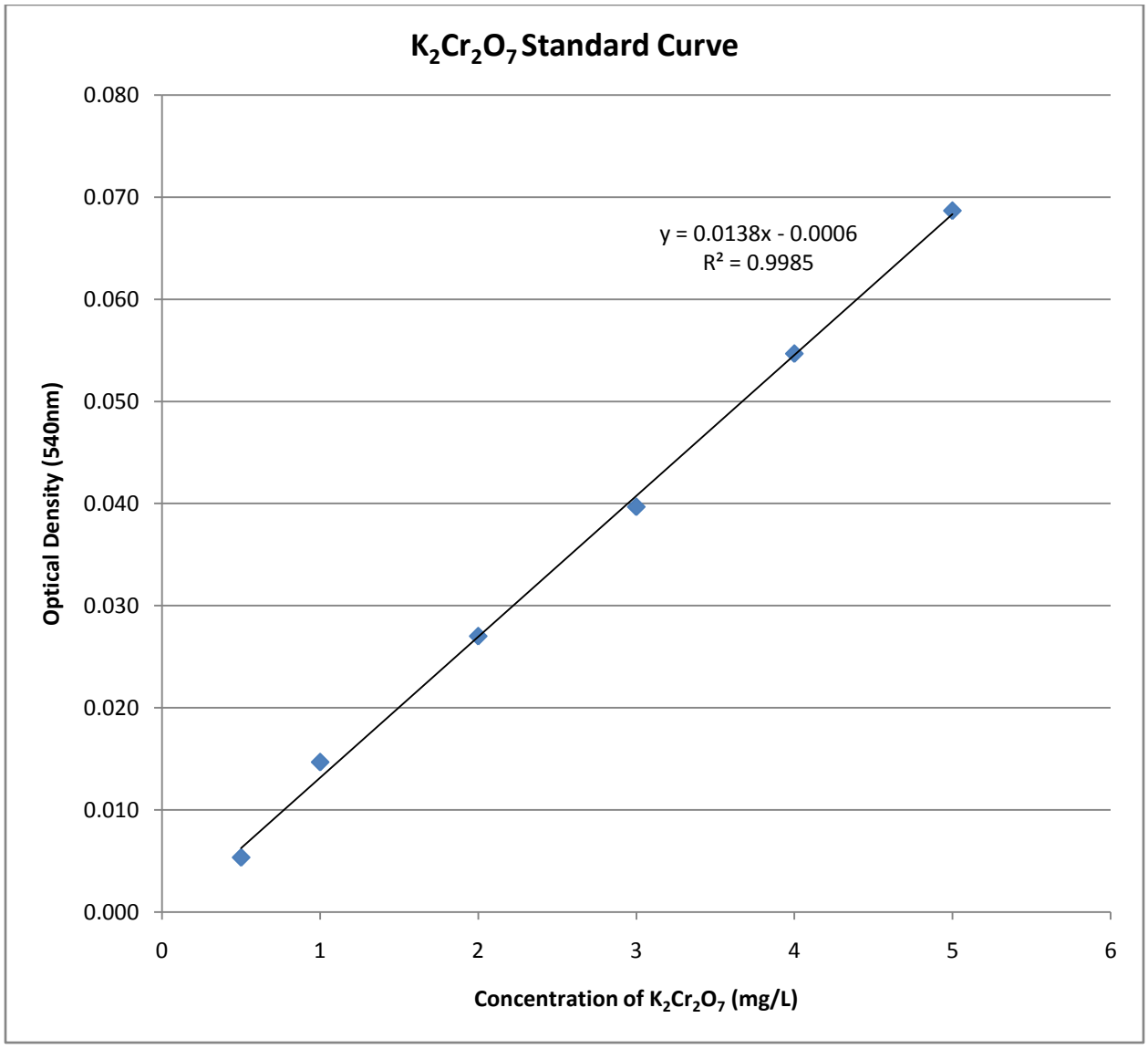

Figure 4. K2Cr2O7 Standard Curve. The plot was created from the averaged optical density values of the standard solutions. The standard curve yields a straight line that runs through almost all the points with equation $y = 0.013x - 0.000$, where $y = absorbance\ value\ at\ 540\ nm$ and $x = concentration\ of$ $K_2Cr_2O_7$.

3.2 Concentration of chromium in the collected soil samples

The average optical density was obtained from the triplicate samples (see Table 3) for each soil sample. During the preparation of the soil samples for the chromium assay, it was noted that all the soil samples were of different yellow hues (some darker or lighter between each sample). To account for potential inconsistency, which might result for each soil sample, 0.5 mL of sample with 9.5 mL of reagent grade water was used as a blank (as noted in the materials and methods section).

