# Development of an Analytical Method for Polymeric Nanoparticle Quantification in Serum by Fourier Transform-Infrared Spectroscopy

By

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Sc.B., Brown University 2018

Thesis

Submitted in partial fulfillment of the requirements for the Degree of Masters of Science in the Department of Biomedical Molecular Pharmacology, Physiology and Biotechnology at Brown University

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# MOTIVATION

In the field of drug delivery, current methods of drug and polymer quantification in systemic circulation involves the use of immunoassays, mass spectrometry, high performance liquid chromatography, or measurements on the effect of the delivered drug [1, 2, 3]. Each technique presents its own unique set of advantages and challenges [4].

Mass spectrometry remains the current gold standard in pharmacologics due to its robustness, ability to distinguish between similar compounds, and sensitivity [5]. However, use of this technology is far from commonplace due to its cost. Furthermore, when applied to the field of polymer-based drug delivery, mass spectrometry presents many challenges in characterizing low molecular weight compounds such as polymers [6].

The purpose of this study was to develop a proof of concept process to quantitatively assess polymer levels in blood using FT-IR. By measuring absorption based on the polymer, this offers a key advantage in providing a means of direct measurement for polymer-based drug delivery systems without the use of reagents. The significance of this work provides insight on a potentially accessible, affordable means of detection of drug delivery vehicles.

# BACKGROUND

*Fourier-Transform Infrared Spectroscopy*

Fourier-transform infrared spectroscopy is a technique widely used in analytical chemistry that is based on measuring the bond interactions between molecular compounds. Bonds between two atoms vibrate and absorb external infrared light at specific frequencies [7, 8]. The Beer-Lambert law is used to determine the degree of absorption at a given wavenumber and is given by the following relationship:

$$A = \mathcal{E}cl$$

where $A$ is the absorbance, $\mathcal{E}$ is the molar absorptivity, $c$ is the concentration of the analyte, and $l$ is the thickness of the sample. When analyzing absorbance, it has been shown that the concentration of an analyte can be determined based on the absorbance of a sample [9]. By analyzing a ratio of two peaks for a given spectrum, one can theoretically remove the contribution of the path length that can vary between different samples due to variations in sample pellet thickness.

*Phase Inversion Nanoencapsulation*

Phase Inversion Nanoencapsulation (PIN) is a technique developed by the Mathiowitz lab designed to encapsulate a drug within a polymer coating as discrete spheres ranging from the micro to nano scale. The key advantage of this method over other encapsulation techniques lies in its ability to spontaneously form nanoparticles [10]. The process involves the preparation of a low concentration polymer-solvent solution with a non-solvent that is miscible to the solvent used to dissolve the polymer of interest. This solution is added drop-wise into a non-solvent, forming a continuous phase that promotes the formation of the nanospheres. Once formed, the nanospheres are collected by filtration and dried via lyophilization. See Figure 1.

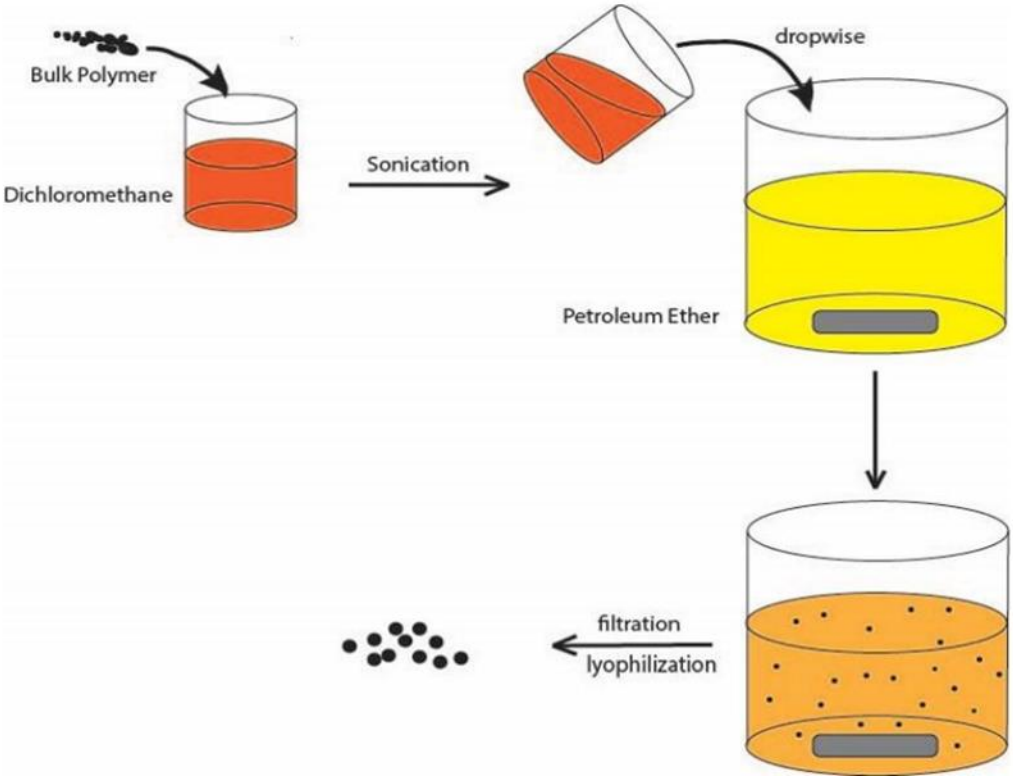



*Figure 1: Procedure to form nanoparticles via PIN [11].*

*Isolated Loop*

The isolated loop is a surgical procedure done to characterize gastrointestinal (GI) uptake of polymeric nanoparticles *in vivo* [12]. In the interest of applications for oral drug delivery, this procedure allows characterization of a drug conjugate of interest in the GI while bypassing the harsh acidic environment of the stomach. A mammalian model is anesthetized while a section of its small intestine is isolated by suture to retain a volume of injected microsphere solution. The mammalian model is maintained under anesthesia for the duration of the experiment. Blood samples and other organs are harvested and preserved for further analysis.

# INTRODUCTION

Previous work has been done to quantitatively analyze biological samples by FT-IR [13]. In the field of polymer-based drug delivery systems, there is a current lack of methods in polymer quantification in blood. This work seeks to elucidate these current gaps in knowledge.

Biologically derived drugs pose challenges to FT-IR based characterization. Figure 2 compares IR spectra of zinc bovine insulin and serum from a SD rat. Without uniquely distinguishable peaks to monitor changes in relative absorbance peak heights, it would be difficult to determine any absorption based on the spectrum of the drug alone. At present, polymer-based drug delivery systems serve as ideal candidates of characterization through this method based the presence of these unique peaks from the serum.

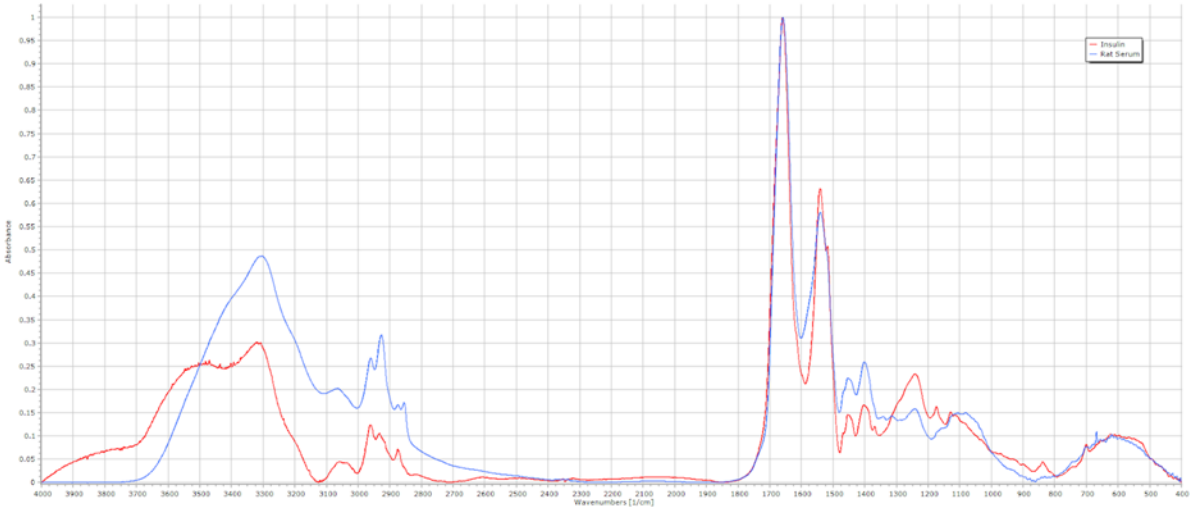



*Figure 2: Insulin Compared to Serum FTIR*

# SPECIFIC AIMS

1. To develop an expedited method of generating a calibration curve for PLA levels in rat serum using aliquots for FT-IR.
2. To find the sensitivity of our calibration curve by assessing its detection limit, quantification limit, and linear range.
3. To evaluate the resolution of the calibration curve by evaluating the standard deviation of a sample with a given concentration over multiple samples.
    a. If resolution size is determined to have a high enough degree of sensitivity, a test involving the addition of a known amount of analyte will be performed to evaluate the viability of quantifying PLA levels outside of the detection and quantification limits.

# MATERIALS AND METHODS

## In Vivo Experiments: Isolated Loop

The isolated loop experiments were performed by Aharon Agazury and others from the Mathiowitz lab. 4 Sprague Dawley (SD) rats were anesthetized using 3% isoflurane (v/v). From each, a 6 cm loop of small intestine was exposed and isolated by suture. 1 mL of blood was drawn to serve as a baseline. A solution containing PLA nanospheres was then injected into the isolated section of the small intestine. An additional 1 mL of blood is drawn after 5 hours of GI exposure to the PLA solution and taken for further processing. Further information regarding this experiment can be found in the List of Tables.

## Calibration Curve Generation

### *PLA Nanoparticle Preparation*

8kDa PLA (donation by Takeda) was used to prepare the nanoparticles via PIN. PLA was dissolved in dichloromethane (DCM) to form a solution that was 1.5% PLA (w/v). Petroleum ether (Fischer Chemical) was poured into a beaker and allowed to stir (Thermolyne SAFE-T SHP9). The PLA-DCM solution was added to the petroleum ether in a ratio of 1:60 (v/v). PLA nanoparticles were harvested after filtration and lyophilized using the BenchTop Pro with Omnitronics VirTis SP SCIENTIFIC.

### *Stock Solution Preparation*

Three separate stock solutions of known concentrations were formed from potassium bromide (KBr), SD rat serum, and 8kDa PLA nanoparticles in suspension. Dry sample mass of each component was first taken and placed into 50 mL falcon tubes. DI water was added to each of the three dry components until the desired mass-to-volume ratios were achieved. All three

solutions underwent vortexing (Talboys standard vortexer speed setting 10) and sonication (Fischer Scientific Ultrasonic Bath) to produce homogenous solutions.

*KBr Pellet Preparation*

The intended mass ratios of the three solution components: KBr, serum, and PLA NP were first determined (see List of Tables). Based on the known mass-to-volume ratios of the stock solutions, the appropriate volume of each component was pipetted into 20mL scintillation vials. The samples were then vortexed, and flash frozen by submersion into liquid nitrogen. Vortexing and immediate flash freezing was done to maximize the homogenous distribution of the analytes in solution and prevent settling. These samples were then lyophilized (BenchTop Pro with Omnitronics VirTis SP SCIENTIFIC) at 78 millitorr, for a minimum of 8 hours. Because of issues with pellet cracking, 25 mg of a sample was mixed with 75 mg of KBr, and pressed with 10 tons of pressure for 3 minutes. Triplicates of the calibration curve pellets were prepared to evaluate precision of the method. See Figure 3.

