# NE 445L/481 Nanoscale Biosystems Lab Report

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University of Waterloo  
4A Nanotechnology Engineering  
Group 7

#### Rajesh Swaminathan Student ID: 20194189 Email: rajesh@meetrajesh.com Phone: 519-590-5439 Lab Partner: Peter Lee (20201956)

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**Lab Instructor**: Lillian Liao

# Lab 1 Polymeric Nanoparticle Formulation for Drug Delivery Applications

### Introduction and Objective

The main objective of this laboratory is to investigate two different encapsulation techniques, namely nano-precipitation and double emulsion. The drugs that will be studied in light of these two encapsulation techniques are docetaxel and doxorubicin respectively. The drugs will be encapsulated in a PLGA-PEG polymer matrix to form nanoparticles of a certain radius. The differences between the two encapsulation techniques will be characterized by looking at the average size and distribution of the resulting particles.

### Experimental Procedure

The procedure for this laboratory was obtained from the NE 454L/481L Nanoscale Biosystems Laboratory 1 part of the Nanotechnology Engineering Program 4A Lab Manuals, 2009. The only deviation from this procedure was that the Branson Dismembrator was turned ON for 1 second and turned OFF for 4 seconds.

### Data Collected

|  |  |
| --- | --- |
| **Recorded Measured Results for Nanoprecipitation** | **Values** |
| Polymer Mass Measured of PLGA-b-PEG MW1 Stock | 150.3 mg |
| Polymer Mass of MW1 – Docetaxel in ACN | 10.1 mg |
| Polymer Mass of MW2 - THF | 10.8 mg |
| Polymer Mass of MW3 - THF | 11.1 mg |

|  |  |
| --- | --- |
| **Recorded Measured Results for Double Emulsion** | **Values** |
| Polymer Mass Measured of PLGA-PEG MW1 | 101.8 mg |
| Total Volume of PVA | 12.57 ml |

Particle effective diameter obtained via nanoprecipitation = **46.1 nm**   
Particle effective diameter obtained via double emulsion = **734.8 nm**

### Discussion and Analysis of Data

**Question 1**

Based on the collective results between groups, the following trends were observed:

1. **Molecular weight**: Increasing molecular weight increases particle size. This can be attributed to a decrease in net shear stress as the molecular weight increases.
2. **Concentration:** As polymer concentration is increased, particle size increases as well. This can be attributed to larger amounts of surface interaction between the polar and non-polar surfaces of the particles, caused by the extra polymer added. The larger surface interaction leads to a decrease in shear stress which also yields larger particles.
3. **Solvent Variation:** Strongly polar solvents were more sensitive to changes in molecular weight of PLGA-PEG. Also, greater solvent hydrophilicity results in smaller nanoparticles due to better solubilisation of the polymer nanoparticles.

**Question 2**

We are basically looking for organic solvents that have similar Hildebrand solubility parameters as that of the four solvents studied in this lab, i.e. within the range of 9-14. For a PLGA-*b*-PEG polymer system with docetaxel, a fifth solvent that could be used in a nanoprecipitation formulation is Dimethylformamide, or simply DMF. Other substitutes are Dichloromethane (DCM) and toluene. All these three suggested solvents, namely DMF, DCM and toluene have Hildebrand solubility parameters close to that of acetonitrile.

Acetonitrile has an experimental Hildebrand solubility parameter of 11.92 as seen in . DMF is the closest with an experimental Hildebrand solubility parameter of 11.97, whereas DCM and toluene have solubility parameters of 10.89 and 9.56 respectively.

|  |  |  |
| --- | --- | --- |
| **Solvent** | **Calculated Solubility Parameter in units of (cal/cc)1/2** | **Experimental Solubility Parameter in units of (cal/cc)1/2** |
| Acetonitrile | 12.49 | 11.92 |
| Tetrahydrofuran | 11.44 | 9.10 |
| Acetone | 10.79 | 9.77 |
| Dimethylformamide | 11.95 | 11.97 |
| Dimethyl sulfoxide | 14.50 | 14.71 |
| Dichloromethane | 12.17 | 10.89 |
| Toluene | 11.56 | 9.56 |

Table 1 Solubility Parameter for Various Solvents

Since DMF has the closest solubility parameter to that of acetonitrile, we would expect it to perform the closest to the performance achieved by acetonitrile in this lab.

