Spring 1999

Separation of L-lysine by Ion-exchange Chromatography

Jennifer Jean Zurawick

University of Tennessee - Knoxville

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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

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College: Engineering
Department: Chemical Engineering

Faculty Mentor: Dr. Paul Frymier

PROJECT TITLE: Separation of L-lysine by Ion-exchange Chromatography

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: __________________________, Faculty Mentor

Date: 5/10/99

Comments (Optional):
Separation of L-Lysine by Ion-Exchange Chromatography

Senior Honors Project
May 10, 1999

Jennifer Zurawick
Faculty Mentor: Dr. Paul Frymier
Abstract

Since the biochemical industry is a new area of growth for chemical engineering graduates, the University of Tennessee is broadening its curriculum to include bioprocesses in the unit operations laboratory. The production and purification of L-lysine is an important industrial process and has been chosen as the bioprocess lab experiment. A senior lab group is running the bioreactor to produce L-lysine by fermentation of the bacteria Corynebacterium glutamicum. The lysine produced in the reactor will be purified by ion-exchange chromatography. The lysine will bind to the negatively charged column and release from the column when a buffer changes the pH of the column, changing the charge on lysine to a neutral charge. After the purified lysine is collected, a reaction catalyzed by saccharopine dehydrogenase will be used to quantify the yield of lysine from the reactor according to absorbance changes in the solution as the lysine reacts.

In order to add the bioseparations process to the current lab curriculum, experiments were conducted to determine the operating conditions of the column and to achieve an effective purification of lysine. The column bed volume, capacity, and resin, as well as the buffer solutions for an effective purification of lysine must be determined. Pump flow rates, loading times, and sample collection times and intervals will also be critical to the operation of the chromatography column. The approach to determining these conditions involved gaining a general understanding of ion-exchange chromatography and running trial and error experiments. This approach was found to be time consuming and resulted in no favorable results. However, ideas to improve this method were developed and future work should proceed at a faster rate.
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Introduction

The biochemical industry is a new area of growth for chemical engineering graduates. In order to prepare students for careers in this industry, the University of Tennessee is broadening its curriculum to include bioprocesses in the unit operations laboratory. Important units of the typical bioprocess include fermentation and bioseparations. The production and purification of L-lysine is an important industrial process and has been chosen as the bioprocess lab experiment.

The bacteria Corynebacterium glutamicum produces L-lysine by fermentation in a bioreactor. To include bioseparations in the lab, an ion-exchange chromatography column will be utilized to purify the lysine produced by the bacteria. The lysine will bind to the negatively charged column and release from the column when a buffer changes the pH of the column, changing the charge on lysine to a neutral charge. After the purified lysine is collected, a reaction catalyzed by saccharopine dehydrogenase will be used to quantify the yield of lysine from the reactor according to absorbance changes in the solution as the lysine reacts.

Dr. David Shonnard and Dr. David Odde at Michigan Technological University have researched the separation of lysine by ion-exchange chromatography. Their method has not been successful. However, this proposed experimental method will be evaluated and improved at the University of Tennessee so that the experiment can be introduced to the senior chemical engineering lab course. The focus of this project is to determine experimental parameters for the bioseparations lab that result in a successful separation of lysine.
Background

Chromatography Principles

Ion-exchange chromatography is a powerful separation method capable of separating components with only minor differences in properties. It depends on the reversible adsorption of a charged molecule in a mobile phase to a stationary substance with the opposite charge. The ionic strength and pH of the mobile phase entering the system control the adsorption of molecules to the stationary phase. This allows selective desorption of molecules according to ionic strength.

In an ion-exchange process, starting conditions are chosen so that the desired solute molecules will bind to the column. The desired solute must have the charge opposite to that of the stationary substance. These solute molecules will bind to the stationary substance or resin in the column while the remaining solution will pass through the column. The desired solute molecules are removed by changing the ionic strength or pH of the entering solution. The change in pH or ionic strength causes the charge of the solute molecule to become neutral. The neutral charge of the molecule prevents it from binding to the stationary phase of the column so the molecule is released from the column. The column resin will release molecules according to their binding strengths; weaker binding molecules will desorb before molecules of stronger binding strength. The resin is regenerated by washing the column with a solution to remove the solute molecules and replace the charged solute particles with the counter-ions originally present in the resin.

The total capacity of the resin is the number of charged ionic substituent groups per milliliter of expanded resin. The available capacity of the resin is the actual amount
of charged groups that can bind to an ion exchanger under a specific set of experimental conditions. Experimental conditions that affect resin capacity include temperature, buffer strength, pH, nature of counter-ions, and process flow rate. Properties of the substance to be separated, such as molecular weight, charge, and pH sensitivity, also affect the capacity of the resin.

**Lysine Properties**

L-lysine is a basic amino acid often used as a food additive. Most synthetic methods of lysine production result in formation of the “D” configuration of lysine or of a mixture of the “D” and “L” forms. Bacteria fermentation is a method of lysine production that results in only the favorable “L” configuration. The structure of L-lysine is shown as Figure 1.

![Figure 1: Structure of L-lysine](image)

Three hydrogen ions dissociate from the amino acid as pH changes. The hydrogen ion will dissociate when the pH reaches the pKa value of the ion. The pKa values for the lysine hydrogen ions are shown in Table 1.
At pH values lower than two, lysine has a charge of +2. However, as pH increases, hydrogen ions dissociate according to their pKa values, and the charge of the lysine decreases to −1 at a pH above 10.53. The charge of lysine can be determined by a pH curve shown in Figure 3. The pI of an amino acid is defined as the pH at which the amino acid has a net neutral charge. For lysine this occurs at the pH that is equal to half the distance between the second and third pKa values and has a value of 9.74. This can be seen by the pH curve and is calculated by the following equation:

\[
pI = \frac{(pK\alpha_2 + pK\alpha_3)}{2}
\]

Theoretically, lysine should bind to a cation exchange chromatography column at any pH below 9.74 and should begin to desorb from the column when the high pH buffer causes the pH to reach this value. Since lysine has a very high pI value and requires a very high pH buffer to be removed from the chromatography column, other amino acids in a mixture should leave the column long before lysine and this method of separation should be very effective.
Figure 2: Lysine pH vs. Ionic Charge