Soil Samples	Trial 1	Trial 2	Trial 3	Average Optical density
SD1	0.009	0.011	0.010	0.010
SD2	0.009	0.009	0.009	0.009
SD3	0.020	0.021	0.021	0.021
SD4	0.014	0.013	0.014	0.014
PSP1*	0.001	0.004	0.005	0.003
PSP2*	0.008	0.006	0.007	0.007
PSP3*	0.005	0.005	0.005	0.005
PSP4*	0.007	0.007	0.006	0.007
WP1	0.029	0.029	0.028	0.029
WP2	0.014	0.014	0.014	0.014
WP3	0.005	0.004	0.006	0.005
WR1	0.009	0.009	0.007	0.008
WR2	0.015	0.016	0.015	0.015
WR3	0.014	0.012	0.013	0.013
WHD1	0.014	0.014	0.014	0.014
WHD2	0.009	0.009	0.008	0.009
WHD3	0.007	0.006	0.006	0.006
WHD4	0.004	0.004	0.005	0.004
WHD5	0.019	0.020	0.019	0.019
WHD6	0.006	0.005	0.005	0.005

Table 3. Optical density values of collected soil samples. Determination of the concentration of chromium in the collected soil samples.

Notes:

* The soil samples from Paint Shop Pond contained a large quantity of leaves and very little soil.

The concentration of $K_2Cr_2O_7$ of each soil sample was calculated from the equation obtained from the standard curve (see Figure 4) using the average optical density. The concentration of chromium in the sample solution was calculated by multiplying the value for the concentration of $K_2Cr_2O_7$ with 0.3535 (since 35.35% of the compound is chromium). Further calculations were made to determine the amount of chromium (μg) in 1 g of dry weight of soil.

Soil Samples	Average Optical density	Concentration of $K_2Cr_2O_7$ (mg/L)	Concentration of Chromium in (mg/L)	Concentration of Chromium (μg) in 1 g of Dry Weight of Soil
SD1	0.010	0.769	0.272	2.719
SD2	0.009	0.692	0.245	2.447
SD3	0.021	1.590	0.562	5.620
SD4	0.014	1.051	0.372	3.716
PSP1	0.003	0.256	0.091	0.906
PSP2	0.007	0.538	0.190	1.903
PSP3	0.005	0.385	0.136	1.360
PSP4	0.007	0.513	0.181	1.813
WP1	0.029	2.205	0.780	7.795
WP2	0.014	1.077	0.381	3.807
WP3	0.005	0.385	0.136	1.360
WR1	0.008	0.641	0.227	2.266
WR2	0.015	1.179	0.417	4.169
WR3	0.013	1.000	0.354	3.535
WHD1	0.014	1.077	0.381	3.807
WHD2	0.009	0.667	0.236	2.357
WHD3	0.006	0.487	0.172	1.722
WHD4	0.004	0.333	0.118	1.178
WHD5	0.019	1.487	0.526	5.257
WHD6	0.005	0.410	0.145	1.450

Table 4. Determination of the concentration of chromium in the collected soil samples.

3.3 Method of Itzhaki for ePL

The optical density of the resulting supernatant for all concentrations of ePL was measured twice, once with a 0.1mM phosphate buffer blank and once with a methyl orange blank (2mL of methyl orange solution with 2mL of phosphate buffer). The method of Itzhaki allows a water-soluble complex to be formed from the interaction between the cationic ePL and the anionic methyl orange. The amount of ePL in the mixture is measured spectrophotometrically at a wavelength of 465 nm. It is assumed that with higher concentrations of ePL, the amount of methyl orange remaining in the supernatant would decrease (lower optical density value). However, the data that will be most useful in observing a decrease in optical density values will be the assays using phosphate buffer as a blank.

Concentration of ε-poly-L-lysine (ePL)	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	-0.040	-0.043	-0.040	-0.041	0.001
500.00 mg/L	-0.043	-0.038	-0.042	-0.041	0.002
250.00 mg/L	-0.067	-0.075	-0.060	-0.067	0.004
125.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
62.50 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
31.25 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
15.63 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
7.81 mg/L	-0.079	-0.071	-0.078	-0.076	0.003
Phosphate Buffer + Methyl Orange	0.000	0.006	-0.002	-0.003	0.002

Table 5. Assay for ePL at 465nm with a methyl orange blank.

Note:
For all assays blanked with methyl orange, the values recorded in the tables represent the actual optical density value and not a change in optical density.

The optical densities observed between 1000.00 mg/L of ePL with 7.81 mg/L of ePL suggest that there is almost no difference or change in the mixture (see Figure 5).