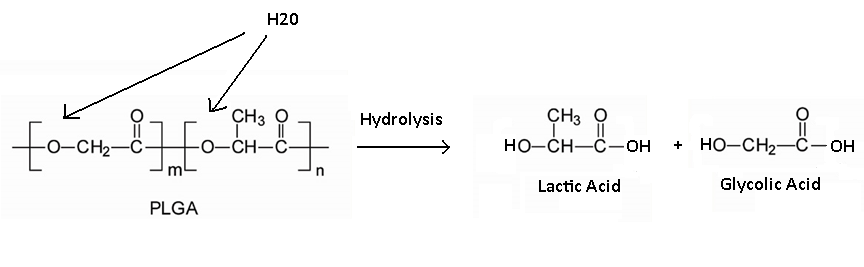
Theoretically, the less soluble the material is in water, the larger the particle size. Therefore, the higher the Hildebrand solubility parameter respective to other solvents, the smaller the mean diameter. Thus, we expect DMF to have about the same particle size relative to acetonitrile, while we expect DCM and toluene to have larger mean particle sizes relative to acetonitrile since these solvents have smaller Hildebrand solubility parameter values.

**Question 3**

The body has the ability to naturally rid itself of PEG (polyethylene glycol) but only when its molecular weight is over 1 kDa. However, bigger PEG molecules are slower to break down than smaller ones.

PLGA (poly-lactic-co-glycolic acid), on the other hand, is broken down using a simple hydrolysis process. This process requires the presence of water which is always present in the body. We also know that PLGA is nothing but just an ester formed by the combination of PGA and PLA. The body already knows how to break down PGA into glycolic acid which can be eliminated from the body through direct excretion via the kidneys as urine. Similarly, the body also knows how to break down PLA into lactic acid which can be easily broken down in H20 and CO2. The body can therefore break down PLGA, a combination of PGA and PLA, using hydrolysis at the ester bond formed between the PGA and PLA molecules.

The process of hydrolysis is simple: the H+ ion from water attaches itself to the C=0—0 ester bond, whereas the OH- ion attaches itself to the carbon of that ester bond, as shown in the figure below. The byproducts of this process are lactic acid and glycolic acid which are easily broken down and eliminated as described in the paragraph above.



**Question 4**

1. *Recombinant human insulin*  
   I would use double emulsion to encapsulate this drug because insulin is large and hard to manufacture. Therefore, we would prefer the technique that has a larger encapsulation efficiency.
2. *Rhodamine labelled dextran*   
   I would use double emulsion to encapsulate this drug as well since this is a non-polar water-oil-water (w/o/w) solution. Such solutions can be very large, usually on the order of 10 – 150 kDa.
3. *Sildenafil citrate (Viagra)*I would use nanoprecipitation to encapsulate this drug since it is non-polar and very small.
4. *Testosterone*  
   I would use nanoprecipitation to encapsulate this drug since it is non-polar and very small, similar to Sildenafil citrate in iii)

### Conclusions and Recommendations

Since our results are dependent on the performance of other lab groups who performed their experiment on the same day as us, it is hard to determine the accuracy of our results. Even if one group had errors in their experimental procedure, it would detrimentally impact the results of all the other groups, including ours.

For instance, in the plot of particle size as a function of polymer concentration in different solvents, we found the particle size in acetonitrile to *decrease* with increasing polymer concentration. This is contradictory to the accepted theory.

Nonetheless, the values obtained for the mean diameter of the nanoparticles synthesized using nanoprecipitation and double emulsion techniques are acceptable and within the bounds anticipated by us given our prior knowledge of the theory behind the two techniques. From our results, we see that the effective diameter obtained via double emulsion is much larger, almost 1500% larger than that obtained via nanoprecipitation. This is reasonable since double emulsion has a second extra encapsulation layer that emulsifies the smaller particles formed in the first step. This results in much larger particle sizes than compared with nanoprecipitation.

A few recommendations can be made based on these new findings: Small, non-polar drugs are best encapsulated using nanoprecipitation, while larger drugs and drugs that are expensive to manufacture and therefore require better encapsulation efficiencies to minimize wastage are best encapsulated using double emulsion.

# Lab 2 Determination of Nanoparticle Encapsulation Efficiency by HPLC and Fluorescence Absorbance

### Introduction and Objective

In the previous laboratory session, we encapsulated two different drugs, namely docetaxel and doxorubicin using nanoprecipitation and double emulsion techniques respectively. In this lab session, we studied the encapsulation efficiency achieved in the encapsulation of these two drugs using these two techniques. HPLC and fluorescence were used to determine the concentration of encapsulated drug. Fluorescence is measured using a microplate reader.