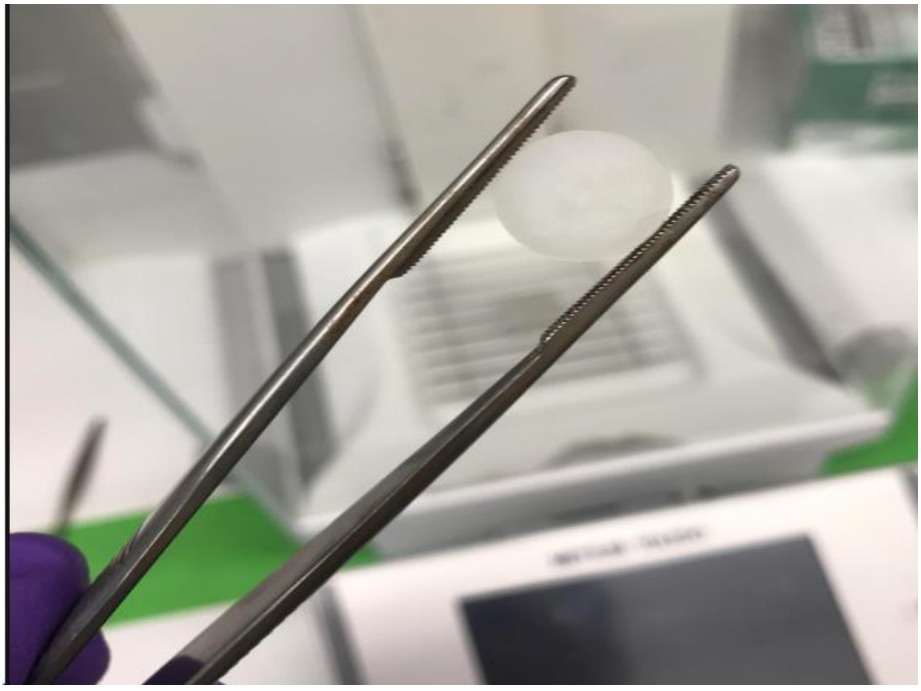


*Figure 3: Typical pellet sample prepared from >99% KBr for FT-IR analysis*

*PLA Nanoparticle Spiking*

PLA NP solutions of known concentration were added to the 0 and 5-hour time point serum samples of all 4 rats from the isolated loop experiment. These were mixed in a ratio of 0.2 mg of PLA to 3.8 mg of lyophilized serum to yield an addition of 5% PLA for each sample. The KBr pellet preparation of these samples proceeded in the same manner discussed above.

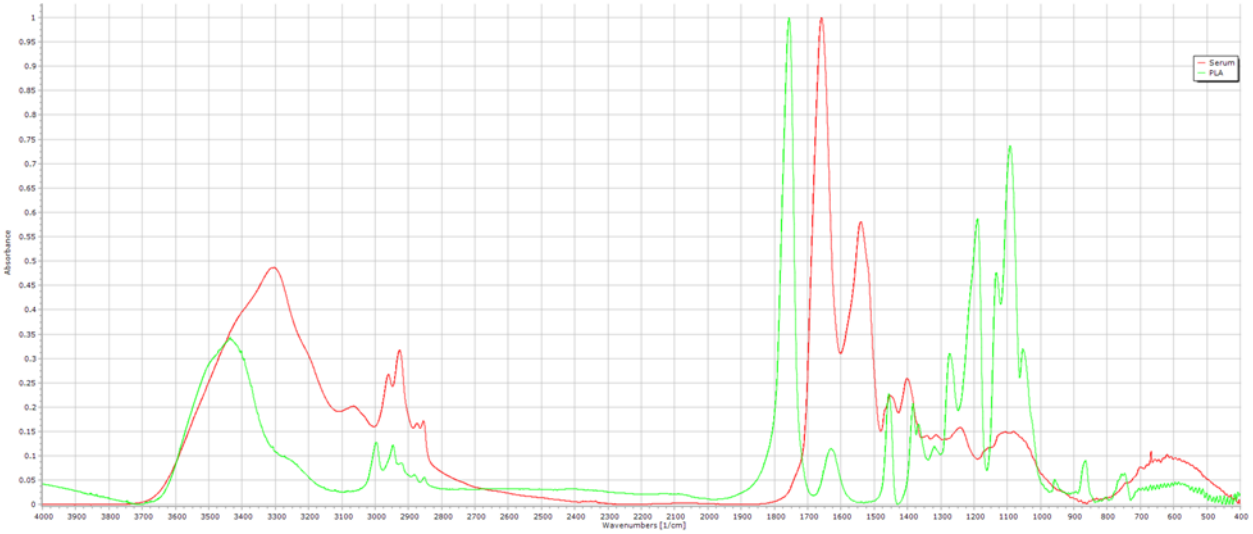
*FT-IR Analysis*

Lyophilization of whole pellet samples was done for a minimum of 12 hours to minimize water interference on the FT-IR. The dried pellets were scanned immediately after lyophilization. The PerkinElmer Spectrum Two FT-IR Spectrometer was used to analyze the pellet samples. 20 scans were taken between 4000 $cm^{-1}$ and 400 $cm^{-1}$.

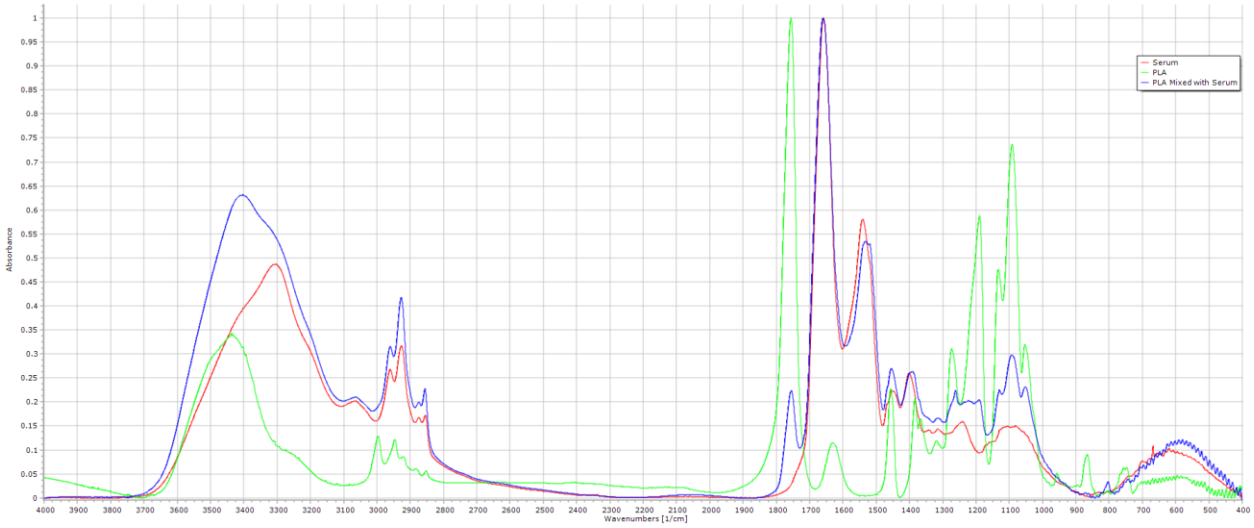
# RESULTS AND DISCUSSION

### Qualitative Analysis of Polymer Absorption in Rat Model via FT-IR

The aim in using FT-IR for quantitative analysis first involves identification of a wavenumber in the two samples that are relatively unique of each other. That is, the most dramatic change in a FT-IR spectrum would come from two individual spectra whose peaks do not coincide with one another. Figure 8 shows an overlay of PLA and serum. In an early assessment, the 1750 $cm^{-1}$ region was deemed a reasonable candidate for peak analysis as a result of the significant contribution that PLA offers. In Figure 4b, a significant peak arises out of a sample of serum that was mixed with a relatively high concentration of PLA (w/w).



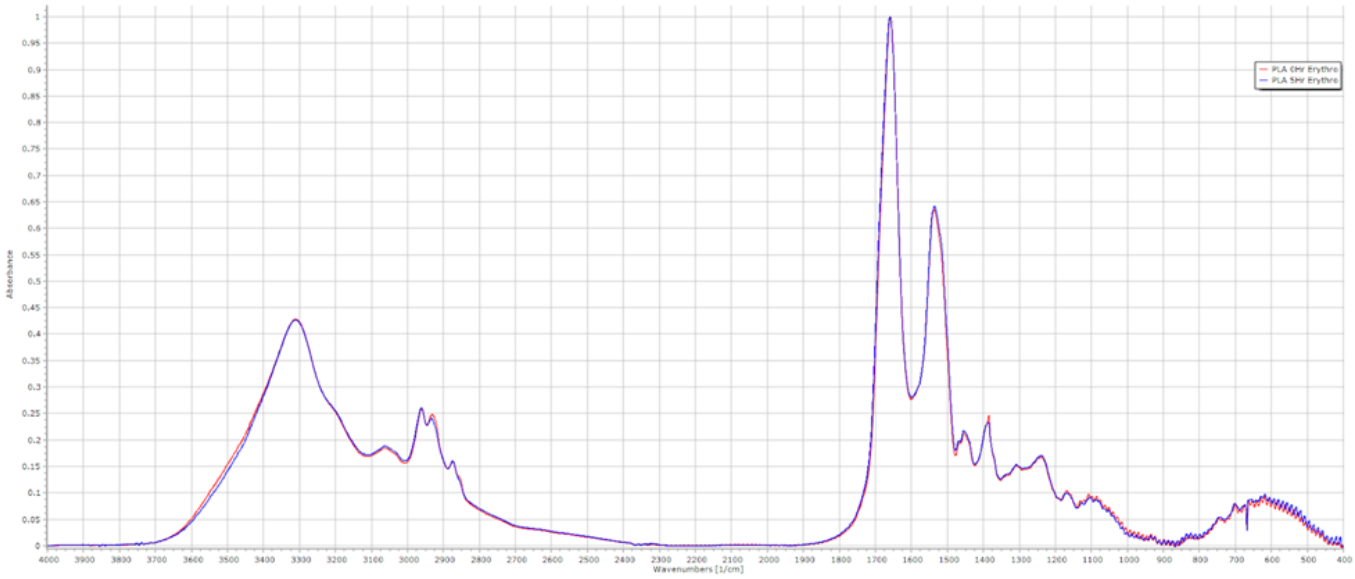

*(a) Comparison of interferograms of PLA NP (green) and SD rat serum (red)*



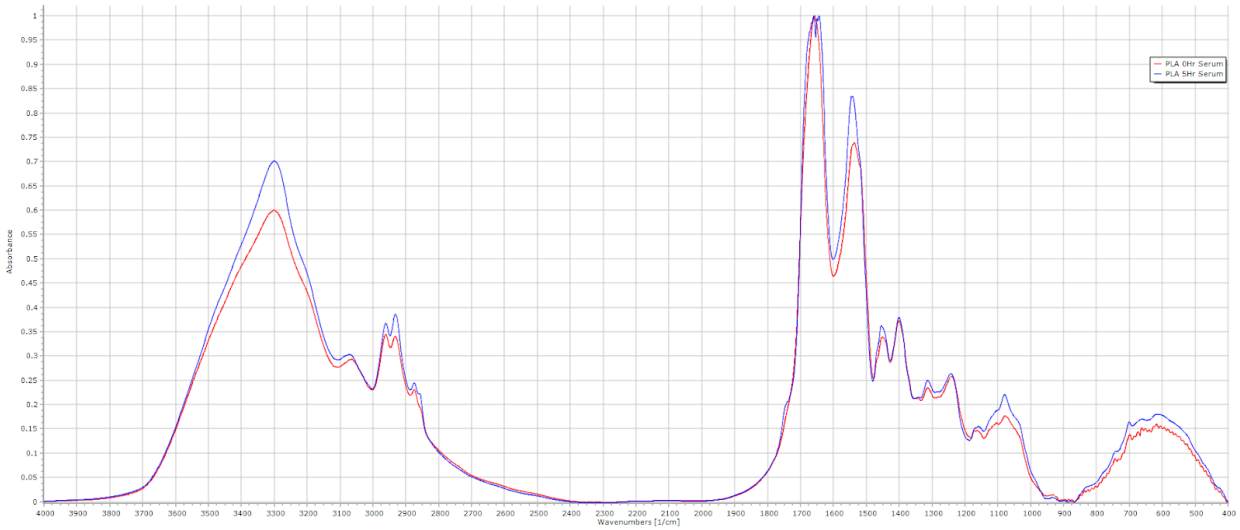

*(b) Interferogram of PLA-Serum mix (blue) compared against baseline PLA and serum interferograms*

*Figure 4: Addition of PLA to SD rat serum results in a change to the resultant interferogram*