Concentration of ε-poly-L-lysine (ePL)	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	1.116	1.114	1.116	1.115	0.001
500.00 mg/L	1.109	1.113	1.109	1.110	0.001
250.00 mg/L	1.081	1.081	1.085	1.082	0.001
125.00 mg/L	1.045	0.995	0.998	1.013	0.016
62.50 mg/L	0.874	0.989	0.981	0.948	0.037
31.25 mg/L	0.965	0.970	0.956	0.964	0.004
15.63 mg/L	0.987	0.973	0.962	0.974	0.007
7.81 mg/L	1.046	1.029	1.038	1.038	0.005
Phosphate Buffer + Methyl Orange	1.148	1.155	1.152	1.152	0.002

Table 6. Assay for ePL at 465nm with a phosphate buffer blank.

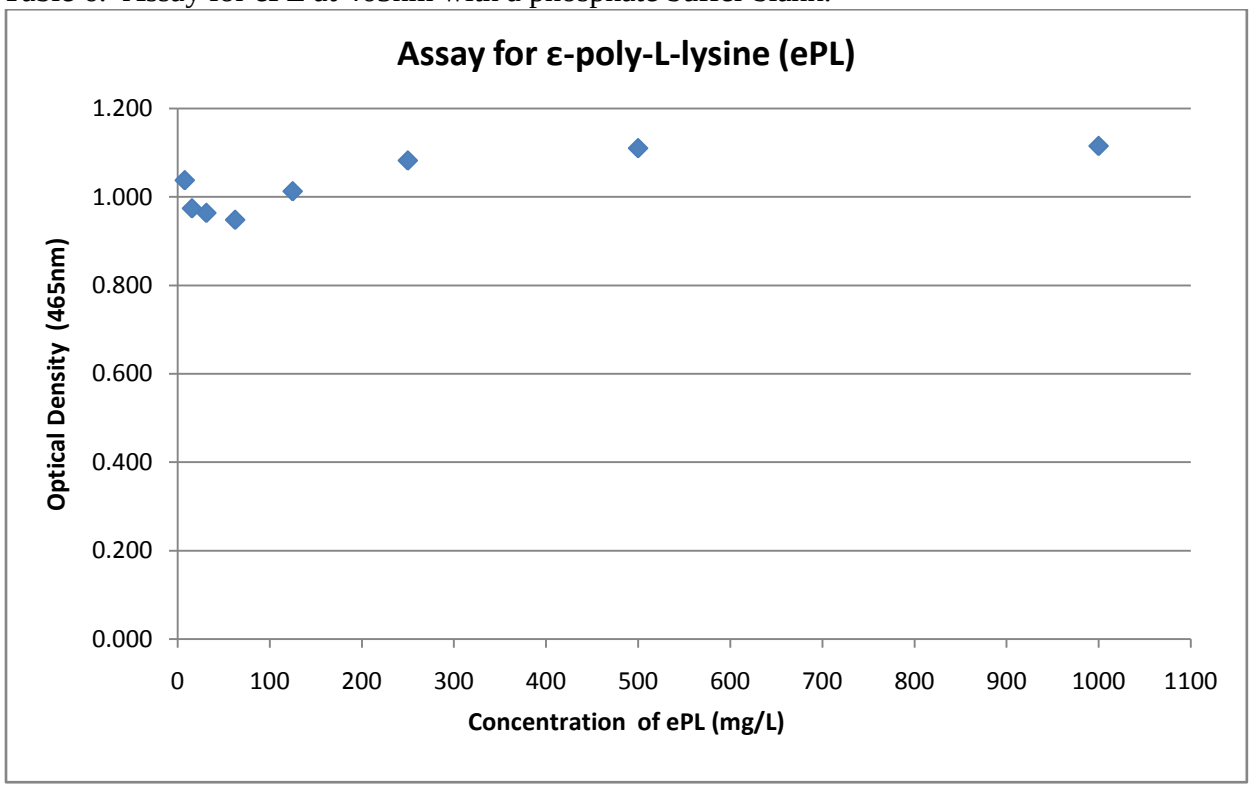

Figure 5. Plot of the average optical density values versus concentration of the resulting supernatant of ε-Poly-L-lysine (see Table 6). From concentrations 62.50 mg/L to 1000.00 mg/L the optical density increases.