- pKa1
- pKa2
- pKa3
Quantification of Lysine

The processes available to determine the amount of lysine produced in a fermentation process are tedious and time consuming. One process for the quantification of lysine is the following reaction catalyzed by saccharopine dehydrogenase.

\[
\text{lysine} + \alpha\text{-ketoglutarate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{Saccharopine Dehydrogenase}} \text{saccharopine} + \text{NAD}^+ + \text{H}_2\text{O}
\]

(Nakatani, 1972)

Since NADH absorbs light at a wavelength of 340 nanometers, the concentration of lysine can be determined by measuring the disappearance of NADH during the reaction.
Experimental Method

Protein Separation

The first experiment performed in the lab involved the separation of anion exchange protein standards according to the procedures listed in the instruction manual for the BioLogic LP Starter Kit. The purpose of this experiment was to gain an understanding of the BioLogic equipment and its capabilities for ion-exchange chromatography. The fraction collector and the main chromatography system were programmed for the desired separation and the resulting data recorded by the Data View Program matched the profile in the Starter Kit.

Previous Lysine Experimentation

Dr. David Odde at Michigan Technological University is presently researching a procedure for the separation of lysine by ion exchange chromatography. His previous work was used as an initial method of separation to be modified and improved after a "clean run" was achieved in this lab. Three lysine separation runs were conducted this semester. All three procedures used the same concentrations of solutions. First, 0.4M lysine was fed to the column. Then a neutral wash, which consisted of a 0.01M K₂HPO₄, 0.15M NaCl solution, was fed to release any excess lysine that did not bind to the column or any other impurities. Feeding of the low pH buffer and then the high pH buffer followed this step. Buffers consisted of potassium tetraborate tetrahydrate solutions of pH=10.2 and pH=11.5. The final step regenerated the resin by feeding the neutral wash to the column to wash the buffers from the column.
Column Resin

The Dianion SK1B resin was chosen for the first two runs of the lysine purification process because it is a cation exchanger that is readily available in the lab. The capacity of the resin was determined by the given capacity of 1.9 meq/mL for the resin and a calculation of the bed volume of the column (Sigma, 1996). The column was packed with the resin and filled with water to keep the resin wet. In an effort to duplicate the method suggested by Dr. David Odde, the Sephadex C-25 resin was ordered and used to pack a column for another run of the lysine purification process. The capacity of this resin is 480 mmol/L (Odde, 1999).

Chromatography Parameters

After the resin capacity had been determined, the flow rates and volumes of lysine, buffers, and wash were determined. A flow rate of 5 mL/min was chosen for the pump. The volume of lysine fed to the column was determined by the capacity of the column. This volume determined the volumes of neutral wash and buffers fed to the system to elute the lysine from the column. Times required to feed the calculated volumes of solutions were determined and programmed into the Biologic LP Chromatography System and the desired fraction collection times were programmed into the BioRad Model 2128 Fraction Collector. A summary of the loading times is located in Table 2. The chromatography process was run and the samples were collected and frozen for a lysine assay analysis at a later date.

For the first run of the lysine purification process, samples were collected only after the addition of the high pH buffer. In the second run of the process, samples were
collected from the last minute of the first wash step to the end of the experiment. The waste solution collected from the start of the experiment was also frozen for analysis.

<table>
<thead>
<tr>
<th>Solution Loaded</th>
<th>Run 1 Loading Times (min)</th>
<th>Run 2 Loading Times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Neutral Wash</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Low pH Buffer</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>High pH Buffer</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Neutral Wash</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 2: Solution Loading Times**

**Lysine Assay**

To quantify the amount of lysine purified by the chromatography separation, the saccharopine dehydrogenase reaction was used. Quantifying the amount of lysine in each fraction collected by the fraction collector also revealed the time the lysine desorbed from the column. NADH and α-ketoglutarate solutions were made according to the concentrations in Table 3, determined by the senior lab group conducting the fermentation process. A 0.1 molar phosphate buffer was used to prepare these samples.

The reaction was prepared by mixing a sample from each fraction collected with both reactants. A control sample was prepared by adding buffer in the place of the sample fraction. Volumes added to the cuvettes are recorded in Table 4. The enzyme was added to the cuvette and the initial absorbance at 340 nm. was read in the Beckman DU 520 Spectrophotometer. This absorbance was recorded for each cuvette as the initial absorbance of NADH in the cuvette. After the reaction had proceeded 30 minutes, a second absorbance reading was taken as the final absorbance of unreacted NADH. The
changes in absorbance of the sample fractions were compared to the change in absorbance of the controlled buffer. The differences were recorded and compared to a calibration curve developed by the senior lab group to correlate the change in absorbance to lysine concentration.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mass (g)</th>
<th>Volume Buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-ketoglutarate</td>
<td>0.0572</td>
<td>4</td>
</tr>
<tr>
<td>NADH</td>
<td>0.011</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3: Assay Solution Concentrations

<table>
<thead>
<tr>
<th>Substance</th>
<th>Volume (microliters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Fraction (or buffer)</td>
<td>100</td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td>100</td>
</tr>
<tr>
<td>NADH</td>
<td>100</td>
</tr>
<tr>
<td>Buffer (0.1M phosphate)</td>
<td>2,000</td>
</tr>
</tbody>
</table>

Table 4: Assay Reaction Components
Results and Discussion

No significant amounts of lysine were found in the samples from the first lysine purification process by the saccharopine assay. In this experiment, only samples leaving the column after the high pH buffer was added were saved for analysis. Since the low pH buffer (pH=10.2) has a higher pH than the pI for lysine, it was hypothesized that the lysine was eluting the column when the first low pH buffer was added to the column. Thus, fractions were collected after the first buffer was added on the second trial run. However, no significant amounts of lysine were found in any of the samples from this experiment. Part of the “waste” or solution that left the column before the first buffer was added (during the lysine loading and neutral wash) was frozen and analyzed for lysine. This sample did have a significant amount of lysine corresponding to 0.1M lysine in approximately 100 mL of solution.

The results of the second lysine purification show that the lysine was not binding to the column. This could be due to an ineffective resin or to the conditions of the process, such as the starting pH or ions present in the neutral wash. The interaction of the ions of the neutral wash or the water present in the column before loading lysine can be significant in determining the degree of separation of a mixture of amino acids in high performance liquid chromatography (HPLC) (Hancock, 1984). These same principles apply to low-pressure ion-exchange chromatography. If the negatively charged phosphate ions in the neutral wash bind to the lysine better than the column binds lysine, these interactions could cause the lysine to leave the column.