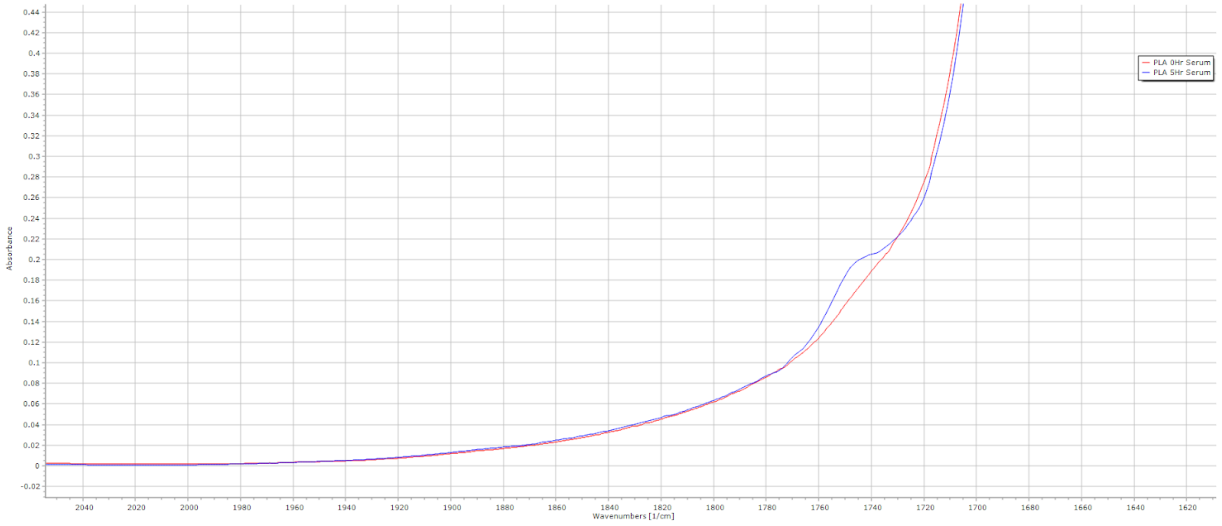
A preliminary study *in vivo* was performed to qualitatively assess PLA nanoparticle absorption in SD rats by isolated loop. PLA was detected in the serum after centrifugation and analysis of both the serum and erythrocyte layers. See Figure 5.




*(a) Erythrocyte layer: 0 Hour (red) and 5 Hour (blue)*




*(b) Serum layer: 0 Hour (red) and 5 Hour (blue)*




*(c) Serum layer: 0 Hour (red) and 5 Hour (blue) at higher resolution*

*Figure 5: Comparison of separated blood components of an SD rat after 5 hour isolated loop*

Analysis of the serum alone offered two key advantages over analysis of the entire blood sample. First, by removing the erythrocyte layer from consideration, we effectively increase the relative ratio of PLA, our analyte of interest, to the biological sample. From this study, the peak near 1750 $cm^{-1}$ was determined as a reasonable candidate for quantitative analysis with applications in vivo.

## Considerations during Method Development

*Aliquot*

When producing 100 mg pellets for FT-IR analysis, KBr and the analyte are typically mixed in a ratio of 99:1. Issues arise in attempting to generate a calibration curve by direct weighing of the analyte. Direct measurements entail that in practice, a small amount of a sample (typically 1 milligram of analyte) must comprise of an accurate ratio of serum and PLA for each calibration curve point. These minute levels can introduce several significant sources of error. In one case, the level of accuracy required for these measurements are beyond the capabilities of the instrumentation available in the laboratory. A more significant source of error comes from weighing out material that has been previously lyophilized. Lyophilization tends to produce powders that are filled with static charge [14]. Attempts to weigh out values as little as 0.01 milligrams of a lyophilized sample (e.g. as needed to correspond to 1% PLA in serum) would oftentimes result in a significant loss of product between transferring the sample between different containers.

There were several advantages that inspired the use of aliquoting from a liquid medium to produce an accurate calibration curve. Firstly, by measuring the necessary masses of the FT-IR component from a stock solution, we minimize the amount of time spent weighing material onto the scale-- a time consuming process that takes up most of the time needed to produce a pellet for FT-IR. Secondly, aliquoting from a liquid allows us to produce measurements of a small quantity with an accuracy that surpasses the capabilities of utilizing scale alone. Finally, having the FT-IR samples in a liquid medium would allow the components to mix these components more uniformly than the standard procedure involving manually mixing with a mortar and pestle.

There were several key criteria that must be met for this method to be feasible: the FT-IR sample components must either dissolve completely, or suspend in liquid solution. This is to ensure that the mass of the components can be reliably measured from pipetting. Any material that quickly settles in solution could lead to inaccurate weighing. In this method, there were three components of interest in preparation of a FT-IR pellet in water: KBr, lyophilized serum powder, and PLA nanoparticles. KBr and dry serum powder are both water-soluble. While PLA nanoparticles are not soluble in water, they are smaller than 10 microns and can stay suspended in water [15]. See Figure 6.

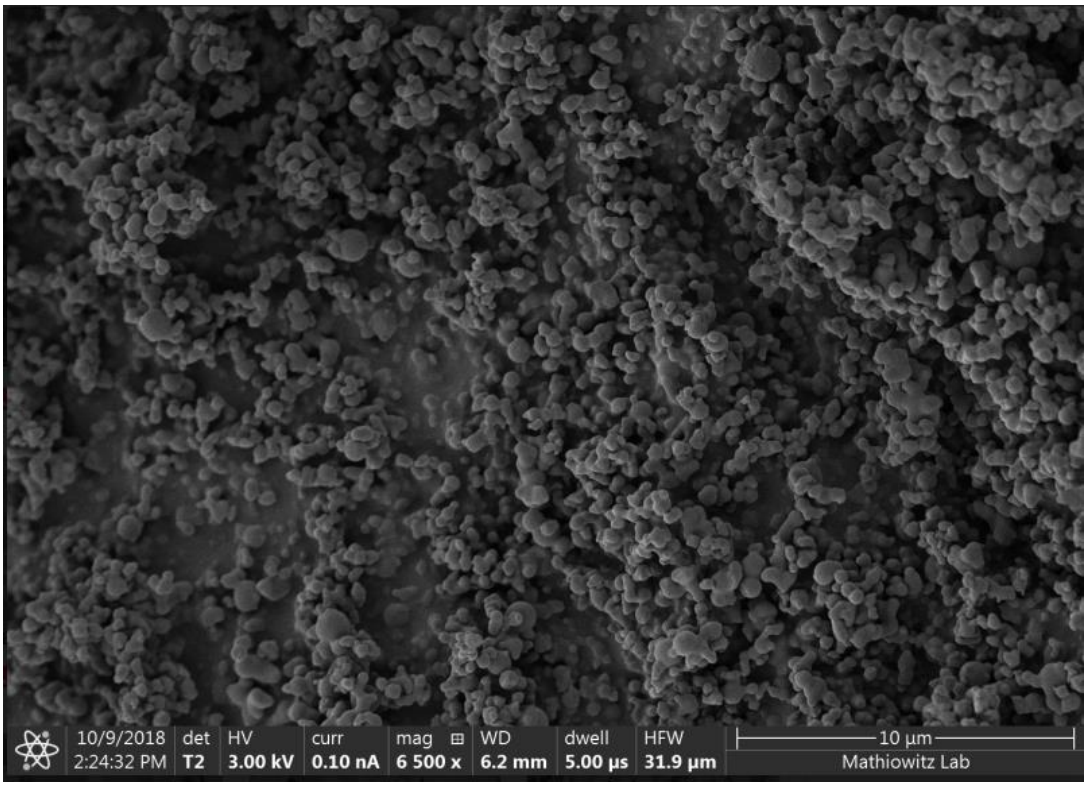



*Figure 6: SEM image of PLA nanoparticles produced by PIN*

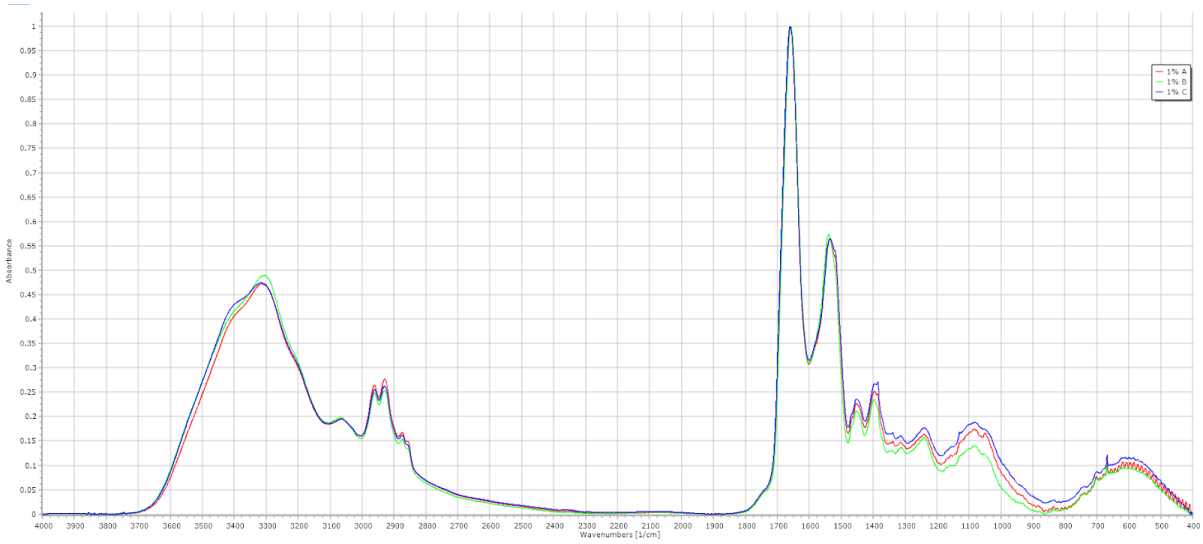
*FT-IR Data Processing*

The spectra were processed using both PerkinElmer Spectrum and Spectragryph. Each sample was baseline corrected first with the *Baseline* function on the PerkinElmer Software. A simple baseline function (with the x-axis point of reference set at 4000 $cm^{-1}$ ) was then applied to each spectrum via Spectragryph. Each spectrum was normalized against the peak near 1650$cm^{-1}$ on Spectragryph. In doing so, all values on the y-axis are converted to peak ratio values compared to the peak near $cm^{-1}$. These values from both the calibration curve and in vivo study at 1750 $cm^{-1}$ were recorded as shown in Table 1.

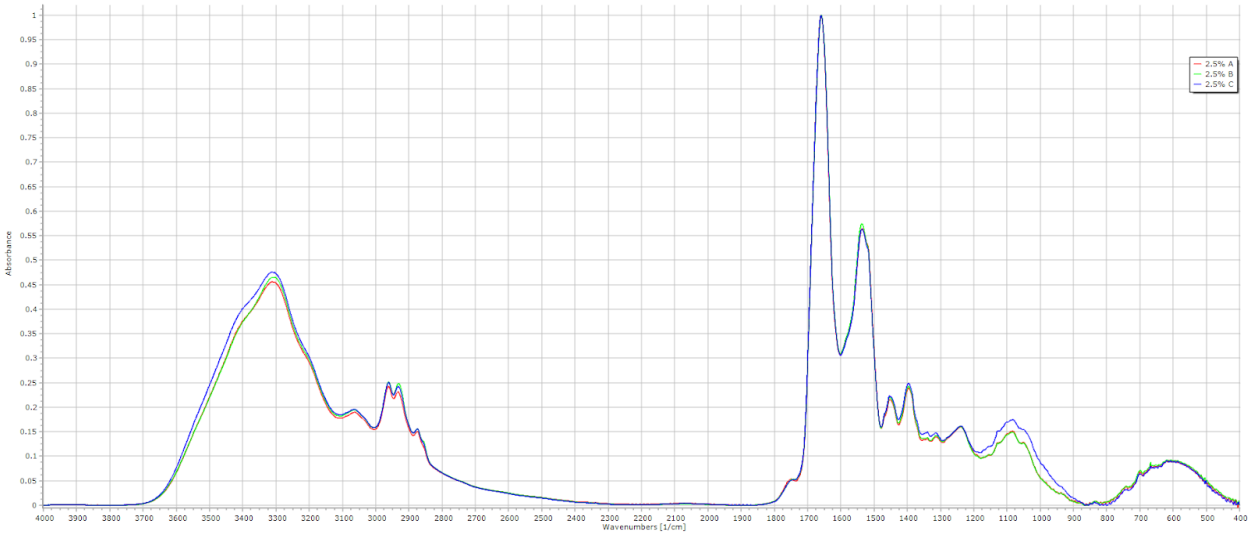
**Table 1: Normalized peak heights near 1750 $cm^{-1}$ for Calibration Curve**

| PLA concentration in serum (w/w) | Normalized peak at 1750 $cm^{-1}$ | Wavenumber of peak $cm^{-1}$ | Mean | Standard Deviation |
|---|---|---|---|---|
| 0.0% | 0.03638 | 1750 | 0.03094 | 0.004715856 |
| | 0.02801 | 1750 | | |
| | 0.02843 | 1750 | | |
| 1.0% | 0.04122 | 1750 | 0.04091 | 0.001518914 |
| | 0.03926 | 1750 | | |
| | 0.04225 | 1750 | | |
| 2.5% | 0.053061 | 1750.8 | 0.052463667 | 0.000722932 |
| | 0.05267 | 1743.5 | | |
| | 0.05166 | 1744.8 | | |
| 5.0% | 0.090811 | 1754.6 | 0.084666333 | 0.005466507 |
| | 0.080343 | 1753.1 | | |
| | 0.082845 | 1746.9 | | |
| 7.5% | 0.11933 | 1754.8 | 0.12155 | 0.004402034 |
| | 0.1187 | 1753.4 | | |
| | 0.12662 | 1754.7 | | |
| 10.0% | 0.16472 | 1755.5 | 0.15164 | 0.011408961 |
| | 0.14646 | 1754.9 | | |
| | 0.14374 | 1754.5 | | |
| 12.5% | 0.22385 | 1755.1 | 0.220853333 | 0.002667065 |
| | 0.21997 | 1754.6 | | |
| | 0.21874 | 1755.3 | | |
| 15.0% | 0.31289 | 1755.8 | 0.29403 | 0.019586495 |
| | 0.29541 | 1756.6 | | |
| | 0.27379 | 1755.9 | | |