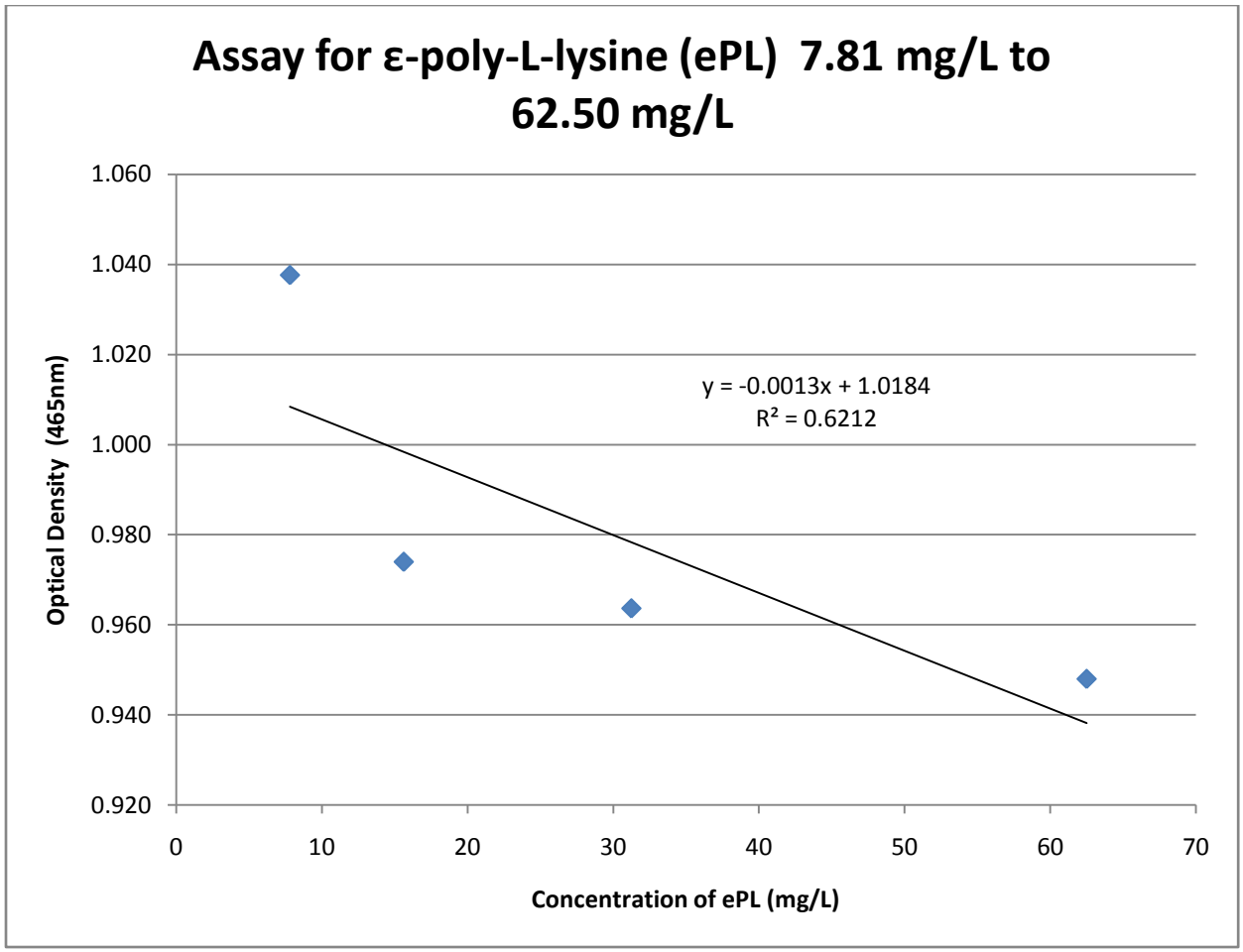

Figure 6. Plot of the average optical density values versus concentration of the resulting supernatant of ε-Poly-L-lysine (see Table 6) from 7.81 mg/L to 62.50 mg/L.

3.4 Method of Itzhaki for poly-L-lysine

The optical density of the resulting supernatant for all concentrations of poly-L-lysine was measured twice, once with a 0.1mM phosphate buffer blank and once with a methyl orange blank (2mL of methyl orange solution with 2mL of phosphate buffer). Poly-L-lysine was used to determine whether the method of Itzhaki would react to other molecules of Lysine and would produce the expected result (higher concentrations of the molecules, lower optical density). Using the procedure described in the materials and methods, the optical densities of poly-L-lysine was measured (see Table 7 and Table 8).

Concentration of poly-L-lysine	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
500.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
250.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
125.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
62.50 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
31.25 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
15.63 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
7.81 mg/L	-0.079	-0.071	-0.078	-0.076	-0.079
Phosphate Buffer + Methyl Orange	-0.002	0.000	-0.003	-0.002	-0.002

Table 7. Assay for poly-L-lysine at 465nm with a methyl orange blank.

Using methyl orange as a blank (see Table 7) meant that most of the optical density values were less than -0.100 since the spectrophotometer could not read values below that. However, when phosphate buffer was used as a blank, the optical density exhibited the expected results, indicating that the method of Itzhaki works for molecules of. At concentrations of greater than 62.50 mg/L of poly-L-lysine the optical density values do not decrease suggesting that the assay for poly-L-lysine levels off at concentrations higher than that.

In Figure 7, the optical densities were plotted versus concentration of poly-L-lysine and the leveling off can be observed for concentrations higher than 62.50 mg/L. In Figure 8, the optical density values were plotted versus concentration of the resulting supernatant of poly-L-lysine (7.81 mg/L to 62.50 mg/L) and demonstrate a straight line curve with an R-value of 0.984.