In order to eliminate the possibility of an ineffective resin causing the column to not bind lysine, the Sephadex C-25 resin suggested by Dr. David Odde was utilized for
the third lysine purification experiment. However, the small size of the resin prevented
the flow of fluids through the column at atmospheric pressure. This resin would be
beneficial for HPLC, but is not effective for our experimental methods and equipment.
Conclusions

Although the results of these experiments were very discouraging, much was learned about the chromatography equipment and many ideas for future work were evident. Dr. Paul Frymier suggested that the resins be tested with a lysine solution before using the chromatography column. This would allow for a quick determination of the effectiveness of the resin in binding L-lysine.

When an effective resin useful for low-pressure chromatography is found, research can begin on other chromatography elements. The ionic interactions of the neutral wash and the effectiveness of the buffers can be analyzed by running the ion-exchange experiments already performed.

After an effective resin is discovered and a “clean run” of the lysine purification process is made, modifications can be made to optimize the performance of the column and to minimize the safety hazards of the buffers. If the low pH buffer removes lysine, the pH values of both buffers can be reduced and a new buffer system would be possible. Biological buffers that can operate at a high pH should be as effective as the tetraborate buffers and much safer. Since lysine is produced as a food additive, the process using the tetraborate buffers would not be possible for industrial use.
Literature Cited


Odde, David. Lab Instructions Fax. 2 February 1999.


*Sigma Chemical Company Biochemicals, Organic Compounds, and Diagnostic Reagents.* Sigma-Aldrich Corporation. 1996.

Appendices
Appendix A: Lab Notebook Pages

Lysine Separation

Bed volume of Qionen 5K16 resin in column:
Capacity: 1.9 meq/mL or 3.6 meq/g (Sigma Chemical Co)

\( h = 12.8 \text{ cm} \times d = 1 \text{ cm} \)
\[ A = \pi r^2 h = \pi (0.5)^2 (12.8) = 10.1 \text{ cm}^3 = 10.1 \text{ mL} \]

Capacity = 1.9 meq/mL (10.1 mL) = 19.2 meq lysine

If 1 meq = 1 mmol

19.2 mmol lysine \( \left( \frac{\text{mL}}{0.4 \text{ mmol}} \right) = 48 \text{ mL} \)

For 5 mL/min Flow rate:

48 mL \( \left( \frac{\text{mm}}{5 \text{ mL}} \right) = 9.6 \text{ mm} \) to load lysine on column

L-lysine monohydrochloride MW 182

\( 0.4 \text{ mol} \left( \frac{\text{L}}{0.1 \text{L}} \right) = 0.4 \text{ mol (182 g)} = 7.28 \text{ g for 100 mL soln} \)

Phosphate buffered saline 0.01 M K₂HPO₄ 0.15 M NaCl

Phosphate \( \frac{0.4 \text{ mol}}{L} \left( \frac{5}{0.1 \text{L}} \right) (174.29 \text{ g}) = 0.00384 \text{ g K₂HPO₄ for 500 mL soln} \)

NaCl \( \frac{0.15 \text{ mol}}{L} \left( \frac{58.44 \text{ g}}{\text{mol}} \right) = 4.35 \text{ g NaCl for 500 mL soln} \)
NAD - amo. weighted = 4.35 g
Kad + l
d = 0.8456 ng
Lysine - amo. weighted = 11.24 g

<table>
<thead>
<tr>
<th>Times</th>
<th>Lysine (C)</th>
<th>PBS (D)</th>
<th>B1 (A)</th>
<th>B2 (B)</th>
<th>PBS (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>7 mm</td>
<td>9 mm</td>
<td></td>
</tr>
</tbody>
</table>

Sample Time:
5 min \( x \) = 4 mL
\( x = 0.8 \) mm

Sample Collection:
T = 16 - 17 mm
T = 17 - 24 mm
T = 24 - 31 mm
Lysine Assay - 1st Run

4/16

\[
\text{mass (g)} \quad \text{Vd buffer (mL)}
\]
\[
\alpha KG \quad 0.0572 \quad 4
\]
\[
\text{NADH} \quad 0.011 \quad 4
\]

actual weights

\[
\alpha KG = 0.0574 \text{ g}
\]
\[
\text{NADH} = 0.0110 \text{ g}
\]

100 mL NADH, \( \alpha KG \), sample

200 mL buffer

blank - use buffer instead of sample

initial reading

add 100 mL enzyme

reading after 30 min.

\( \text{Dabs sample - Dabs blank} \)

Reaction

\[
\text{Lys} + \text{NADH} + \alpha KG \rightarrow \text{SACR} + \text{NAD}^+
\]

absorb

no absorption

4/16 - samples were taken only after high pH buffer was added - lys. conc. was much lower than expected so an assay was not run
PBS wash - Samples 1-2
B1 samples 3-11
T = 39 min
T = 24

Dilution: 10 times

A = Samples 3-11 (1/10 each) (50uL sample + 900uL buffer)
B = samples 1-2
C = C1
D = C11

Need to dilute more (100 times)

B: B - samples 5-6, 10uL each + 1300uL buffer

0.590 abs

Dilution: 50 times - all samples (Sample 2 - no dilution

300uL each sample (60uL total sample volume)

2940uL buffer

<table>
<thead>
<tr>
<th></th>
<th>Init.</th>
<th>%0</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sample</td>
<td>0.694</td>
<td></td>
<td>0.663</td>
</tr>
<tr>
<td>A so</td>
<td>0.682</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
<td>B so</td>
<td>0.714</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
<td>C so</td>
<td>0.705</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
<td>D so</td>
<td>0.695</td>
<td>0.446</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.699</td>
<td>0.535</td>
<td></td>
</tr>
</tbody>
</table>

1.035
Read sample from Pin Frymer at 1:40 → 0.382 0.054

Air gas 0.0583g

Sample: E = 10mL of sample 17.218 mL 990mL buffer
W = 10mL water 4 990mL buffer

<table>
<thead>
<tr>
<th>No Sample</th>
<th>T=0</th>
<th>T=30min</th>
<th>∆</th>
<th>∆-∆blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.724</td>
<td>0.204</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>FeCl₃</td>
<td>0.732</td>
<td>0.479</td>
<td>0.253</td>
<td>0.228</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>0.733</td>
<td>0.673</td>
<td>0.06</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Water is near 0.001 M. undiluted = 0.1 M
Sephadex resin - new column
0.9 x 30 cm column

\[ V = \frac{\pi r^2 h}{\pi} = \frac{\pi}{\pi} \left( \frac{0.2}{2} \right)^2 (15) = 0.365 \text{ h cm}^3 = \text{mL} \]

h = height of packing = 15 cm  V = 5.78 cm^3 = 5.78 mL

0.73516 g lysine for 100 mL soln.