No peak ratio value could be determined for PLA concentrations of 0 and 1%. The values listed were taken from the normalized absorbance heights (post processing) at 1750 $cm^{-1}$. Qualitative analysis of the region near 1750 $cm^{-1}$ points to a limit of detection of 1%. Peak ratio values were available closer to 2.5%, suggesting a limit of quantification at this concentration. Refer to Figure 7.




*(a) 1% PLA (w/w)*




*(b) 2.5% PLA (w/w)*

*Figure 7: Calibration Curve Interferograms. Peaks at the 1750 $cm^{-1}$ region were only visible with a minimum PLA concentration of 2.5%.*

The upper limit of the linear region was also assessed. The differences between 10-12.5% and 12.5-15% seem to increment at similar values, but both differences are greater than those within the range of 2.5-10%. See Figure 8.

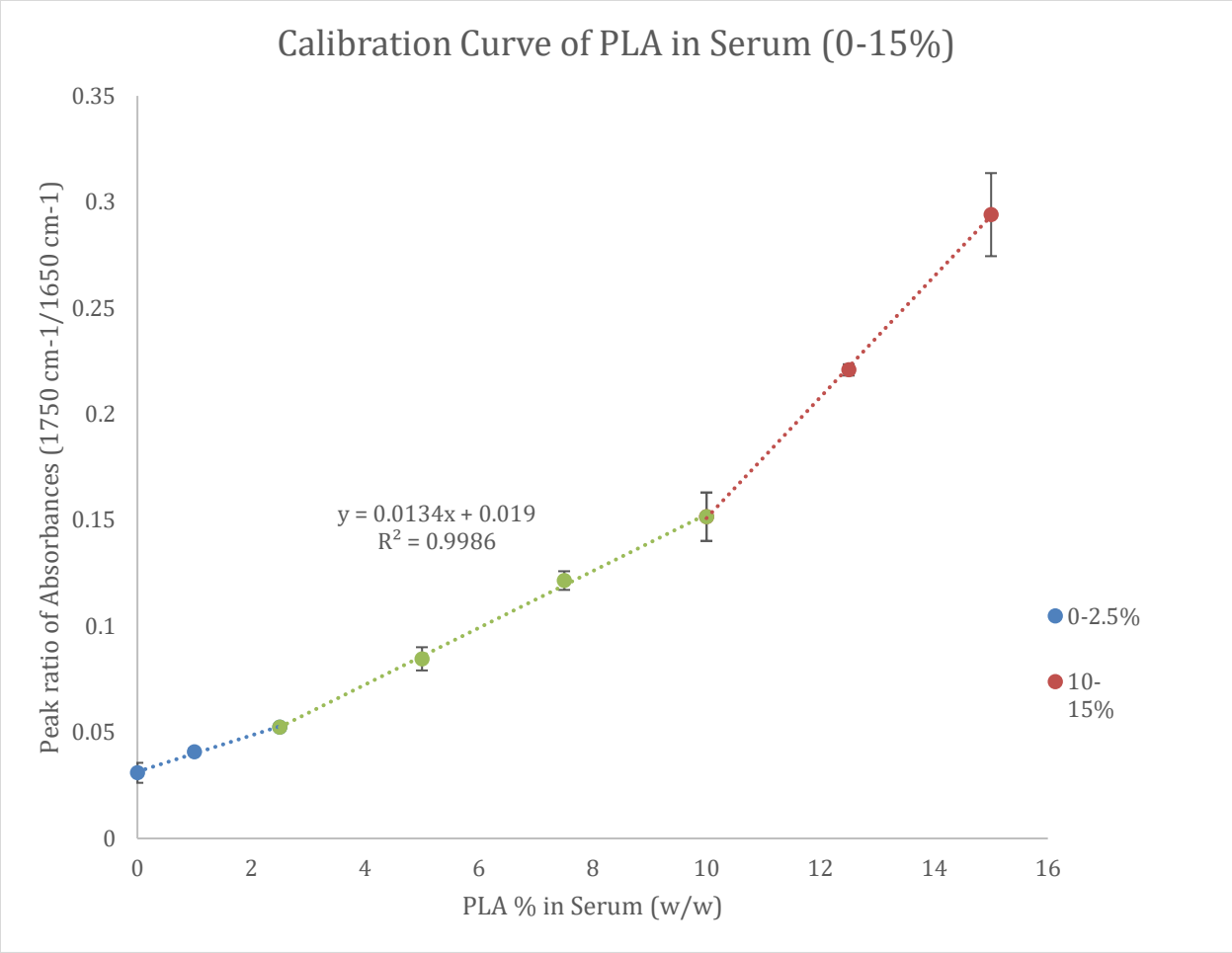



*Figure 8: Calibration Curve of PLA-Rat Serum. The linear range appears to be between 2.5 and 10% PLA (w/w)*

**Modification of the Calibration Curve by Comparing Differences from Baseline**

*Generation of a Calibration Curve Using Differences from Baseline*

Comparison of the changes in the peak ratio values from baseline was performed to calculate nanoparticle absorption *in vivo*. Concentration values within the linear range are assumed to behave according to the following relationship:

$$y_1 = mx_1 + b$$

For a given curve and for any two points within the linear region, there is a direct relationship that can be found between the concentration of PLA and the peak ratio value at 1750 $cm^{-1}$ as shown:

$$y_2 - y_1 = (mx_2 + b) - (mx_1 + b)$$

$$\Delta y = m\Delta x$$

Table 2 lists the differences in PLA concentrations and peak ratios after subtracting the lowest value of the linear region (2.5%) from each value within our desired range (2.5-10%). The modified calibration curve generated from these values (see Figure 9) maintains linearity ($R^2$ = 0.9986) and is intended for quantification of PLA so long that the difference in the peak ratios from baseline (Δy) correspond to a concentration of PLA within the range of 0-7.5% (w/w).

**Table 2: PLA Concentration Values Compared to Peak Ratio Differences (Δy)**

| PLA concentration | Peak ratio difference from baseline (Δy) | Standard deviation |
|---|---|---|
| 0 | 0 | 0.00102238 |
| 2.5 | 0.032019333 | 0.005514103 |
| 5 | 0.068903 | 0.004461001 |
| 7.5 | 0.098993 | 0.011431843 |

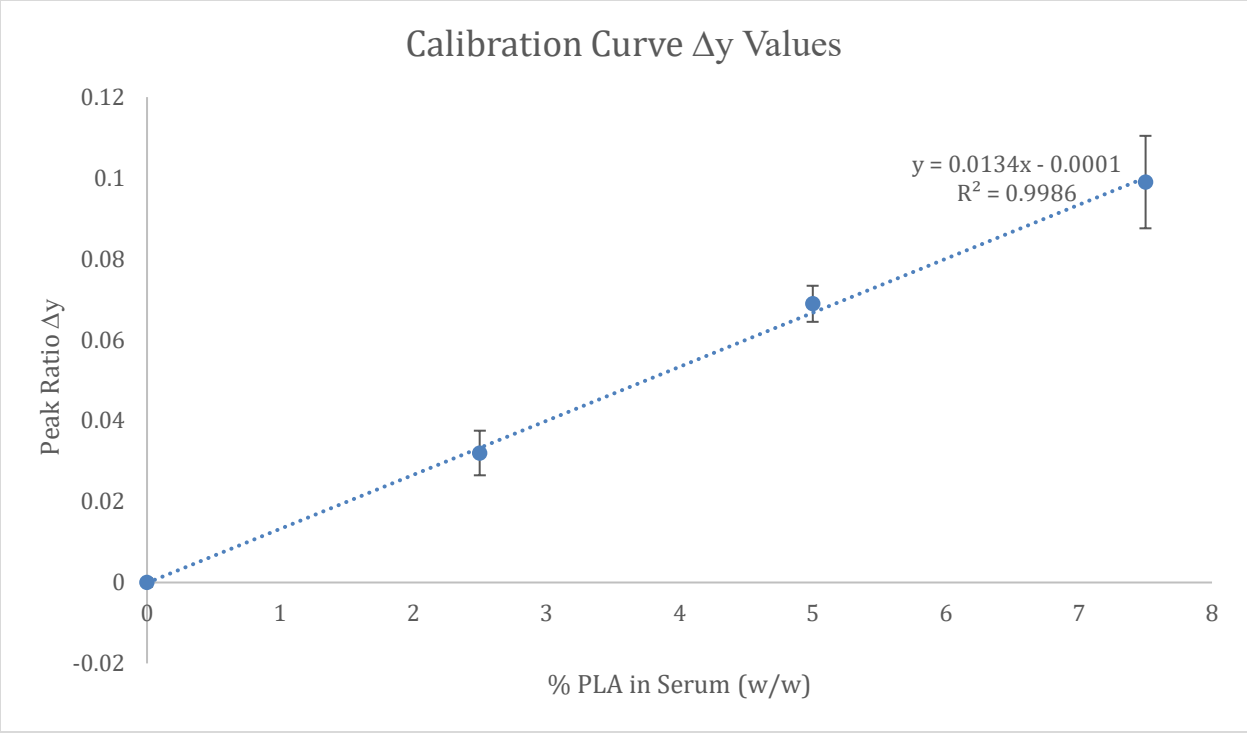



*Figure 9: Calibration Curve Generated from Differences in the Linear Region*

*Application of the calibration curve*

The results from the preliminary study demonstrated a noticeable change in the peak at 1750 $cm^{-1}$ compared to baseline. However, the change did not result in a signal that was strong enough to correlate to a value within the limit of quantification.

The standard deviations of the peak ratio values did not signficantly overlap between different PLA concentrations. As a result, the resolution of the calibration curve was deemed sensitive enough that spiking could be applied to calculate PLA concentrations originally out of the quantification limit. Table 3 details the normalized peak heights at 1750 $cm^{-1}$ after 5% spike addition to the 4 rats. It is important to note here that the spikes of 5% did not provide peak ratios comparable to those found in the calibration curve. This point is elaborated further discussed in the conclusion.