*Encapsulation efficiency* is defined as the ratio between the masses of the original drug added to solution and the drug encapsulated in the nanoparticle formulation.

### Experimental Procedure

The procedure for this laboratory was obtained from the NE 454L/481L Nanoscale Biosystems Laboratory 2 part of the Nanotechnology Engineering Program 4A Lab Manuals, 2009.

The following deviations from the lab manual were observed: Exercise 1 was skipped and replaced with another dilution experiment provided by the lab instructor. Also, since we ran out of doxorubicin half-way through the lab, we needed an extra 0.5 mg/mL doxorubicin via to complete all the specified dilutions.

### Results and Data

## *In Lab Exercise 1*

|  |  |
| --- | --- |
| **Bromothymol Blue Dilution** | **Absorbance at 609 nm** |
| Distilled Water | 1.007 |
| 10x | 0.437 |
| 100x | 0.191 |
| 1000x | 0.088 |

## *Docetaxel Encapsulation Efficiency using HPLC*

Mass of Docetaxel Sample with MW1 = **8.8 mg**  
Mass of Original Docetaxel Sample with MW = **10.1 mg**

Our solution was prepared with 1 ml of 10 mg/ml MW1 in acetonitrile and 0.1 mg/ml of docetaxel. This adds up to a total of 10.1 mg. Thus the yield percentage is calculated as follows:



The yield percentage obtained above is 12.9% short of 100%. There are many reasons for this. Centrifugation and filtration contribute to some of the drug losses. Transferring between vials also causes some loss as there will always be some solution left behind in the vial at the end of the transfer. Some of the original material could have been stuck to the paper that was used to measure out the material. There could also be a certain amount of instrumental error in the mass balance and when using the volumetric pipette to measure out the drug. Also, there could be visual and parallax errors involved in reading the blue line on the pipette which may cause inaccurate amounts of liquid to be transferred.

## *Doxorubicin Encapsulation Efficiency by Fluorescence*

Mass of Doxorubicin Sample with MW1: **46.2 mg**Mass of Original Doxorubicin Sample with MW1: **51.4 mg**

In the preparation of our solution, we took 101.8 mg of MW1 and added it to 400 l of the 2.5 mg/mL doxorubicin solution which has a mass of 0.4 ml \* 2.5 mg/mL = 1mg. This yields a total mass of 101.8 mg + 1 mg = 102.8 mg. We also observe that we used only half the retenate after centrifugation. Thus, the final mass used was 102.8 mg / 2 = 51.4 mg. We may calculate the yield percentage again similar to how we calculated it in the previous section:



Explanations for why the yield is off from 100% by 10.1% are similar to that explained the previous section: instrumental errors and transfer left-overs.

## *Results Obtained from Fluorescence Procedure*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| **A** | Blank |  |  | 0.1 g/l | 0.025 g/l | 0.0025 g/l | 0.0005 g/l | 1000x Dil | 10x Dil |
| **B** | Blank |  |  | 0.1 g/l | 0.025 g/l | 0.0025 g/l | 0.0005 g/l | 1000x Dil | 10x Dil |
| **C** | Stock |  |  |  |  |  |  |  |  |
| **D** | Stock |  |  | 0.05 g/l | 0.0075 g/l | 0.001 g/l | 10000x Dil | 100x Dil |  |
| **E** | 0.25 g/l | 0.25g/l |  | 0.05 g/l | 0.0075 g/l | 0.001 g/l | 10000x Dil | 100x Dil |  |

Table 2: Micro plate Layout

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| **A** | 0.018 |  |  | 1.672 | -0.055 | -0.155 | -0.201 | -0.258 | -0.065 |
| **B** | -0.019 |  |  | 1.404 | -0.042 | -0.052 | -0.1 | -0.234 | -0.028 |
| **C** | 3.093 |  |  |  |  |  |  |  |  |
| **D** | 3.098 |  |  | 0.629 | -0.152 | -0.056 | -0.208 | -0.218 |  |
| **E** | 2.998 | 3.027 |  | 0.618 | -0.099 | -0.081 | -0.237 | -0.245 |  |