50 mL x 0.14 mL (22/1490 minutes) = 0.4 mL - bid volume

Vol. Lysine Column can hold

\[ \frac{450 \text{ mmol}}{\text{L}} \left( \frac{1 \text{ mL}}{1000 \text{ mmol}} \right) \left( \frac{578 \text{ mL}}{1 \text{ L}} \right) \left( \frac{0.001 \text{ L}}{\text{mL}} \right) \left( \frac{1000 \text{ mL}}{1 \text{ L}} \right) = 6.944 \text{ mL} \]

7 mL lysine \( \left( \frac{1 \text{ mL}}{5 \text{ mL}} \right) \) = 1.4 mL

15 mL wash = 3 min
15 mL B1 "
15 mL B2 "
20 mL wash 4 min

Column not draining fast enough for flow rate

7 mL lysine 9 mm
15 mm other steps
Samples 12-01-20

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Diluted 100 times</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>15</td>
<td>18</td>
<td>14</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>18</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.011 g NADH needed

Actual weight = 0.01100 g

340 nm

<table>
<thead>
<tr>
<th></th>
<th>T=0</th>
<th>T=2</th>
<th>T=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.924</td>
<td>0.868</td>
<td>0.875</td>
</tr>
<tr>
<td>A</td>
<td>0.995</td>
<td>0.944</td>
<td>0.880</td>
</tr>
<tr>
<td>B</td>
<td>0.928</td>
<td>0.861</td>
<td>0.836</td>
</tr>
<tr>
<td>C</td>
<td>0.892</td>
<td>0.844</td>
<td>0.816</td>
</tr>
<tr>
<td>D</td>
<td>0.891</td>
<td>0.844</td>
<td>0.821</td>
</tr>
<tr>
<td>E</td>
<td>0.892</td>
<td>0.853</td>
<td>0.831</td>
</tr>
</tbody>
</table>
TO: Paul Frymier  
Chem Eng. Dept.  
Univ. of Tenn., Knoxville  
Phone: 423-974-7076  
Fax: 423-974-7076

FROM: D. Shonnard  
Phone: (906) 487-  
Fax: (906) 487-3213

MESSAGE: Paul,  
proposal is enclosed. Missing pages are just administrative junk.  

Good luck!

[Signature]
course, 20% offered a biochemical engineering course with a laboratory component, and 32% had adopted a designed biochemical engineering curricula (Monbouquette, 1995). One of these programs, University of California at Davis (UCD), recently created a new undergraduate major titled "Chemical/Biochemical Engineering" which combines fundamental education in chemical engineering with key coursework in the life sciences and specialized applications in bioprocess engineering (McDonald, 1994). Three new courses were developed in the chemical engineering department at UCD for this major covering the fundamentals of biochemical engineering, bioseparations, and a bioprocess engineering laboratory. Seventeen lecture or laboratory credits related to biotechnology in addition to these three courses are required and are offered by other departments on campus. Tri-State University has adopted a biochemical engineering lecture/laboratory course featuring a term-long, project-oriented laboratory approach with four separate experiments (Hooker, 1994). This approach overcame the time constraints of long duration biochemical engineering experiments which are difficult to fit into the usual 1-3 hour laboratory periods. At Cornell University, a bioreactor experiment was incorporated into the existing senior-year unit operations laboratory in such a way that students without previous biology coursework experience could participate and the laboratory experiments could be completed in the normal three-hour class period (Shuler et al., 1994).

2.2 Biochemical Engineering Option. Before describing the proposed experiments in more detail, we first present a set of elective courses designed to prepare chemical engineering students for careers in biotechnology and bioprocess engineering. This set of courses is called the "biochemical engineering option". The concept of an option within the curriculum of chemical engineering is an extension to the curricular ideas expressed by D.R. Woods of McMaster University who argued that the educational experience is derived first from the required fundamental topics and then augmented with elective courses based on faculty expertise (Woods, 1992). In addition to providing an effective means of providing this experience, an option can provide students with official recognition of their achievement in a subdiscipline of chemical engineering, without undermining the requirements of the degree itself. Therefore, we are
2. NARRATIVE
2.2 Current Situation

2.2.1 Michigan Technological University. Michigan Tech began in 1885 as the Michigan School of Mines. It was established with a charter which directs its vision to support the industries of Michigan and the upper midwest. As the industries of Michigan developed and expanded into manufacturing and chemical production, so did the mission and capability of the university. By the 1960's the university was renamed Michigan Technological University (MTU) to reflect the diversity of engineering and technology programs present on campus. Today, Michigan Tech is the 12th largest engineering school based on undergraduate enrollment. It is in the enrollment of women in engineering, and features strong graduate programs (M.S. and Ph.D.) in all engineering and science fields.

2.2.2 Department of Chemical Engineering. Approximately 70% of the university's 6,300 students are in engineering disciplines. The Chemical Engineering program has nearly 500 majors and graduates over 100 students annually, ranking it 10th nationally in number of undergraduates. With strong programs in process design, advanced control, and process safety, and with particularly strong backgrounds in communications and pilot-scale laboratory facilities, graduates of the Chemical Engineering program are well equipped to participate in a global engineering workforce. The department has recently made a new commitment to expanding its efforts into the growing biochemical arena by the addition of Drs. David Shonnard and David Odde, both with expertise in biochemical engineering. Correspondingly, the department is developing its biochemical engineering emphasis with CM450, Biochemical Processes, (3 cr, quarter system: taught by Dr. David Shonnard) offered annually and a new course CM495, Engineering Analysis of the Cell, (3 cr; taught by Dr. David Odde) which was well-received and will be offered annually. A fundamental vision of the department is to implement curricula and develop facilities which provide a stronger transition from the classroom to the workplace. The Process Simulation and Control Center (PSCC) facility, a key feature of the senior Chemical Engineering Laboratory course sequence (CM401, CM402, and CM403), is intended to do this. Integrating advanced instrumentation and control equipment into full pilot scale production facilities the PSCC provides
a real-life environment in which students learn not only chemical engineering fundamentals, but also safe operating procedures, operation of state-of-the-art process equipment, and learn to deal first-hand with production problems. The PSCC facilities were designed and constructed with over $1.3 million in resources from industry and foundations. In addition to these multi-million dollar facilities, the Subsurface Remediation Laboratory (SRL) expands laboratory and course experiences into the Bioremediation area. The SRL was designed and constructed primarily with university and NSF funds.