**Table 3: In vivo peak values near 1750 $cm^{-1}$ after spike addition of 5% PLA to serum samples (w/w)**

| Rat 1 5% Spike | | | | |
|---|---|---|---|---|
| Timepoint | Normalized peak near 1750 cm-1 | Wavenumber of peak (cm-1) | Mean | Standard deviation |
| 0 Hr | 0.083194 | 1750.8 | 0.078287333 | 0.00424936 |
| | 0.075857 | 1749.2 | | |
| | 0.0775811 | 1752 | | |
| 5 Hr | 0.071431 | 1746.6 | 0.076330333 | 0.006814164 |
| | 0.073448 | 1751.4 | | |
| | 0.084112 | 1751.7 | | |

| Rat 2 5% Spike | | | | |
|---|---|---|---|---|
| Timepoint | Normalized peak near 1750 cm-1 | Wavenumber of peak (cm-1) | Mean | Standard deviation |
| 0 Hr | 0.061719 | 1749.4 | 0.060403333 | 0.001227076 |
| | 0.05929 | 1753.3 | | |
| | 0.06201 | 1753.6 | | |
| 5 Hr | 0.06613 | 1747.04 | 0.072441667 | 0.006450198 |
| | 0.072173 | 1751.3 | | |
| | 0.079022 | 1751.5 | | |

| Rat 3 5% Spike | | | | |
|---|---|---|---|---|
| Timepoint | Normalized peak near 1750 cm-1 | Wavenumber of peak (cm-1) | Mean | Standard deviation |
| 0 Hr | 0.06088 | 1746.59 | 0.058889333 | 0.003717862 |
| | 0.0546 | 1744.8 | | |
| | 0.061188 | 1750 | | |
| 5 Hr | 0.083485 | 1752.3 | 0.089045667 | 0.005997615 |
| | 0.088251 | 1752.3 | | |
| | 0.095401 | 1753.1 | | |

| Rat 4 5% Spike | | | | |
|---|---|---|---|---|
| Timepoint | Normalized peak near 1750 cm-1 | Wavenumber of peak (cm-1) | Mean | Standard deviation |
| 0 Hr | 0.069336 | 1746.3 | 0.067880667 | 0.00267952 |
| | 0.064847 | 1748.8 | | |
| | 0.069459 | 1751.7 | | |
| 5 Hr | 0.069391 | 1751.5 | 0.069244667 | 0.002491725 |
| | 0.066683 | 1749.8 | | |
| | 0.07166 | 1751.8 | | |

The differences in the means between the 0 and 5-hour time points of each rat are then calculated to determine the level of PLA NP absorption based on the calibration curve. These values are shown in Table 4. Based on the predicted values of calculated PLA NP in serum from spiking, there are variable degrees of nanoparticle absorption among the 4 subjects.

**Table 4: Predicted Levels of PLA in Serum from Peak Differences**

| Subject | Peak Difference | Standard deviation | Calculated PLA in Serum (%) | Standard Deviation (%) |
|---|---|---|---|---|
| Rat 1 | -0.001957 | 0.00803056 | -0.146044776 | 0.599295489 |
| Rat 2 | 0.012038333 | 0.00656879 | 0.898383085 | 0.489990968 |
| Rat 3 | 0.030156333 | 0.007056478 | 2.250472637 | 0.526602862 |
| Rat 4 | 0.001364 | 0.00362144 | 0.101791045 | 0.227025669 |

# CONCLUSION

In this study, PLA offers challenges because the peak that showed a significant rise in absorbance at higher concentrations, around 1750 $cm^{-1}$, also coincides with a peak that arises due to ester bonds in lipids [16, 17, 18]. This may explain some of the results in the *in vivo* study, where a shoulder is visible in some rats at the 1750 $cm^{-1}$ region even before administration of the PLA NP solution. Future studies could explore lipid levels in blood and their relationship with FT-IR readings at 1750 $cm^{-1}$. Other peaks besides the one at 1750 $cm^{-1}$ could lead to more accurate predictions of blood PLA concentrations and future studies could explore this.

In conclusion, use of FT-IR spectroscopy to quantify polymer levels in biological samples demonstrates promise and broad applications in the field of drug delivery as a fast, low cost, nondestructive, and convenient method. The work presented in this thesis has demonstrated precision in the method. Future work is required to further validate this method.

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# List of Tables

**Table 5: Aliquot Preparation for Calibration Curve**

| Material weight (mg) | | |
|---|---|---|
| PLA | Serum | KBr |
| 0.150 | 0.850 | 99.000 |
| 0.125 | 0.875 | 99.000 |
| 0.100 | 0.900 | 99.000 |
| 0.075 | 0.925 | 99.000 |
| 0.050 | 0.950 | 99.000 |
| 0.025 | 0.975 | 99.000 |
| 0.010 | 0.990 | 99.000 |
| 0.000 | 1.000 | 99.000 |

| Stock solution concentrations (w/v) | | |
|---|---|---|
| Analyte | Weight (mg) | Water (mL) |
| PLA | 10 | 10 |
| Serum | 50 | 50 |
| KBr | 6000 | 30 |

| Ratios for Standards | | | | |
|---|---|---|---|---|
| Concentration | PLA (mL) | Serum (mL) | KBr (mL) | Total volume (mL) |
| 15.0% | 0.30 | 1.70 | 0.99 | 2.99 |
| 12.5% | 0.25 | 1.75 | 0.99 | 2.99 |
| 10.0% | 0.20 | 1.80 | 0.99 | 2.99 |
| 7.5% | 0.15 | 1.85 | 0.99 | 2.99 |
| 5.0% | 0.10 | 1.90 | 0.99 | 2.99 |
| 2.5% | 0.05 | 1.95 | 0.99 | 2.99 |
| 1.0% | 0.02 | 1.98 | 0.99 | 2.99 |
| 0.0% | 0.00 | 2.00 | 0.99 | 2.99 |

**Table 6: Spiking Calculations**

| Material weight (mg) | | |
|---|---|---|
| PLA | Sample | KBr |
| 0.050 | 0.950 | 99.000 |
| 0.000 | 1.000 | 99.000 |

| Stock solution concentrations (w/v) | | |
|---|---|---|
| Analyte | Weight (mg) | Water (mL) |
| PLA | 10 | 20 |
| Serum | 20 | 20 |
| KBr | 10000 | 20 |

| Ratios for Spiking | | | | |
|---|---|---|---|---|
| Concentration | PLA (mL) | Serum (mL) | KBr (mL) | Total volume (mL) |
| 5% Spike | 0.40 | 3.80 | 0.792 | 4.992 |
| 0% Spike | 0.00 | 4.00 | 0.792 | 4.992 |

**Table 7: Preliminary Isolated Loop Study**

| Subject | Sex | Weight (g) | Polymer dosage (mg) | Remaining polymer after administration (mg) | True dosage (mg) |
|---|---|---|---|---|---|
| Rat 1 | N/A | 766 | 84.6 | 0 | 84.6 |

**Table 8: Isolated Loop with PLA NP**

| Subject | Sex | Weight (g) | Polymer dosage (mg) | Remaining polymer after administration (mg) | True dosage (mg) |
|---|---|---|---|---|---|
| Rat 1 | Female | 367 | 110.2 | 19.2 | 91.0 |
| Rat 2 | Female | 270 | 110.3 | 33.2 | 77.1 |
| Rat 3 | Male | 450 | 110.4 | 43.9 | 66.5 |
| Rat 4 | Male | 510 | 110.8 | 35.8 | 75.0 |