Table 3: Absorption Values obtained from Microplate

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| **A** | -4 |  |  | 3814 | 895 | 240 | 40 | 6 | 1276 |
| **B** | 4 |  |  | 4180 | 1144 | 287 | 55 | 12 | 1690 |
| **C** | 4637 |  |  |  |  |  |  |  |  |
| **D** | 4692 |  |  | 3415 | 906 | 131 | -1 | 160 |  |
| **E** | 4593 | 4723 |  | 3689 | 908 | 129 | 0 | 172 |  |

Table 4: Fluorescence Values from Microplate

## *Results Obtained from HPLC Procedure*

Table 5 HPLC Procedure Results

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Peak at** | **Retention Time** | **Area Under Curve** |
| **Standard Docetaxel 25 ppm in ACN** | 22.7 nm | 1.703 minutes | 791.3 |
| **HPLC Sample with Docetaxel** | 22.7 nm | 1.683 minutes | 882.5 |

### Discussion and Analysis of Data

**Question 1**

***Docetaxel***

From Table 5, we see that the 22ppm docetaxel standard in ACN has an area-under-curve (AUC) of 791.3. Our own sample solution with docetaxel encapsulated in it has an AUC of 882.5. We use the fact that AUC is directly proportional to concentration. Thus, the ratios of AUCs must equal the ratios of concentrations of the sample and standard. We can use this fact to determine the concentration (in ppm) of our sample in the following manner:

Therefore, the concentration of the HPLC Sample is **27.88 ppm**

Using the fact that ACN has a density of 0.786 g/mL, we can convert the concentration of our sample from ppm (parts per million) to the more familiar units of g/L:

At a volume of 500 l, we obtain a mass of:

We can now proceed to calculate the encapsulation efficiency of docetaxel which was encapsulated using nanoprecipitation. We observe that the total mass of drug added initially was 0.1 mg; however, only 87.1% was actually put to use as seen in Lab 1. Thus, only 0.0871 mg of docetaxel was put to use. The encapsulation efficiency of docetaxel is therefore:

***Doxorubicin***

The following graph plots fluorescence as a function of concentration. We observe a linear relationship between the two variables, i.e. fluorescence intensity increases with concentration. A linear trendline was generated in Excel, and the slope was determined to be 119451.

Using this slope, we can obtain the concentration of our sample as shown in the following table:

|  |  |  |
| --- | --- | --- |
| **Fluorescence Value** | **Calculated Concentration of Dilution (mg/mL)** | **Calibrated Concentration (mg/mL)** |
| 160 | 0.00134 | 0.13395 |
| 172 | 0.00144 | 0.14399 |
| 1276 | 0.01068 | 0.10682 |
| 1690 | 0.01415 | 0.14148 |

The average of the four calibrated concentrations is 0.13156. Using the fact that the original doxorubicin concentration used was 0.225 mg/mL, we can now calculate the encapsulation efficiency as follows by dividing the two numbers as shown below:

***Literature Values and Comparison***

The literature values found in the pre-lab for encapsulation efficiencies using nanoprecipitation and double emulsion techniques were 17%-23% and 67% respectively. The encapsulation efficiencies we obtained in the lab were 12.6% and 58.5% respectively. Thus, the encapsulation efficiencies we obtained are about 10% lower than that obtained in the literature.

***Ways to Improve Encapsulation Efficiency***

Encapsulation efficiency can be improved by making few errors in procedure and by using more precise equipment. We had a leaky microplate which resulted in significant contamination and reduced efficiencies. So using unbroken equipment might yield better results. Another way of improving encapsulation efficiency is to use a better solvent. What constitutes as a better solvent? A solvent in which the polymer is less soluble in would constitute as a better solvent. A polymer is likely to have much improved encapsulation if it is not as soluble in the solvent.

**Question 2**

*Explain the phenomenon of decreasing fluorescence intensity present in the higher concentrations (0.1, 0.25, 0.5 mg/mL) of doxorubicin standards.*

Higher concentration implies more particles in the same volume of liquid. This increased number of particles can absorb and scatter light produced from Raman and Rayleigh scattering more, thus resulting in reduced light intensity in florescence spectroscopy at higher concentrations of doxorubicin standards. Absorption of emitted florescent photons by other molecules in solution is also a contributing factor. At higher concentrations, more absorption can happen resulting in fewer photons actually making it to the detector and decreased fluorescent intensity.