2.3 Curricular Need. Historically, chemical engineers have been employed in the chemical and petroleum processing areas. However, the percentage of new chemical engineers in these sectors has steadily decreased. According to a recent survey conducted by AIChe, the percentage of chemical engineers initially employed in the chemical and fuels area decreased from 65% in 1991 to 39% in 1995, while the combined percentage in the food/consumer, biotechnology, pulp&paper, and other industry areas jumped from 21% to 32% over the same period (Graham, 1996). To prepare chemical engineering graduates for the diverse industrial markets of the future, chemical engineering education must broaden its curriculum to include industrial sectors beyond the traditional ones. In response to this need, we propose an integrated pair of senior-year laboratory experiences in support of a biochemical engineering option within the Department of Chemical Engineering at Michigan Technological University. This biochemical engineering option is a proposed set of elective courses to be offered by the Department of Chemical Engineering and the Department of Biology at MTU and is intended to prepare graduates for careers in bioprocess industries.

2.b Development Plan
2.b.1 Previous Biochemical Engineering Curriculum Development. To place our proposal in proper context and highlight the intended improvements, we briefly review recent biochemical engineering curricular initiatives at other chemical engineering departments which are relevant to our initiative. A recent survey of U.S. chemical engineering departments showed that, of those who responded (77% response rate), 71% offered at least one biochemical engineering
developing a biochemical engineering option within chemical engineering which is comprised of a set of free elective courses. These courses are

<table>
<thead>
<tr>
<th>Course Code</th>
<th>Course Title</th>
<th>Credits</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL104</td>
<td>Principles of Biology</td>
<td>4 credits</td>
</tr>
<tr>
<td>BL321</td>
<td>Microbiology</td>
<td>4 credits</td>
</tr>
<tr>
<td>BL401</td>
<td>Biochemistry I</td>
<td>4 credits</td>
</tr>
<tr>
<td>CM450</td>
<td>Biochemical Processes</td>
<td>3 credits</td>
</tr>
<tr>
<td>CM451(proposed)</td>
<td>Bioseparations</td>
<td>3 credits</td>
</tr>
<tr>
<td>CM452*</td>
<td>Engineering Analysis of the Cell</td>
<td>3 credits</td>
</tr>
</tbody>
</table>

*currently offered under CM495, "Current Topics in Chemical Engineering"

In addition to enrolling in the courses listed above, students participating in the option will be required to complete the proposed fermentation and the bioseparation experiments discussed in this proposal. BL104 is an introductory biology course for nonmajors and BL321 is an introduction to the principles and techniques involved in studying microorganisms. Both courses are taught by faculty in the Biology Department. BL401 presents an introduction to important classes of biomolecules and fundamental elements of enzyme kinetics and cellular metabolism. This material is the foundation for CM450 which introduces fundamental engineering analysis of fermentation systems and industrial applications of fermentation technologies. The proposed CM451 course will introduce bioseparations and purification of fermentation products. Finally, Engineering Analysis of the Cell (CM452) will provide training in the mathematical modelling of cellular and molecular processes. Together, these courses will only require 3-6 credits beyond the existing requirements for a degree in chemical engineering due to elective flexibility in the curriculum. As proposed, this list of courses for the biochemical engineering option will present a logical sequence of biological sciences and engineering fundamentals for the production and purification of industrially important biomolecules derived from fermentation processes.

The biochemical engineering option will accomplish several important goals in preparing chemical engineering students for careers in biochemical engineering. First, through their technical content, these courses will serve to prepare chemical engineering students for entry-level positions in the biotechnology industry in the areas of the design, operation, and control of bioprocesses. Second, the option will prepare students for interaction between chemical engineering and microbiology faculty and students (especially in BL321). Third, students completing the option
courses and laboratory requirements will be much better prepared to pursue chemical engineering graduate programs in departments offering biochemical engineering.

2.b.3 Proposed Enhancements. To effectively complement the biochemical engineering courses outlined above and meet the need for hands-on laboratory experience in bioprocessing fundamentals, we propose an integrated set of bioprocess engineering experiments within the existing senior-year Chemical Engineering Laboratory course sequence (CM401, CM402, CM403). Two new experiments will be based on an important industrial bioprocess for the production and purification of L-lysine, an essential amino acid found only in low concentrations in plant protein (making it a widely used livestock feed supplement). These experiments will complement the lecture material presented in CM450, CM451, and CM452 which will be offered to senior-level chemical engineering majors. The experiments will also broaden the current traditional technological applications within the Chemical Engineering Laboratory to include fermentation and bioseparation unit operations. In addition, the data obtained by students will be archived using the existing networked PSCC facilities for complete integration into our student computing network.

2.b.4 Fermentation and Bioseparation Experiments. To develop an effective biochemical engineering laboratory, we established three primary objectives. First, experiments should be integrated such that all important units in a typical bioprocess are present, and the students participate in the conversion of raw materials to fermentation product and through a series of bioseparations to a purified form. Second, the experiments should be integrated into the senior-year Chemical Engineering Laboratory experience so that a common senior-year experience can be assured for all graduates of our department. In addition, this will allow us to make full utilization of the advanced data archival capabilities in the existing process management hardware and software. Third, the proposed experiments should expose the students to new types of separation and reactor technologies not currently found in the Chemical Engineering Laboratory.
Fermentation Experiment. The proposed experiments will be based on production and purification of L-lysine using an auxotrophic strain of *Corynebacterium glutamicum* (Nakayama, 1972; Nakayama et al., 1976). With this organism, a typical fermentation using cane sugar molasses yields 40-50 g lysine/liter with a conversion rate of sugar to lysine of 30-40% (Nakayama, 1972).