# List of Figures

*Calibration Curve Interferograms*

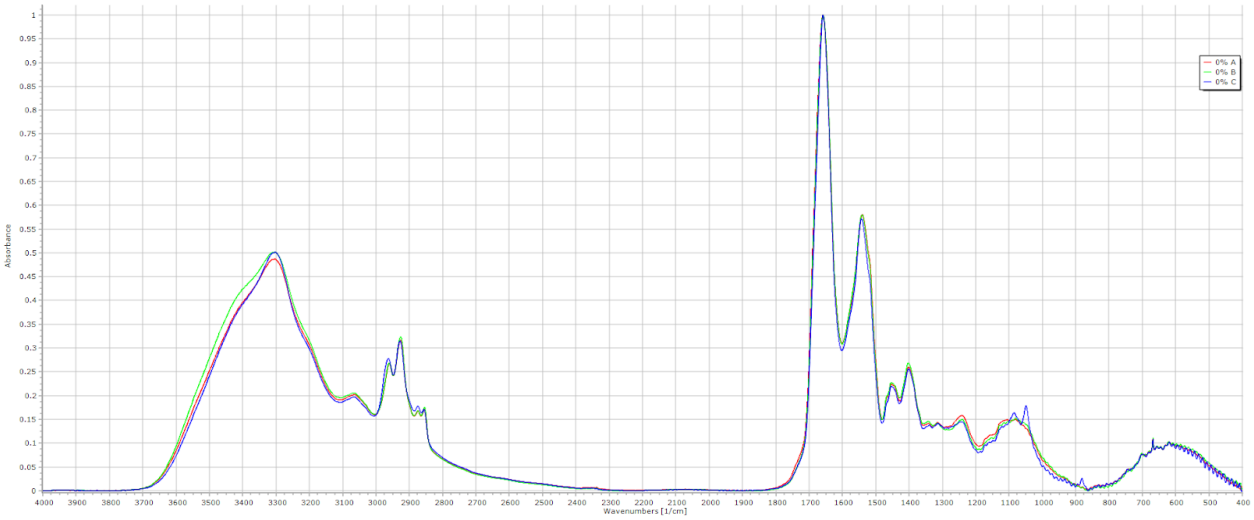


*Figure 10: 0% PLA in serum (w/w)*

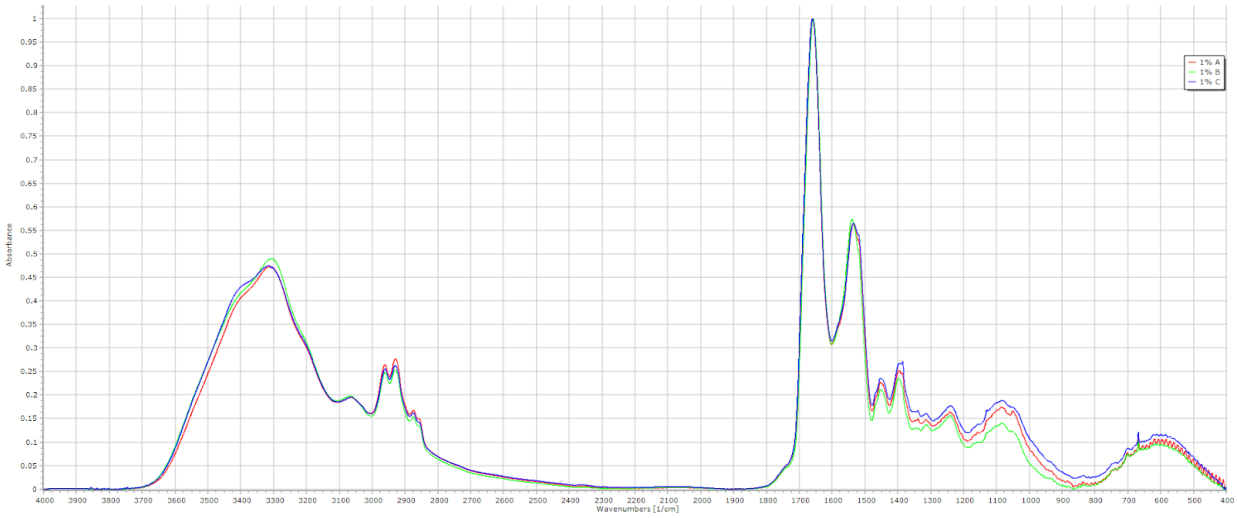


*Figure 11: 1% PLA in serum (w/w)*

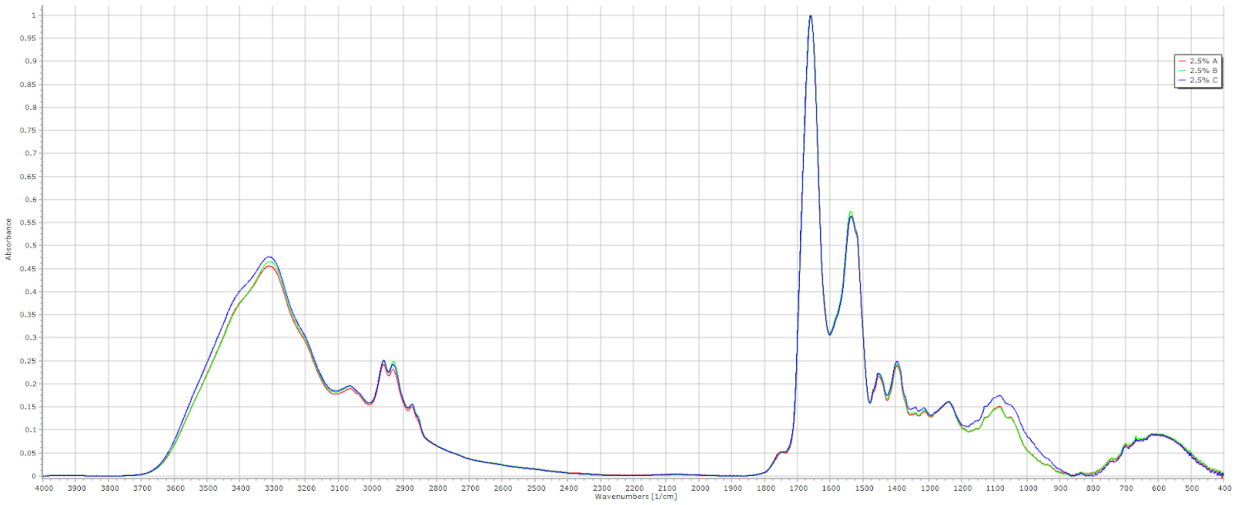


*Figure 12: 2.5% PLA in serum (w/w)*

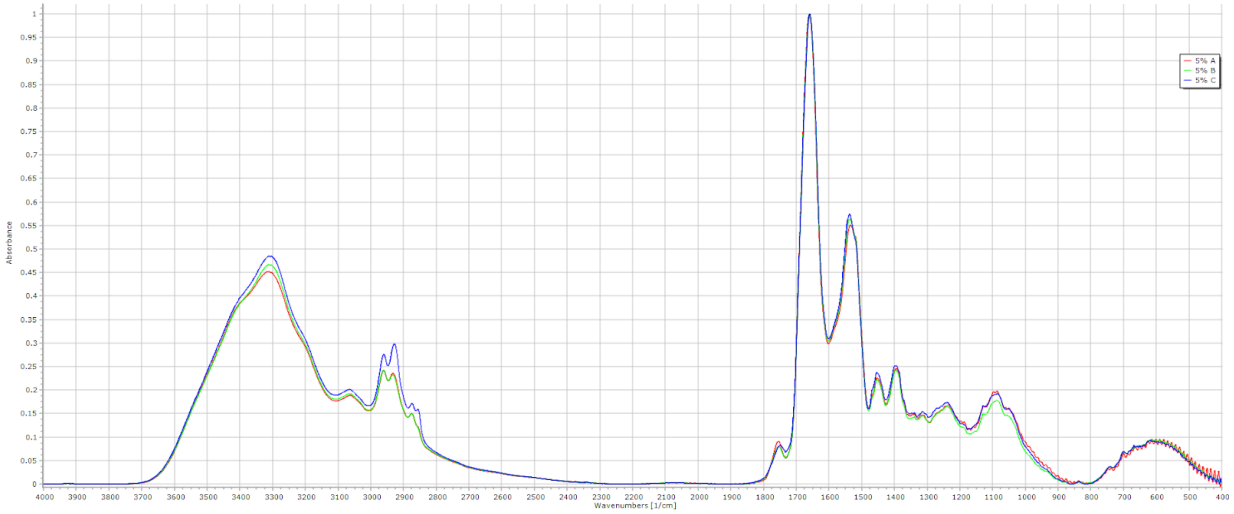


*Figure 13: 5% PLA in serum (w/w)*

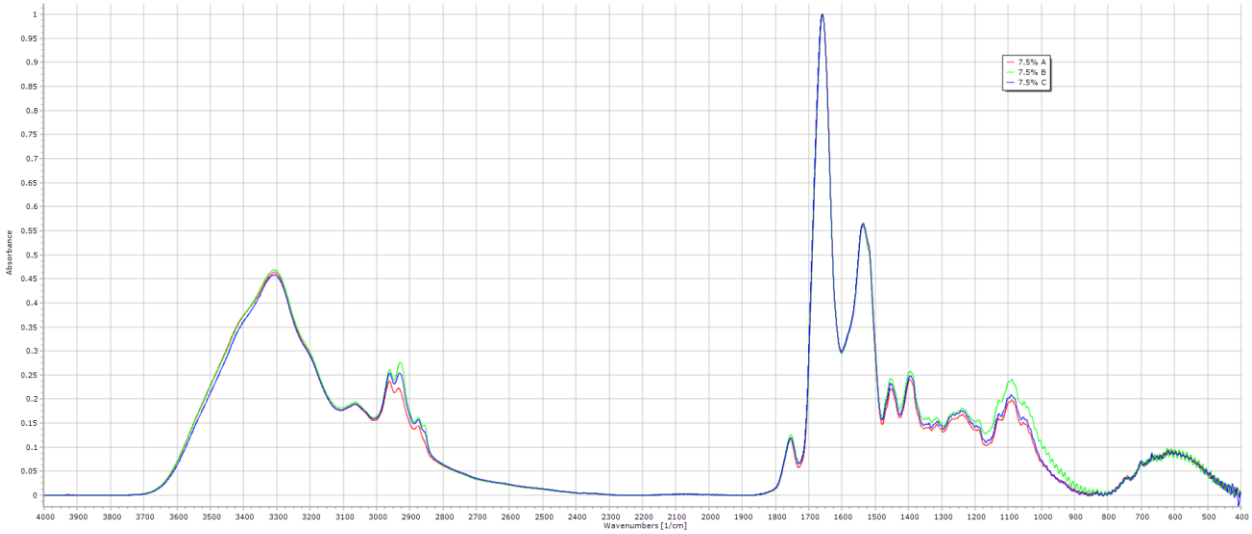


*Figure 14: 7.5% PLA in serum (w/w)*

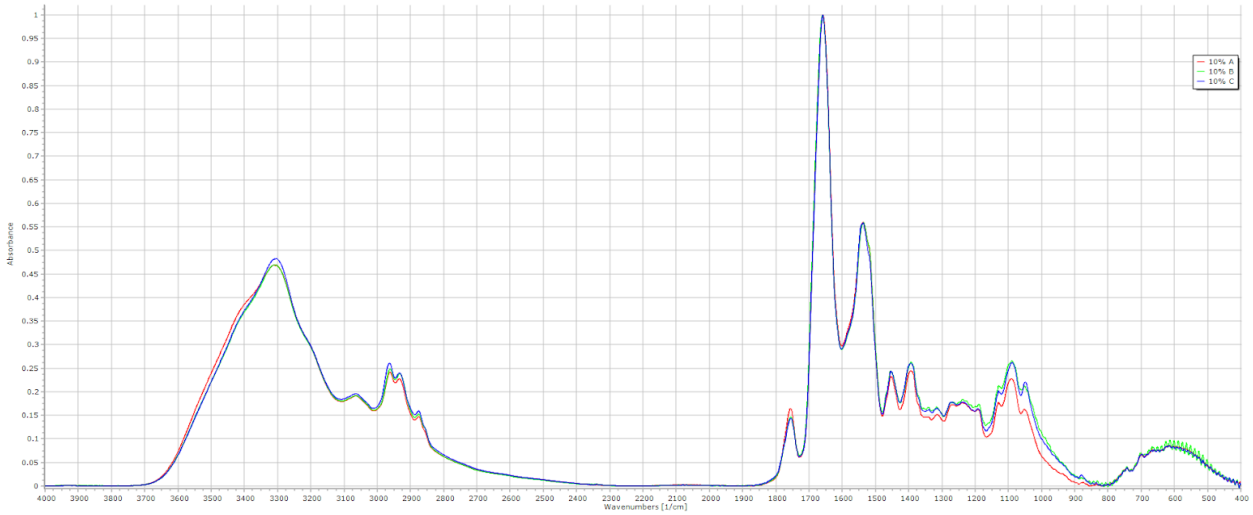


*Figure 15: 10% PLA in serum (w/w)*