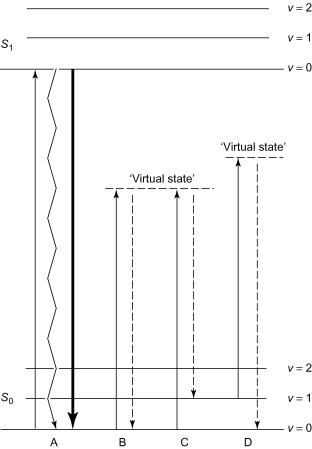
**Question 3**

*In general, fluorescence spectroscopy is more sensitive than absorbance spectroscopy. Why? Include an energy diagram.*

In absorbance spectroscopy, white light from the visible spectrum is shined through the sample, and the spectrum is obtained by detecting whatever light makes it through the sample, i.e. isn’t absorbed by the sample. This technique is usually performed on simple molecules.

In fluorescence spectroscopy, on the other hand, UV light is shined through the sample. Some of this UV light is absorbed by the molecules and compounds in the sample putting them at a “virtual” state. These molecules then relax to the ground state by re-emitting the absorbed UV photons as lower energy photons in the visible light spectrum. The fluorescence spectrum is therefore obtained by detecting these lower-energy re-emitted photons. It is therefore more sensitive than absorbance spectrum.

The energy diagram for the energy interactions undergone by the sample molecules during fluorescence spectroscopy is shown below:

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### Summary and Conclusions

Due to the presence of a damaged microplate that leaked, a lot of our results were thrown off significantly due to the resulting contamination and changes in concentrations. Contamination was observed when several cells in our microplate turned bluish in colour. We attribute all our discrepancies in results to this broken microplate. To obtain realistic results, we would need to repeat the experiment with a proper, new, and clean microplate.

Nonetheless, we were able to use the remaining data to obtain values for encapsulation efficiencies of our two drugs by fitting them to the theoretical model. The values we obtained for the encapsulation efficiencies of docetaxel and doxorubicin were reasonable enough and fairly close to literature values.

# Lab 3 Calcium Alginate Hydrogel Encapsulation of Insulin and Enzyme Linked Immunosorbent Assay (ELISA)

### Introduction and Objective

The objective of this third and last section of the NE 481 lab is to encapsulate human insulin using alginate gel formations created using alginate and calcium ions. We then attempt to determine insulin levels in our created alginate sample and in an unknown sample using a modern technique known as Enzyme Linked Immunosorbent Assay (ELISA).

### Experimental Procedure

The procedure for this laboratory was obtained from the NE 454L/481L Nanoscale Biosystems Laboratory 3 part of the Nanotechnology Engineering Program 4A Lab Manuals, 2009. Because our group’s results were completely inaccurate and skewed due to several errors in procedure, we had to obtain and use results from another group (Group 3) for the purposes of data analysis and discussion in the laboratory report.

### Results and Data Collected

The reported values below are our measured results. These values are not applicable to ELISA since we used a different group’s results.

Mass of alginate added: **0.0212 g**

Mass of insulin added: **0.0113 g**

Mass of CaCl2 added: **0.1667 g**

Mass of EDTA added: **0.0496 g**

Molar mass of CaCl2 = 110.98 g/mol  
Unknown sample number provided to us by lab instructor: **#4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ELISA Plate Layout (One strip per group) | | | | | |
|  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|  | 1 | 2 | 3 | 4 | 5 |
| A | Blank | 5uU/mL | 10uU/mL | 20uU/mL | 5uU/mL |
| B | Blank | 5uU/mL | 10uU/mL | 20uU/mL | 5uU/mL |
| C | 2uU/mL | 50uU/mL | 100uU/mL | 200uU/mL | 100uU/mL |
| D | 2uU/mL | 50uU/mL | 100uU/mL | 200uU/mL | 100uU/mL |
| E | Sample | Sample | Sample | Sample | Sample |
| F | Sample | Sample | Sample | Sample | Sample |
| G | Unknown | Unknown | Unknown | Unknown | Unknown |
| H | Unknown | Unknown | Unknown | Unknown | Unknown |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 |  |
| A | 2.452 | 2.282 | 1.874 | 1.972 | 2.049 |  |
| B | 2.47 | 1.923 | 1.738 | 1.876 | 2.078 |  |
| C | 2.083 | 1.744 | 2.308 | 2.306 | 3.086 |  |
| D | 3.182 | 1.733 | 3.132 | 2.366 | 2.432 |  |
| E | 1.919 | 1.697 | 1.919 | 1.989 | 2.062 |  |
| F | 2.335 | 1.862 | 1.755 | 1.891 | 2.4 |  |
| G | 2.627 | 1.961 | 1.779 | 3.102 | 3.047 |  |
| H | 3.216 | 2.615 | 1.802 | 2.036 | 2.66 |  |