A flow diagram of both the lysine fermentation and bioseparation experiments is shown in Appendix 4. The bioreactor assembly will be mounted on a console which allows for automatic control of fermentation temperature, agitation rate, aeration rate, pH, and dissolved oxygen. Personal computer interfacing will allow for process control and data acquisition using a software program purchased with the fermenter. The computer will also be connected to the Chemical Engineering Laboratory data acquisition and control network which will allow for easy sharing and manipulation of the acquired experimental data and for archiving of previous data. Before batch fermentation, the reactor vessel will be filled with mineral growth media and then sterilized in a mobile autoclave. The bioreactor medium will be formulated according to previously published procedures (Crueger and Crueger, 1984). To avoid carmelizing the sugar cane molasses, a concentrated solution of the growth substrate (dissolved in distilled water) will be filter sterilized into the autoclaved vessel before fermentation. A flask-grown inoculum of the bacterium will then be added to the sterile vessel. Fermenter conditions will be a temperature of 28°C, pH of 7.0, duration of fermentation 60-70 hours, impeller speed of 150 rpm, and aeration rate of 0.6 reactor volumes per minute. Several experimental objectives will be assigned to each laboratory group.

Each group will determine the oxygen transfer rate in the fermenter media as a function of several agitation rates, and from these measurements calculate the volumetric mass transfer coefficient for oxygen. Next, they will measure the mass of sugar consumed, the mass of lysine produced, and the ratio of lysine produced to sugar consumed. This ratio is an indication of the efficiency of the fermentation which can have a large impact on the economic viability of the process. The conversion of sugar to lysine is largely controlled by the amount of homoserine added to the medium. This intermediate will be converted to the amino acid threonine and ultimately be incorporated into proteins, enzymes, and new biomass. Threonine also participates in multivalent
feedback inhibition (along with lysine) of the enzyme aspartokinase in the pathway for lysine, methionine, and threonine biosynthesis. Thus, by comparing the homoserine-dependent conversion efficiency of sugar to lysine with previous results stored on the data archival module of the laboratory computer network, each group will be able to assess the impact of feedback inhibition on lysine production.

**Bioseparations Experiment.** The second phase of the bioprocess experiment will be a bioseparation using column chromatography. Typically, industrial processes have used cation exchange chromatography to purify lysine from the product fermentation broth, which exploits the cationic nature of lysine (Azaki and Ozeki, 1992). To recover lysine, students will first clarify the fermentation broth by ultrafiltration through a 0.22 μm filter unit. This will effectively remove the cells while the dissolved biomolecules, including lysine, will pass through the membrane. The clarified broth will then be loaded at neutral pH onto a cation exchange column using a double-reciprocating pumping system. The adsorbed cations, including lysine, will then be displaced by the gradient in either the pH or the ionic strength, both of which will effectively strip the lysine off the column. These gradients will strip the weakest binding molecules first and the strongest binding molecules last and discrete fractions will be collected. Lysine has an isoelectric point of 9.6 so that it will start to desorb and enter the liquid phase when the pH approaches this point. The students will then identify the lysine-containing fractions by using the ninhydrin reaction to react with the primary amines in lysine, thus producing a colored product which can be measured using a spectrophotometer in the visible range. Thus, students will be able to quantitatively measure the amount of amine-containing material recovered. By combining this with an enzyme-based measurement of lysine concentration, the students will measure the purity of the product as well (Nakatani et al., 1972). The new principles that will be taught are the fundamental mechanisms of adsorptive separation processes, which are typically characterized in terms of an isotherm. Students will perform binding experiments prior to running the column to measure the isotherm (specifically the capacity and the affinity of the cation exchange resin) as a function of pH and ionic strength using the ninhydrin assay. This will give them predictive tools to run the column and...
investigate the underlying phenomena and principles involved in column operation. Students can use this information to make predictions regarding the effectiveness of various elution gradients and in turn experimentally assess their effects on product recovery and purity.

To teach these principles, we will use the established approach for the senior Chemical Engineering Laboratory. Specifically, the supervising faculty member provides a group of 4 students with a set of objectives and a packet of background material relevant to the experiment. The students then write a laboratory proposal and "check-in" with the faculty supervisor. After the first laboratory period (the students are allowed to work all day in lab), the students meet with the faculty member for a debriefing. If necessary, the students spend another day in the lab and then summarize their results in a written report. This approach requires students to demonstrate technical skill in integrating previous coursework, communication skills both oral and written, and teamwork skills in effectively carrying out a project. The bioprocessing experiment will be performed in place of two of our traditional laboratories and completed over a single quarter. It will provide a significant enhancement to the students' education by teaching them biochemical processing in a hands-on environment, which will complement the lecture-based curriculum development described above. In addition, it will provide students with both a reactor and a separation laboratory experience, two areas which are currently underrepresented in the curriculum. Preference for access to this equipment and for these experiments will be given to groups whose students are signed-up for the biochemical engineering option. Each group will conduct the fermentation experiment first, save their product solution in a refrigerator, and then perform the bioseparation ion exchange experiment using their stored solution.

2.c. Equipment
2.c.1 Equipment Requested. In this section, the purpose of each piece of equipment will be described and a correlation made between that piece of equipment and specific concepts taught in the new courses proposed for the biochemical engineering option. The equipment in the fermentation section of the proposed experiments will reinforce concepts taught in the CM450 Biochemical Processes course. This course deals with important aspects of bioreactor modeling.
configuration, operation, and application in the bioprocessing industries. The bioreactor equipment will provide hands-on experience in batch fermentation of an important amino acid with additional activities in reactor control, data acquisition, and oxygen transfer. The mobile autoclave equipment will allow the students to practice proper media and equipment sterilization before the fermentation step. In the procedures for the fermentation experiment, students will practice correct sterile technique for the introduction of aeration gases and making other fluid connections. In order to avoid caramelize during autoclaving, a sugar cane solution will need to be passed through a sterilizing filter system. The bioseparation equipment consists of a membrane microfiltration unit for separating cells from the aqueous solution and a chromatography system. This system will include dual double-reciprocating pumps, fraction collector, uv-vis detector, chart recorder, and programmable controller. This system will allow students to learn fundamentals of adsorptive separations, in this case an ion exchange-based bioseparation. In addition, a uv-visible spectrophotometer will be used for analysis of lysine concentration by ninhydrin reaction which will allow students to quantify the yield of the lysine product.