Concentration of poly-L-lysine	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	0.330	0.327	0.327	0.328	0.001
500.00 mg/L	0.371	0.376	0.377	0.375	0.002
250.00 mg/L	0.370	0.368	0.364	0.367	0.002
125.00 mg/L	0.403	0.399	0.417	0.406	0.005
62.50 mg/L	0.527	0.533	0.534	0.531	0.002
31.25 mg/L	0.768	0.783	0.777	0.776	0.004
15.63 mg/L	0.959	0.964	0.982	0.968	0.007
7.81 mg/L	1.067	1.077	1.069	1.071	0.003
Phosphate Buffer + Methyl Orange	1.148	1.155	1.152	1.152	0.002

Table 8. Assay for poly-L-lysine at 465nm with a phosphate buffer blank.

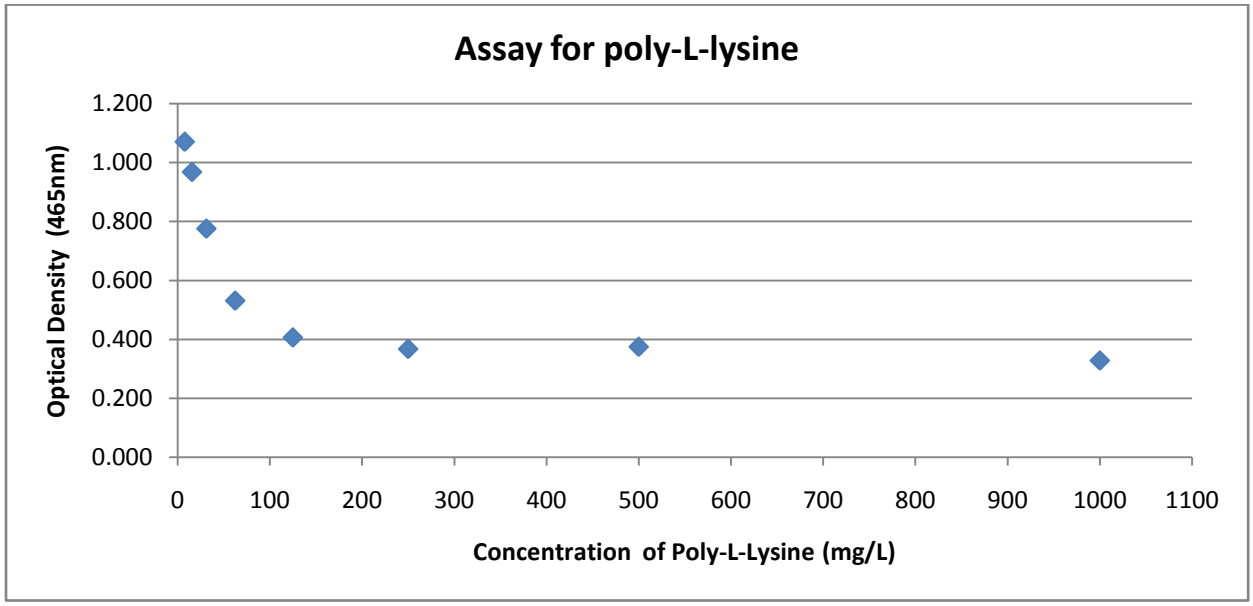

Figure 7. Plot of the average optical density values versus concentration of the resulting supernatant of Poly-L-Lysine (see Table 8). The optical density values level off from 125.00 mg/L to 1000.00 mg/L.