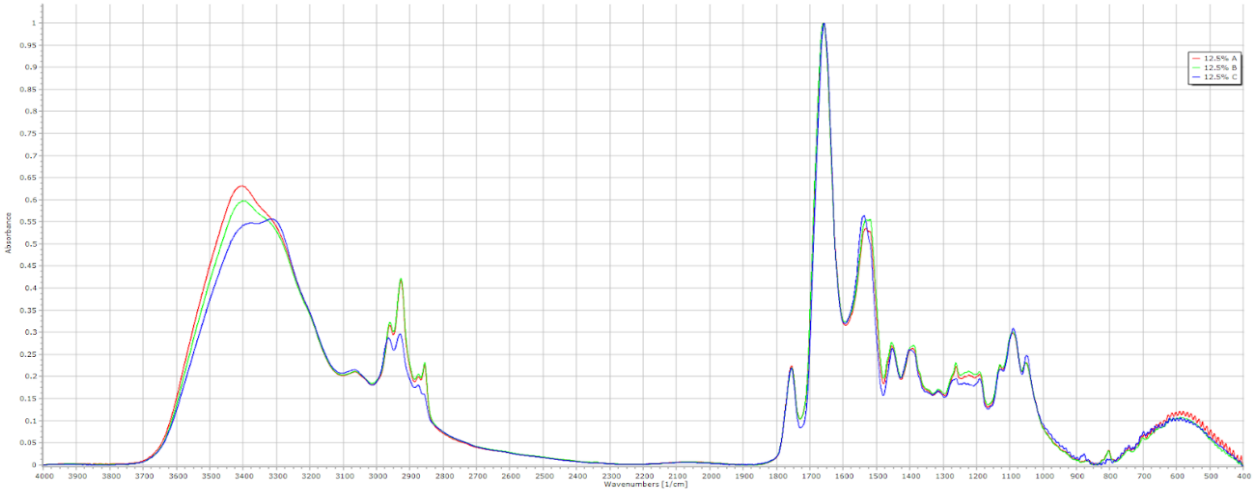


*Figure 16: 12.5% PLA in serum (w/w)*

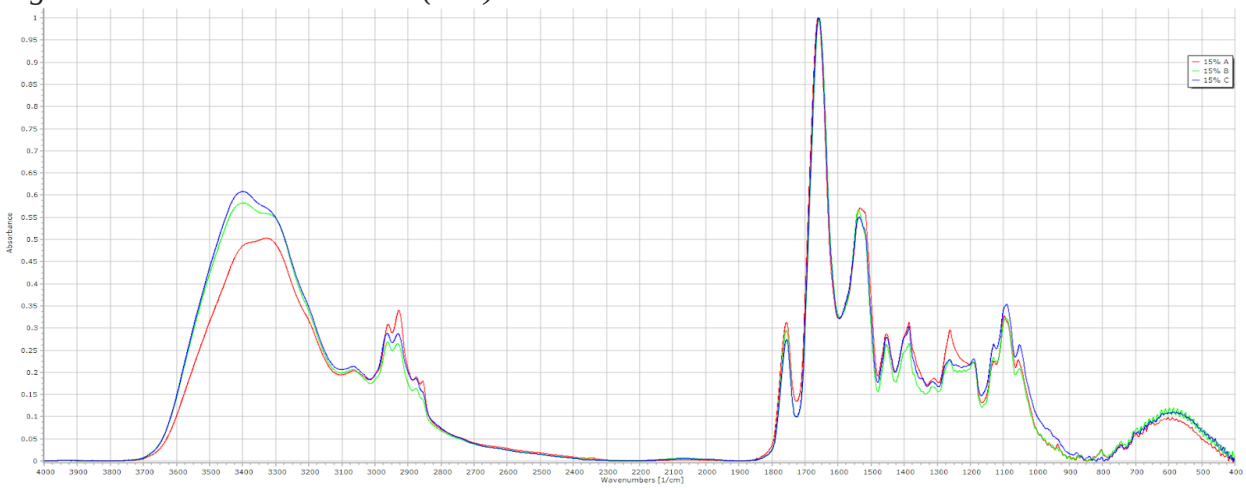


*Figure 17: 15% PLA in serum (w/w)*

*In Vivo Data*

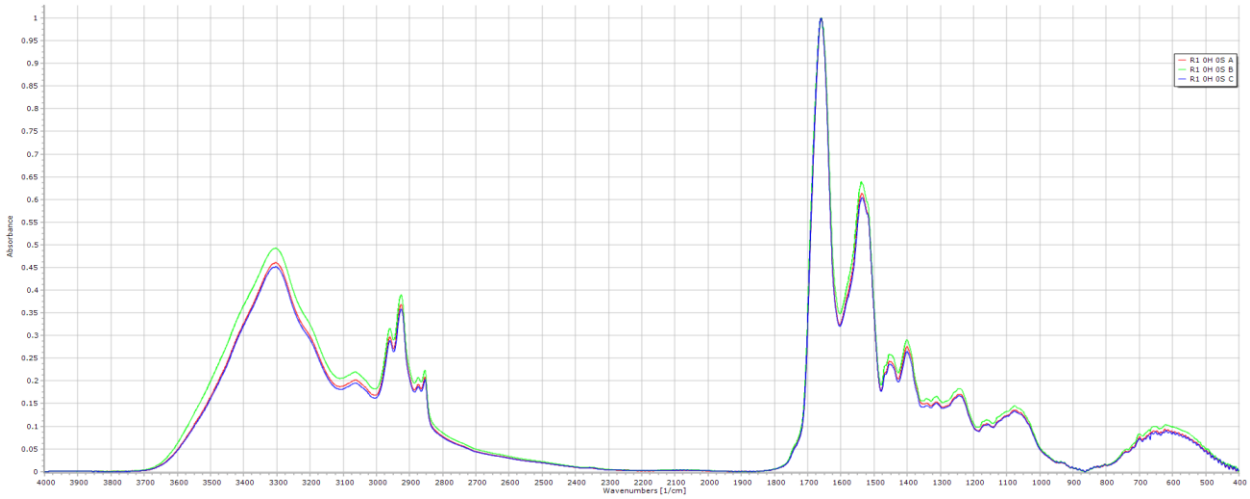


*Figure 18: Rat 1—0 Hour 0% Spike*

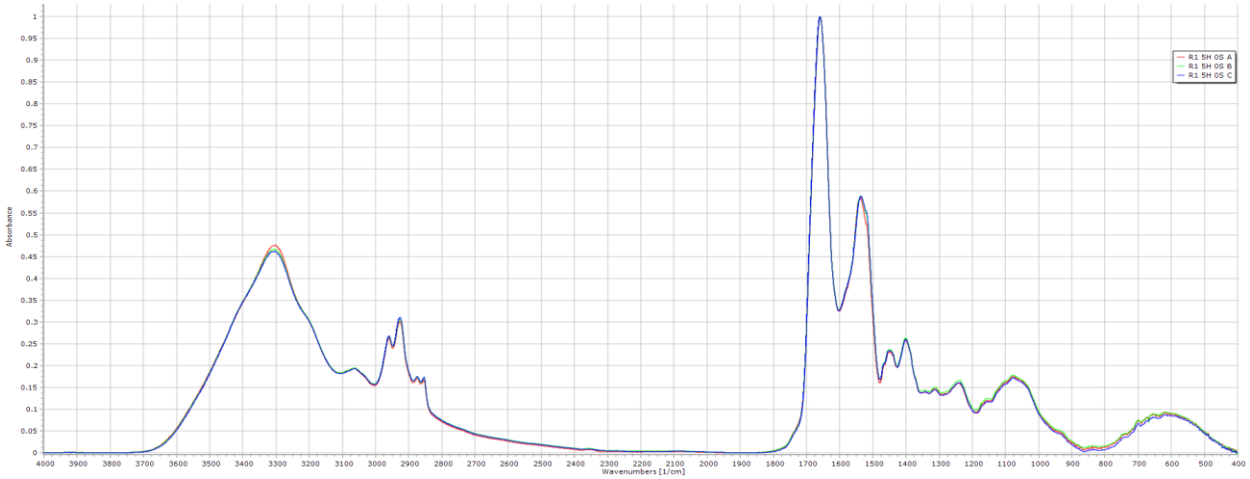


*Figure 19: Rat 1—5 Hour 0% Spike*

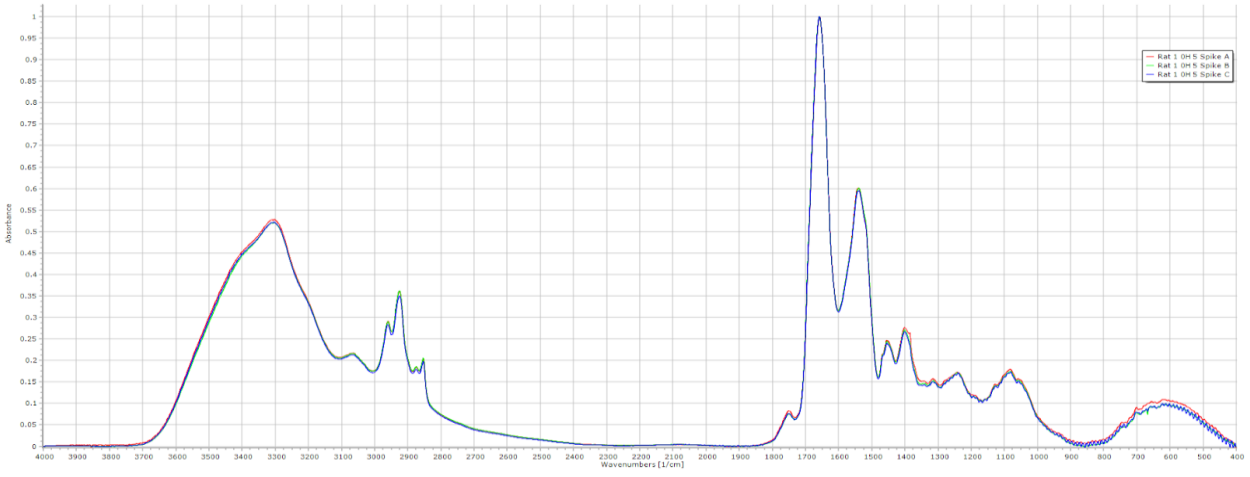


*Figure 20: Rat 1—0 Hour 5% Spike*

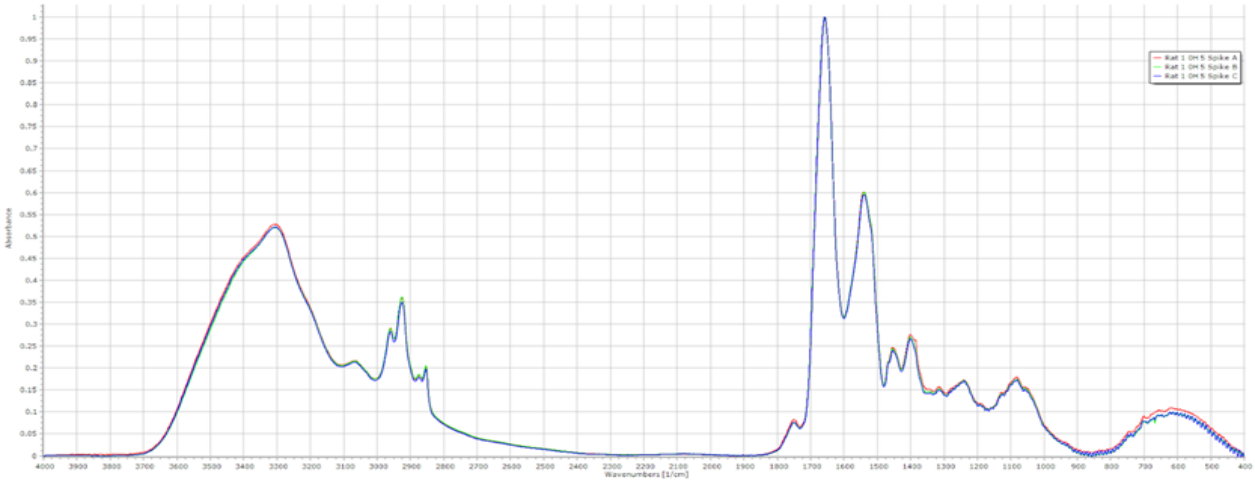


*Figure 21: Rat 1—5 Hour 5% Spike*

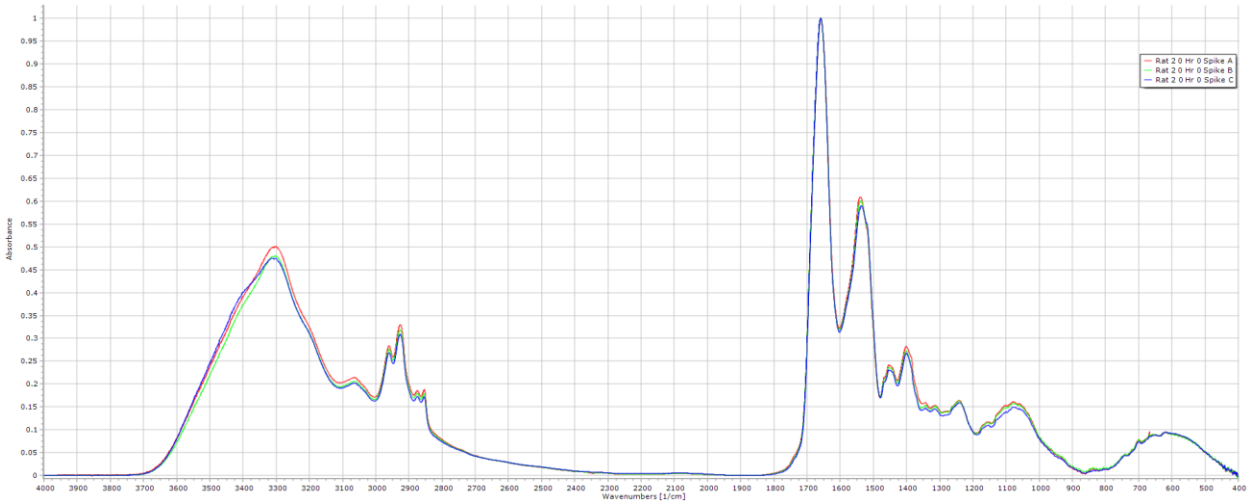


*Figure 22: Rat 2—0 Hour 0% Spike*