2.c.2 Equipment On Hand for the Project. We have already acquired key pieces of equipment needed to carry out the project: a 5 liter bioreactor and the associated temperature, pH, and dissolved oxygen probes needed for control and monitoring the bioreactor experiment. In addition, we have the biochemical engineering laboratory infrastructure needed to maintain and propagate bacterial cultures including a laminar flow hood, shaker incubator, water-jacketed incubator, pH meter, analytical balance, refrigerator, and freezer. Also, the PSCC facility will provide state-of-the-art capability for data archiving from the bioprocess laboratory. This will allow us to build an on-line database of bioprocess experiments for access by students in their analysis. In addition, it will foster the integration of the laboratory into a completely networked, integrated system that fully utilizes the department’s state-of-the-art PSCC facilities. The department has thoroughly surveyed its laboratory equipment and a summary of the equipment is given in Appendix 1.
2.c.3 Implementation and Equipment Maintenance. The Chemical Engineering Department at Michigan Technological University employs a permanent staff in support of the Chemical Engineering Laboratory whose members are funded on the university general account. Mr. David Caspary is the Manager of Laboratory Facilities. He is in charge of all equipment improvements in the Chemical Engineering Laboratory including upgrades in the laboratory computer network (process control and data acquisition). He has been involved from the beginning of the Process Simulation and Control Center (PSCC) in the Chemical Engineering Laboratory with conceptual design, installation, operation, and maintenance of the experiments. He has also designed and installed equipment in other departmental teaching and research laboratories, such as the new SRL. Mr. Timothy Gasperich is a research associate and is responsible for routine maintenance of the experimental equipment in the Chemical Engineering Laboratory. In the recent past he has helped construct two new pilot experiments in the PSCC and has installed much of the new equipment in the SRL. Mr. Richard Richards is a master machinist with over 40 years experience and is in charge of the chemical sciences machine shop. He is available to construct and repair equipment for departmental research and educational needs. These three individuals have several decades of combined experience in the construction and maintenance of laboratory facilities.

2.d Faculty Expertise. The expertise of the principal investigator, David Odde, is in cellular and molecular bioengineering. Dr. Odde has worked in a number of bioengineering-related areas starting with membrane-based separations and sensor development while at Honeywell, Inc. (Minneapolis, MN). This research, performed while an undergraduate chemical engineering student at the University of Minnesota, resulted in the patenting of a membrane-based dehumidification process. In addition, while at the University of Minnesota, Dr. Odde studied extensively in the biochemical engineering area including the bioseparations course taught by Dr. Edward Cussler. From there he worked for a year in industry developing novel biomaterials for a start-up company, Lai Laboratories, Inc. (Burnsville, MN) after which he entered the graduate Chemical and Biochemical Engineering program at Rutgers University. Working for Dr. Martin
Yarmush, Dr. Odde completed a Master’s thesis entitled “Activity and Stability of an Immobilized Monoclonal Antibody,” an analysis of affinity-based bioseparations. Working for Dr. Helen Buettner, he then completed his Doctoral dissertation entitled “Experimental and Theoretical Investigation of Nerve Growth Mechanisms: Contribution of Microtubule Dynamics,” which investigated the molecular basis of nerve growth. While at Rutgers, Dr. Odde was also a participant in the NIH-sponsored Predoctoral Biotechnology Training Program, a highly interdisciplinary program that educates science and engineering graduate students in a broad range of biotechnologies. Dr. Odde has continued his bioengineering research at Michigan Tech and last year taught a new course entitled “Engineering Analysis of the Cell,” which was well-received (student rating 4.5 out of 5.0) and attracted a broad range of science and engineering students.

Dr. Shonnard’s teaching and research interests are in the areas of biochemical engineering (CM450 Biochemical Processes), subsurface remediation (CM/GE496 Fundamentals of Subsurface Remediation and CM/GE497 Subsurface Remediation Laboratory), and environmental transport and impacts of pollutants (CM498 Pollution Assessment and Prevention). He has helped develop new multidisciplinary lecture and laboratory courses as part of a NSF project (see prior NSF results), has a publication in press on initial curricular development results, and has presented on this activity at several meetings and at an international workshop. In addition, by the experience gained in a postdoctoral position and also currently in his NSF-supported research program, Dr. Shonnard has expertise in all facets of bioreactor operation, including assembly, sterilization, data acquisition, and bioreactor control. This experience extends to both batch and fluidized bed bioreactor operations.

Dr. Fisher has been in a leadership position in academia for over twenty years; eight as Associate Dean of Engineering at Wayne State University and twelve years as Professor and Chair of Chemical Engineering at Michigan Tech. He has been directly involved with major curricular improvements including providing the leadership in the development and implementation of the $1.5 million PSCC automated pilot plant facilities in the department. He was instrumental in recruiting Drs. Shonnard and Odde to Michigan Tech and is committed to the major laboratory and
curriculum improvements in Bioprocess Engineering outlined in this proposal. Dr. Fisher is also a Principal Investigator of a curriculum development effort to develop and implement a Chemical Engineering Technology: Advanced Process Operations degree program supported by NSF/ATE.

2.e Dissemination and Evaluation. Four avenues will be used to disseminate results at the institutional, regional, national, and international levels. First, we will present the results at a national meeting of the American Society for Engineering Education (ASEE). Second, we will submit an article describing the laboratory to Chemical Engineering Education, a leading journal in the area of chemical engineering pedagogy. Third, we will add a description of the facility to our departmental homepage on the world-wide web, with photographs and process flow diagrams included and data made available to the public. This will provide information on the facilities, their use in the senior Chemical Engineering Laboratory, and their integration into the PSCC. Fourth, we will write articles for distribution in our departmental and college alumni newsletters. In addition, we will write a laboratory manual, available on-line to the public, for a more complete description of the facility. Together, these approaches will provide a wide range of interested audiences with the news of the integrated bioprocessing laboratory at Michigan Tech.