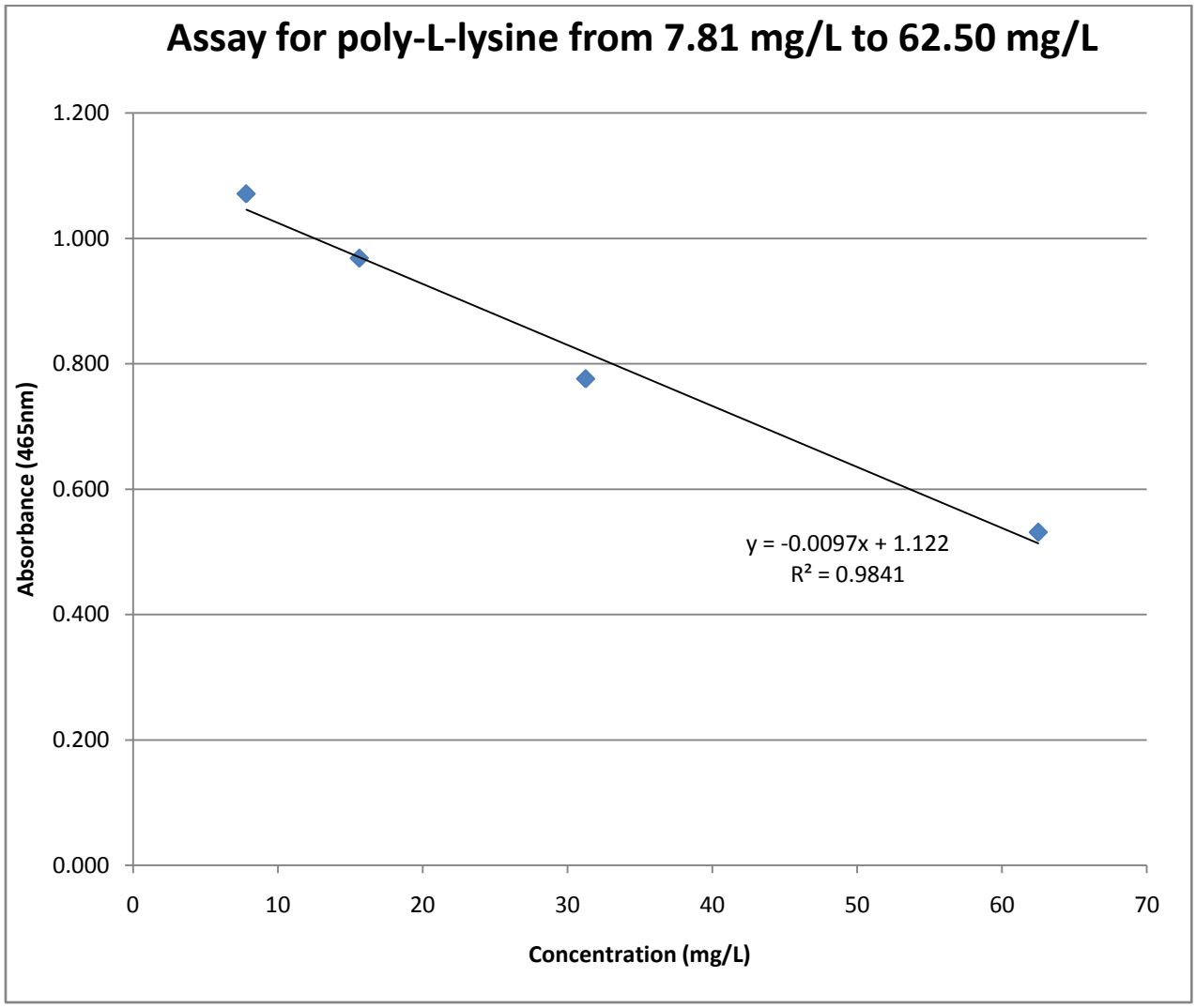

Figure 8. Plot of the average optical density values versus concentration of the resulting supernatant of poly-L-lysine (Only values ranging from 7.81 mg/L to 62.50 mg/L were used, see Table 8).

3.5 Method of Itzhaki for L-lysine

The optical density of the resulting supernatant for all concentrations of L-lysine was measured twice, once with a 0.1 mM phosphate buffer blank and once with a methyl orange blank (2 mL of methyl orange solution with 2 mL of phosphate buffer). Using methyl orange as the blank (see Table 9), meant that most of the optical density values were less than -0.100 since the spectrophotometer could not read values below that.

Concentration of L-lysine	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
500.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
250.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
125.00 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
62.50 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
31.25 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
15.63 mg/L	< -0.100	< -0.100	< -0.100	< -0.100	n/a
7.81 mg/L	-0.079	-0.071	-0.078	-0.076	0.003
Phosphate Buffer + Methyl Orange	-0.002	0.000	-0.003	-0.002	0.001

Table 9. Assay for L-lysine at 465 nm with a methyl orange blank.

When the optical density values for L-lysine were measured against a phosphate buffer blank, the results (see Table 10) again indicate that the optical density values do not change with concentration. In fact, all the optical density values of L-lysine were between the ranges of 1.100-1.200.

Concentration of L-lysine	Trial 1	Trial 2	Trial 3	Average	Standard Error
1000.00 mg/L	1.150	1.165	1.173	1.163	0.007
500.00 mg/L	1.155	1.151	1.159	1.155	0.002
250.00 mg/L	1.170	1.167	1.160	1.166	0.003
125.00 mg/L	1.163	1.166	1.160	1.163	0.002
62.50 mg/L	1.158	1.166	1.160	1.161	0.002
31.25 mg/L	1.164	1.159	1.164	1.162	0.002
15.63 mg/L	1.159	1.157	1.163	1.160	0.002
7.81 mg/L	1.170	1.170	1.161	1.167	0.003
Phosphate Buffer + Methyl Orange	1.159	1.145	1.149	1.151	0.004

Table 10. Assay for L-lysine at 465 nm with a phosphate buffer blank.