Conversions used in our calculations:

Insulin: **28.9 IU/mg**  
1 U/mL = **35 pg/mL**

### Discussion and Analysis

**Question 1**

*Calculate means and coefficients of variation (CV) for all duplicates.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|  | Blank | 5uU/mL | 10uU/mL | 20uU/mL | 5uU/mL |
| Mean | 2.461 | 2.1025 | 1.806 | 1.924 | 2.0635 |
| STDEVP | 0.009 | 0.1795 | 0.068 | 0.048 | 0.0145 |
| CV | 0.003657 | 0.085375 | 0.037652 | 0.024948 | 0.007027 |
|  |  |  |  |  |  |
|  | 2uU/mL | 50uU/mL | 100uU/mL | 200uU/mL | 100uU/mL |
| Mean | 2.6325 | 1.7385 | 2.72 | 2.336 | 2.759 |
| STDEVP | 0.5495 | 0.0055 | 0.412 | 0.03 | 0.327 |
| CV | 0.208737 | 0.003164 | 0.151471 | 0.012842 | 0.118521 |
|  |  |  |  |  |  |
|  | Sample | Sample | Sample | Sample | Sample |
| Mean | 2.127 | 1.7795 | 1.837 | 1.94 | 2.231 |
| STDEVP | 0.208 | 0.0825 | 0.082 | 0.049 | 0.169 |
| CV | 0.09779 | 0.046361 | 0.044638 | 0.025258 | 0.075751 |
|  |  |  |  |  |  |
|  | Unknown | Unknown | Unknown | Unknown | Unknown |
| Mean | 2.9215 | 2.288 | 1.7905 | 2.569 | 2.8535 |
| STDEVP | 0.2945 | 0.327 | 0.0115 | 0.533 | 0.1935 |
| CV | 0.100804 | 0.14292 | 0.006423 | 0.207474 | 0.067811 |

The above means and coefficients of variation (CV) were obtained with the help of the following built-in Excel spreadsheet functions:

* The AVERAGE() function was used to calculate the mean for all duplicates.
* The STDEVP() function was used to calculate the standard deviation for all duplicates
* The coefficient of variation, CV, was calculated by dividing the result obtained from STDEVP() by the result obtained from AVERAGE(). Thus CV = STDEVP() / AVERAGE()

**Question 2**

*Plot a standard curve of the standards, fit a line to it. Fit data onto the model. Does the result match up with what was expected? Does the result match with the absorbance at 280nm?*

The following graph is a plot of absorbance vs. concentration. Since our data was all over the place, the lab instructor asked us to use the data from a different group that performed their lb on a different day. Unfortunately, their data wasn’t any better either. The graph below shows the best-fit line from which it can be seen how poor the correlation is. There are just too many outliers to form any trend.

We used the calibration curve and our own group’s data (Group 7) to fit the data on to the model. The following table shows our results:

|  |  |  |
| --- | --- | --- |
|  | **Group 7 Sample** | **Group 7 Unknown** |
| **Mean** | 1.2225 | 4.0035 |
| **STDEVP** | 0.0605 | 0.0605 |
| **CV** | 0.049489 | 0.015112 |
| **Conc (U/mL)** | 784.1451 | 2214.234 |

We don’t believe these results to be correct or accurate in any way since we could not obtain a proper trend line.

However, we can still proceed with the data analysis by ignoring certain outlier points the deviate too much from a trend. We consequently omitted 4 concentration data points and kept the remaining 5 data points. We also subtracted the *lowest* data point in the set of blank standards from the 5 sample data points to avoid negative results. The chart below shows the graph obtained after such excessive data manipulation:

This chart has a better-looking trend line with fewer outlier data points. The slope of the trend line was found to be 0.0101. Using this slope, we can now proceed to fit the data to the ELISA model and obtain the following values for mean, standard deviation and CV:

|  |  |  |
| --- | --- | --- |
|  | **Group 7 Sample** | **Group 7 Unknown** |
| **Mean** | 1.2225 | 4.0035 |
| **STDEVP** | 0.0605 | 0.0605 |
| **CV** | 0.049489 | 0.015112 |
| **Conc (U/mL)** | 784.1451 | 2214.234 |
| **Modified Conc (U/mL)** | 120.5668 | 394.8377 |

These results are probably incorrect due to the heavy data manipulation explained in the previous paragraphs. However, if the experiment is repeated, the new numbers can simply be plugged into this model and the same steps can be followed to obtain more realistic results.