Success of the project will be evaluated primarily using the University's course evaluation system. Using a series of questions, the evaluation asks students to rate the effectiveness of the instructor and the course. In addition, the students are given the opportunity to write comments on the form if they wish. The success of the project will also be evaluated in the longer term by a proposed plan for assessment being developed as part of MTU's reaccreditation process. Specifically, we plan to conduct surveys of alumni two and five years post B.S. degree, which will provide an opportunity to poll graduates who participated in the bioprocess laboratory about the effectiveness of the project and the impact it has had on their career.
3. REFERENCES CITED


TO:  Prof. Paul Frymier  
Univ. of Tennessee - Knoxville

Phone:  
Fax:  423-974-7076

FROM:  Prof. David Odde

Phone:  (906) 487-704-3213
Fax:  (906) 487-3213

MESSAGE:

Paul,

These were the objectives I gave each of two groups last spring.  The flow rate was too high and the packing compressed.  One group even had the top fittings pop off!  I suggest 1-3 m/min. Good luck.

Dave
MEMORANDUM

Department of Chemical Engineering
Michigan Technological University

Subject: Bioseparation Experiment: Recovery of an Amino Acid

To: Unit Operations Laboratory Group

From: David Odde

Date: April 22, 1998

Ion exchange is a separation technique that discriminates between molecules on the basis of their electric charge. In the biochemical engineering laboratory we have a cation exchange column that can be used to adsorb cations, such as the amino acid L-lysine. One way of recovering the adsorbed molecules is by altering the pH so that the net charge on the molecule is neutralized. Your objective is to develop a detailed procedure for the recovery of L-lysine from a model fermentation broth. Specifically consider the following system:

Equipment (specifications and manuals are in laboratory)
Pharmacia Gradifrac Programmable Controller (includes pump, mixer, valves, and fraction collector)
Sephadex C-25 Cation Ion Exchange Column (capacity=480 mmol/L, bed volume=530 ml)

Chemicals
Model fermentation broth: 0.4 M L-lysine
Alkaline wash: Tetraborate buffer (TB10.2) 0.1 M K$_2$B$_4$O$_7$, pH 10.2
Alkaline eluent: Tetraborate buffer (TB11.5) 0.1 M K$_2$B$_4$O$_7$, pH 11.5
Neutral wash: Phosphate-buffered saline (PBS) 0.01 M K$_3$HPO$_4$, 0.15 M NaCl, pH 7.0

Operation
Flow Rate: 5.0 ml/min

<table>
<thead>
<tr>
<th>Time</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>start loading lysine</td>
</tr>
<tr>
<td>160 min</td>
<td>start PBS wash</td>
</tr>
<tr>
<td>270 min</td>
<td>start TB10.2 wash</td>
</tr>
<tr>
<td>380 min</td>
<td>start TB11.5 elution</td>
</tr>
<tr>
<td>490 min</td>
<td>start collecting 10 ml fractions</td>
</tr>
<tr>
<td>599 min</td>
<td>stop collecting 10 ml fractions</td>
</tr>
<tr>
<td>600 min</td>
<td>shutdown</td>
</tr>
</tbody>
</table>

In your proposal describe the basic principles of ion-exchange and how the procedure outlined above should lead to a purified lysine product. I am still in the process of developing the procedure for quantifying the lysine concentration and hope that you will be able to validate the procedure by measuring the lysine concentration in the recovered fractions. In addition to the handouts enclosed, see sections 5.4-5.8 of the Gradifrac User Manual. Because this is a new experiment, I will work closely with you in the operation and meeting the objectives.
Appendix C: Calculations

Determine capacity of packing Diamon SK18 1.9 mL or 3.6 mg Sigma Chemical Co.

conversion of mg to moles lysine

Make lysine and neutral wash

Load resin into column
Cal 0.7 kHz 10 cm.

L-lysine monohydrate chloride MW 182

\[
\text{0.44 moles} \quad \frac{\text{L}}{0.12L} = 0.08 \text{ moles} \quad \frac{(182\text{g})}{\text{mol}} = 14.56 \text{ g for 200 mL soln.}
\]

\[
\frac{3.03g}{11.32g} = \frac{14.25g \text{ lysine}}{}
\]

Phosphate Buffered Saline 0.01M K2HPO4 0.15M NaCl

Phosphate \[
\frac{0.01 \text{ moles}}{L} \quad \frac{0.2L}{0.002 \text{ moles}} \quad \frac{(123.12g)}{\text{mol}} = 0.3484 \text{ g K2HPO4}
\]

make 300 mL = \[0.55\text{ g}\]

\[
\text{NaCl} \quad \frac{0.15 \text{ moles}}{L} \quad \frac{0.3L}{0.055 \text{ moles}} \quad \frac{(58.44g)}{\text{mol}} = 2.63 \text{ g NaCl}
\]

\[2.63g \text{ weighed}\]
Lys+ NADH + αKG \rightarrow SACR + NAD

calibration done

Lys diluted 100X

max molarity = 0.002 M
\[0.5 \text{ ml (1 min)} = 0.5 \text{ ml lysine}\]

The flow rate of 5 mL/min (160 mm) = 800 mL

\[\frac{800}{0.5} = 1600\]

\[\text{PBS} \quad \frac{5 \text{ mL}}{\text{min (110 mm)}} = 550 \text{ mL} \quad \text{PBS} \quad \frac{550}{1600} = 0.34 \text{ mL}\]

\[\text{TB10.2} \quad \frac{5 \text{ mL}}{\text{min (110 mm)}} = 550 \text{ mL} \quad 0.34 \text{ mL} \quad \text{TB10.2}\]

\[\text{TB11.5} \quad \frac{5 \text{ mL}}{\text{min (110 mm)}} = 350 \text{ mL} \quad \frac{350}{1600} = 0.22 \text{ mL} \quad \text{TB11.5} \quad 1 \text{ mL}\]

They collect 200 mL out of 2250 mL lysate and PBS wash, collect 745 mL total

\[23 \text{ mL} \quad \frac{2 \text{ mL}}{\text{min}}\]

\[110 \text{ mL lysine} \rightarrow 22 \text{ mm lysine}\]

\[\frac{220}{110} = 2.0 \quad \text{PBS} \quad \frac{220}{5.5} = 40 \text{ mL}\]

\[81 \quad \text{PBS}\]

\[T=54 \text{ mm} \quad \text{B2}\]

\[\text{PBS}\]

\[C=\text{lysine} \quad A=\text{B1} \quad B=\text{B2} \quad 0=\text{wash} \quad \text{Lysine program}\]