To illustrate that the results obtained in Table 10 show no decreasing trend in the data, a plot of optical density values versus concentration of L-lysine was drawn. The

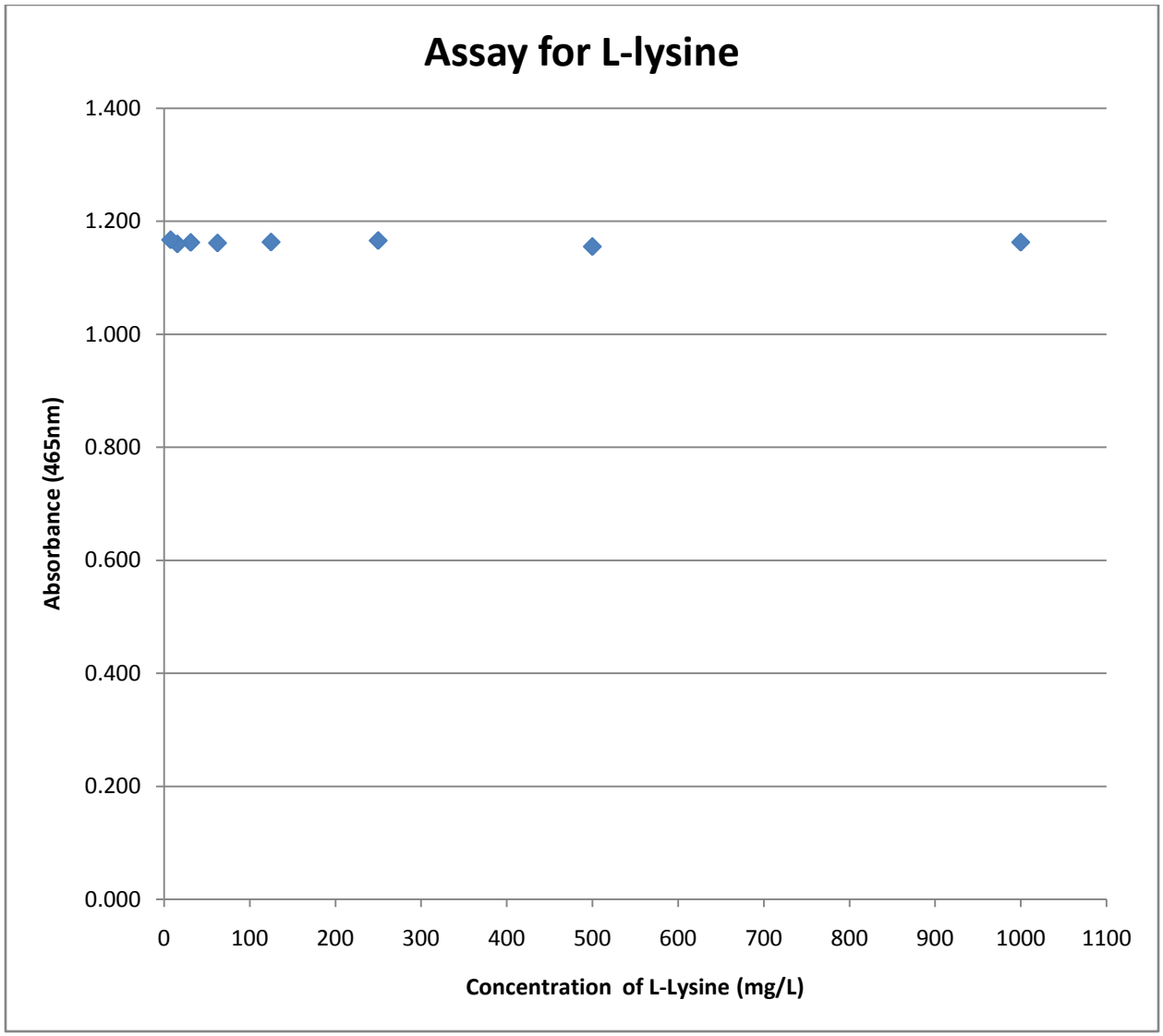

Figure 9. Plot of the average optical density values versus concentration of the resulting supernatant of L-lysine (see Table 10).

3.6 Determination of ePL in *Streptomyces albulus* Routien

Using the procedure described in the materials and methods section, the *Streptomyces albulus* Routien strain was prepared and the supernatant analyzed for ePL or poly-L-lysine molecules. The purpose of this was to see whether the positive control strain would produce any ePL or any poly-L-lysine molecules. From Table 11, it is unclear whether the *Streptomyces albulus* Routien produced any polylysine molecules. Since the data (see Table 6) for the analysis and determination of the ePL powder did not yield any conclusive results it was also not possible to estimate how much ePL the strain may have produced.