To convert concentration values from uU/mL to g/mL, we use the fact that 1U/mL = 35 pg/ml or 35E-6 g/ml. Thus the concentrations in units of g/mL are as follows:

|  |  |  |
| --- | --- | --- |
|  | **Group 7 Sample** | **Group 7 Unknown** |
| **Conc (ug/mL)** | 0.027445 | 0.077498 |
| **Modified Conc (ug/mL)** | 0.00422 | 0.013819 |

Do these ELISA results match up with what was expected? No, the results do not match up with what was expected at all. We expected fewer outliers, and even the outliers were expected to not deviate from the trend *that* much. We expected more linearity in the trend between absorbance values and concentration. This was however not the case with our results.

The experiment may be deemed a failure for all practical purposes and needs to be repeated to say anything decisive about the trends.

**Question 3**

*Discuss any errors; contamination, high background, high CV.*

We had a lot of sources of errors in our lab. This is obvious from the number of outliers in the data that do not conform to any trend.

There were two cases of solution “splashing” on to other rows while snapping the plate row into the plate shaker. This resulted in contamination of the solutions by effectively changing their concentrations. It could also be possible that the plates weren’t washed cleanly before use. We didn’t was them ourselves; we just trusted they were clean before we used them. The pipette tips could also be dirty to begin with, or we may have accidentally used the same tip twice when we shouldn’t have.

What about high background related errors? We had lots of errors with our background readings being too high. This was observed both with our own data as well as Group 3’s data given to us by the lab instructor. Since the background was too high, sometimes even higher than the numbers recorded for standards *with insulin*, we would often obtain negative values when the background readings were subtracted from the standards with insulin. This did not make sense at all, so we concluded there was something wrong with the background readings.

Lastly, since the coefficient of variation (CV) represents the variation between the two results, high CV values can bias a certain data point on the plot by affecting the slope of the trendline on the ELISA plot.

**Question 4**

*What would be the effect on encapsulation efficiency if a higher concentration of sodium alginate was used, for example, 2.0% w/v? Why?*

If a higher concentration of sodium alginate was used, particle solidification will proceed at a much faster rate thereby reducing the amount of time for diffusion to happen. Higher concentration of sodium alginate will also make the polymer more viscous also resulting in reduced drug diffusion back into the solution. Both these factors will contribute to **increased** encapsulation efficiency.

**Question 5**

*What is the difference between internal gelation and external gelation? Which method was used in this lab? Name one advantage of this method.*

**Internal Gelation**: When the gelation process resulting in gel formation starts from the inside and then grows outward, it is called internal gelation. Some gels are formed with the help of a pre-cursor molecule, which then grows outward from the center. This is an internal gelation process.

**External Gelation**: While internal gelation starts from the inside and then grows outward, external gelation is different in that it forms an outside surface first and then proceeds to solidify inwards.

The method used in this lab was *external gelation*. The process was achieved by dropping alginate droplets into a calcium ion bath which immediately diffuse into the alginate as soon as the alginate droplet hits the surface. The diffusion results in a solidification of the polymer surface first which then continues inwards.

One advantage of this external gelation method over the internal gelation method is that once the alginate is placed in the calcium bath, we witness the formation a shell at the surface of the droplet. This shell has the ability to trap the drug within the droplet before it diffuses away from the droplet’s vicinity. This improves the encapsulation efficiency vastly.

**Question 6**

*Encapsulated insulin hydrogels are leaky due to large gel porosity; suggest one method on overcoming this? Support with literature.*

One way of overcoming leaky encapsulated insulin hydrogels is to increase the concentration of the polymer. Increasing the concentration of the polymer will result in quicker solidification of the encapsulated gel because of a steeper concentration gradient at the phase boundary between the polymer and the organic solvent. This quicker solidification of the gel helps us achieve lesser porosity, resulting in a better encapsulation efficiency.