	Blank with Phosphate Buffer	**Blank with Phosphate Buffer + Methyl Orange**
Trial 1	1.074	-0.039
Trial 2	1.080	-0.040
Trial 3	1.069	-0.026
Average	1.074	-0.035
Standard Error	0.003	0.005
2 mL Phosphate Buffer + 2 mL Methyl Orange	1.107	0.000

Table 11. Assay for the resulting supernatant produced by the Streptomyces albulus Routien strain using the method of Itzhaki.

DISCUSSION

4.1 Presence of Chromium in Soil Samples

This part of the project sought to establish that chromium was present in the soil samples collected (which would be used in a larger project involving the isolation and fermentation of ε-poly-L-lysine producing bacteria or fungi from those soils). According to the U.S. Environmental Protection Agency (EPA), the maximum contaminant level (MCL) for chromium is 0.1 ppm in drinking water (U.S. Environmental Protection Agency, 2006). This means that all the collected soil samples are above the limit set by the EPA (1 ppm = 1 μg/g, see Table 4).

Almost all of the soil samples collected measured at least ten times the set limit of chromium in drinking water. The exception was one of the samples from Paint Shop Pond even though it is reported to have high concentrations of chromium ((U.S. Environmental Protection Agency, 2002). The low amount of chromium detected in the Paint Shop Pond samples could be due to the fact that during the preparation of the soil samples, there happened to be a large quantity of leaves in the soil. This means that in 1g of dry weight of soil, a large amount of leaves was weighed in the preparation process.

The method used to measure the chromium in the soil samples may not have been the best method to use since the protocol was slightly altered to measure soil samples and not groundwater. It would be beneficial to conduct more tests using the diphenylcarbazide method to measure soil samples that are unlikely to contain any heavy metals or chromates.

Since all the soil samples contained chromium, this suggests that the organisms growing in those soils would have been challenged environmentally in the presence of chromium. This would indicate that the organisms have been able to survive in an environment containing chromium, thus may indicate a higher chance of isolating ePL producing bacteria.

4.2 Method of Itzhaki to Detect and Quantify ePL

The method of Itzhaki was tested using three different molecules: ePL, poly-L-lysine, and L-lysine. According to the data for ePL (see Table 6) there appears to be an initial range where the ePL exhibited behavior similar to the poly-L-lysine (a linear range). However, when the optical density values were plotted separately for concentrations ranging from 7.81 mg/L to 62.50 mg/L the line obtained had an R value of 0.621. Therefore, the method of Itzhaki did not appear to work as it should (higher concentrations of the molecules, lower optical densities) for ePL. It is possible that the dextrin in the powder form of ePL (50% ePL and 50% dextrin) received from Chisso Corporation, may have interfered with the assay.

It is suggested that in future experiments, a purification of the sample be carried out prior to using the assay to determine the amount of ePL. Therefore, there was no standard to use to measure the amount of ePL produced by the *Streptomyces albulus* Routien strain and thus it is uncertain whether it can serve as an ePL producing positive control strain. However, the optical density values obtained from performing the assay on the supernatant of the fermentation of that strain suggests that the values were close to baseline (see Table 11). It is possible that this strain of *Streptomyces albulus* is not capable of producing ePL or produces far less than other strains reported.

The method of Itzhaki worked well with molecules of poly-L-lysine, producing a straight line at concentrations of 7.81 mg/L to 62.50 mg/L with an R value of 0.984 (see Table 8). At higher concentrations (above 62.50 mg/L) the optical density values reached a leveling off point. This suggests that for poly-L-lysine, the assay is out of the linear range at concentrations above 62.50 mg/L (see Figure 8).

For L-lysine, the methyl orange dye did not seem to work for L-lysine molecules, indicating that the method of Itzhaki does not work for this molecule (see Table 10). The optical density values for L-lysine did not change according to the different concentrations, rather all the values seemed to stay within the optical density range of 1.100-1.200. For the purposes of this project, this is a good indication that he molecules of interest (ePL and/or polymers) will work with the method of Itzhaki and that monomers like L-lysine will not.

4.3 Future Directions

The method of Itzhaki may work better if the molecules were purified before the assay was performed. Purification of the ePL powder to remove the dextrin may help yield results that should follow the expected trend (higher concentrations of the molecules, lower optical densities) as opposed to simply measuring the resulting supernatant as described in the materials and methods.

The *Streptomyces albulus* strain obtained from the ATCC, may not be an ePL producing bacteria and thus produced the results observed in Table11. It is suggested that *Streptomyces albulus* Strain 410 (Kahar et al., 2001) be obtained since it has been recorded to produce ePL for further tests to use as a control. *Streptomyces albulus* Strain 346 is a mutant strain that can also be used and has been reported to produce high quantities of ePL (Chisso Corporation, 2007).

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