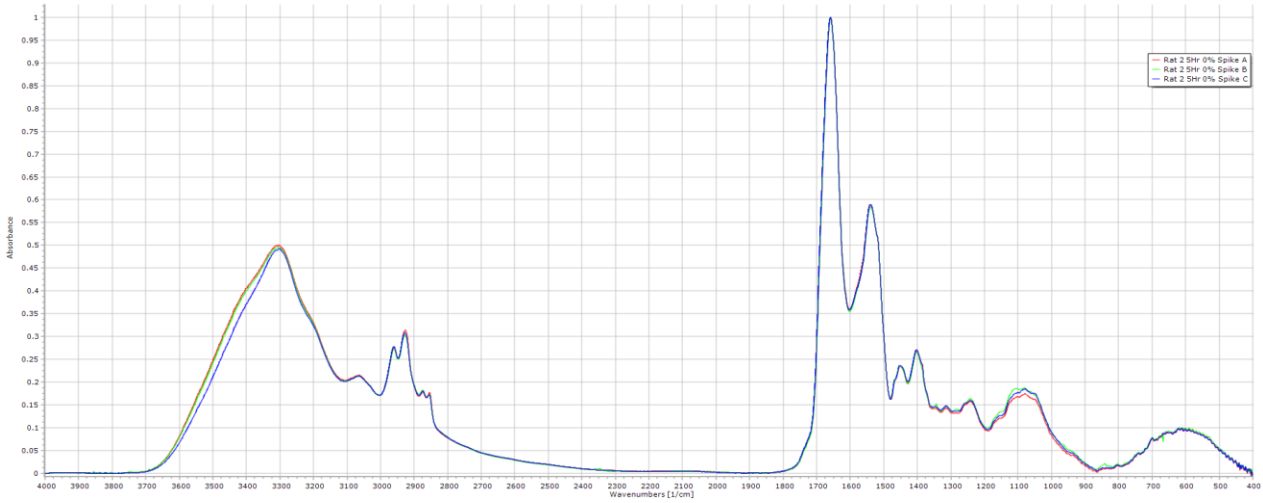


*Figure 23: Rat 2—5 Hour 0% Spike*

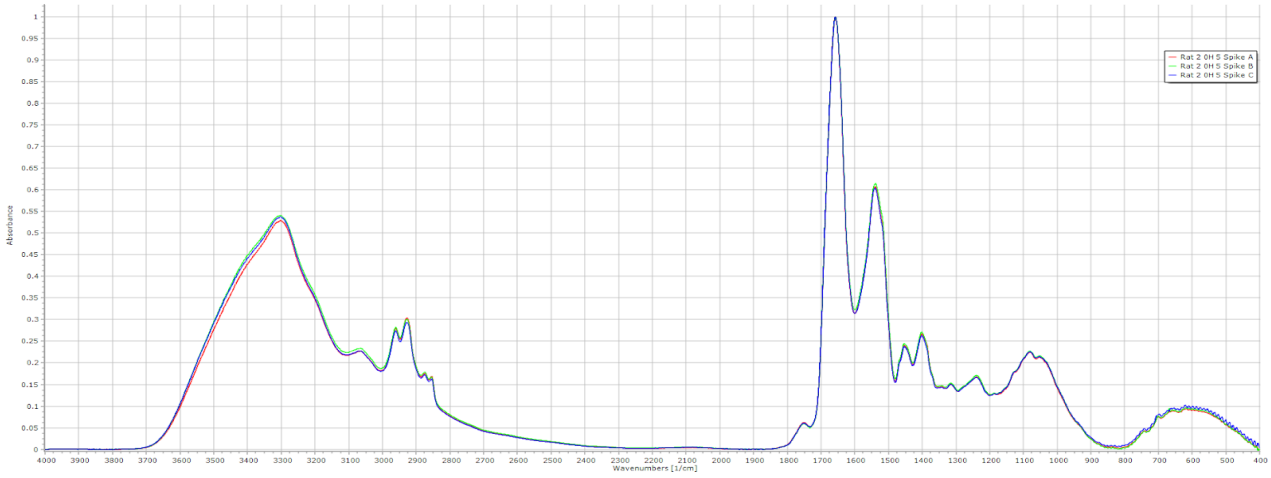


*Figure 24: Rat 2—0 Hour 5% Spike*

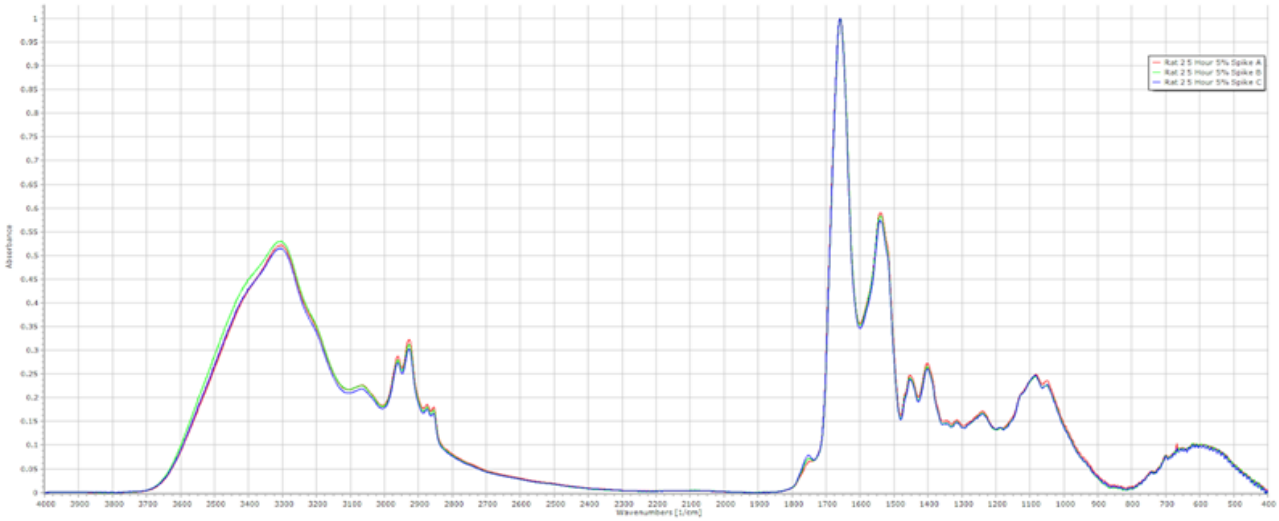


*Figure 25: Rat 2—5 Hour 5% Spike*

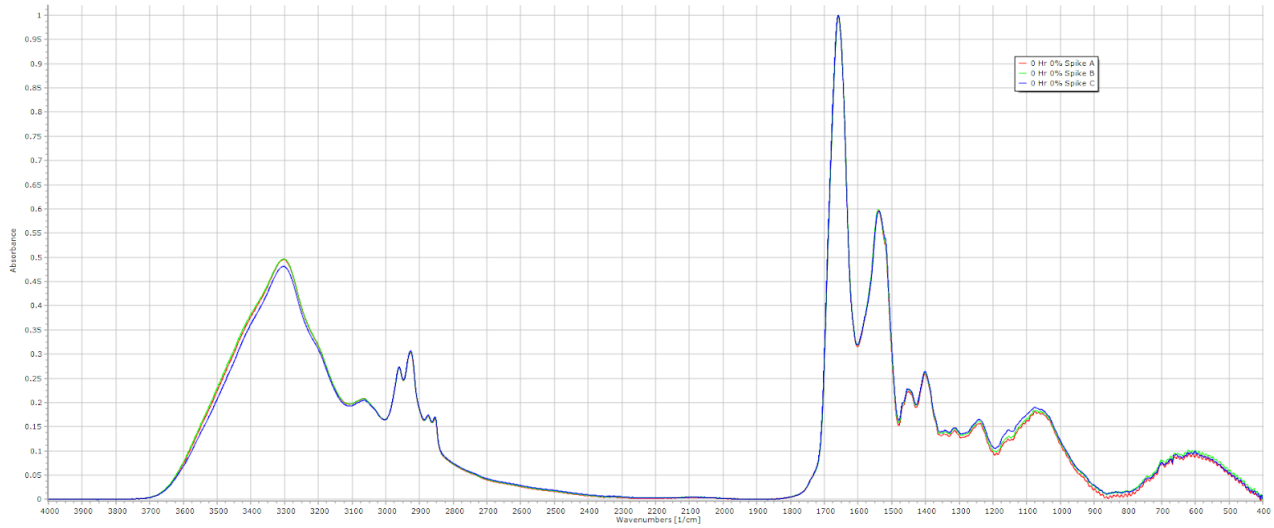


*Figure 26: Rat 3—0 Hour 0% Spike*

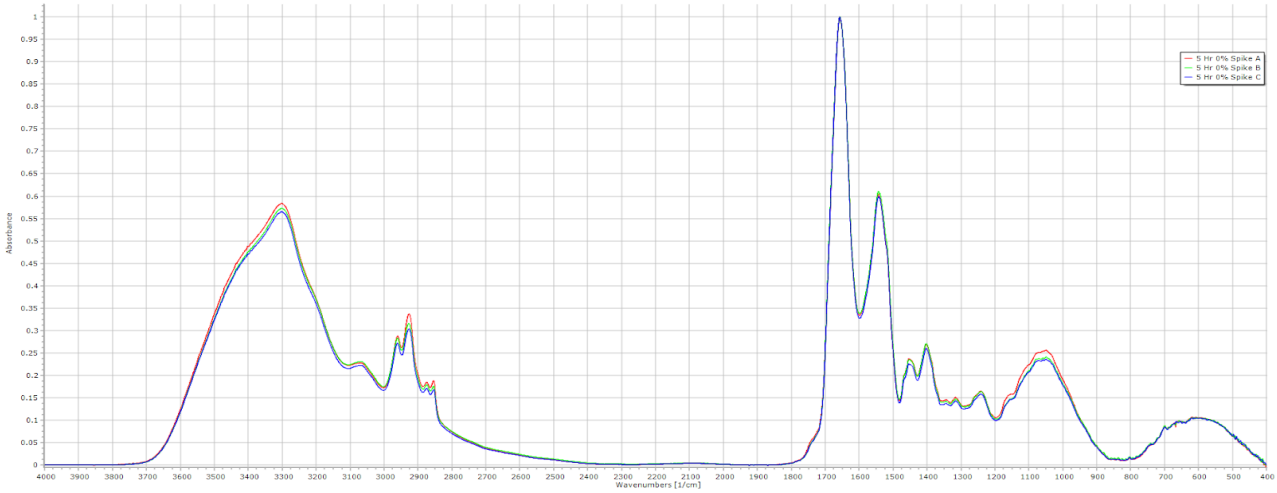


*Figure 27: Rat 3—5 Hour 0% Spike*

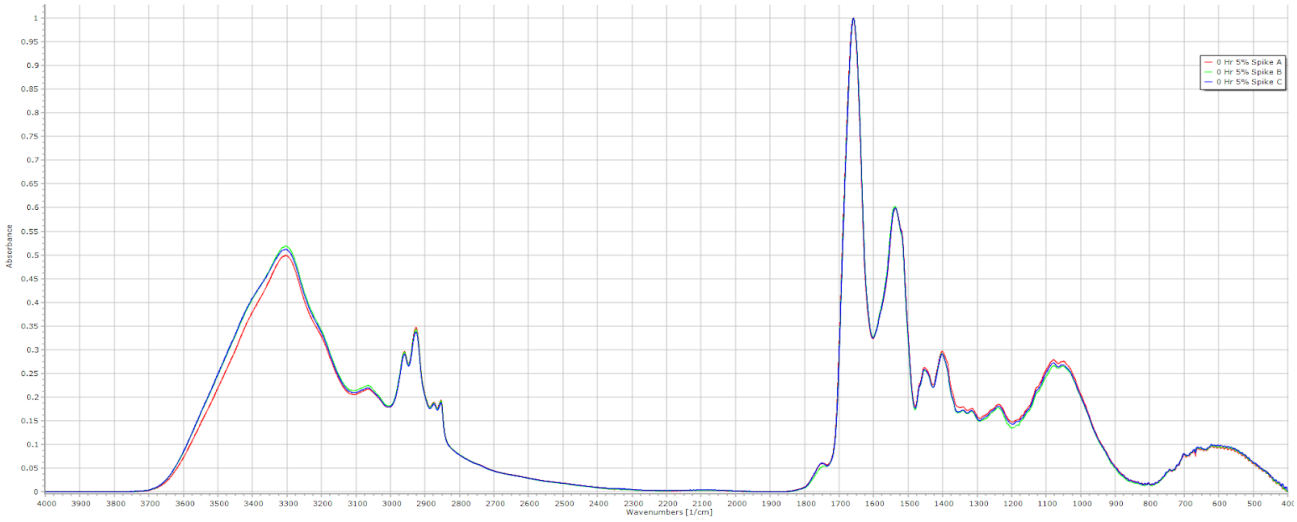


*Figure 28: Rat 3—0 Hour 5% Spike*

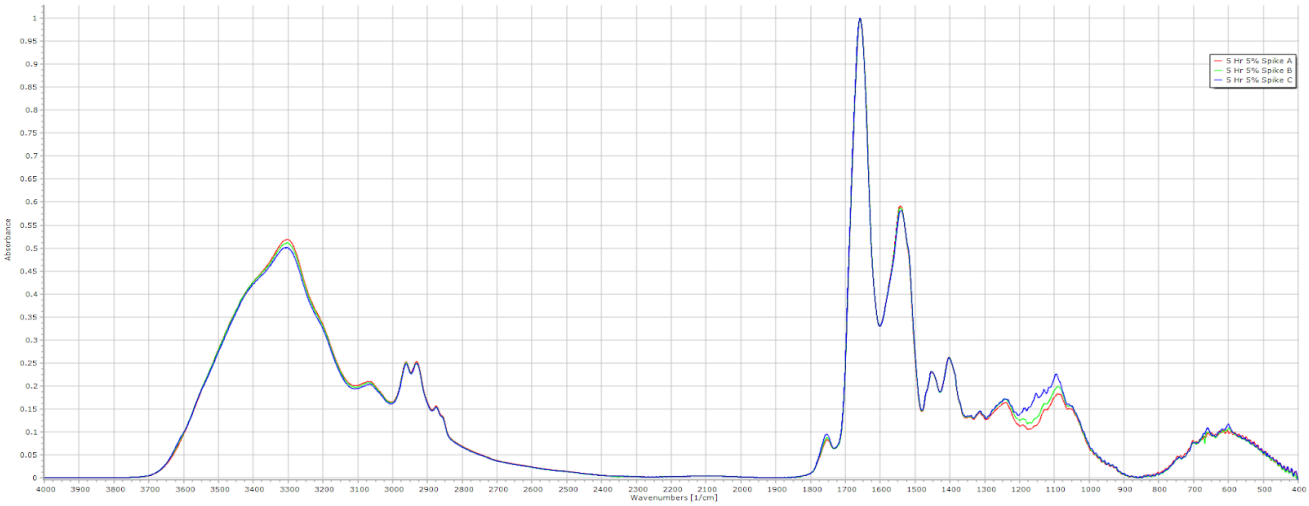


*Figure 29: Rat 3—5 Hour 5% Spike*

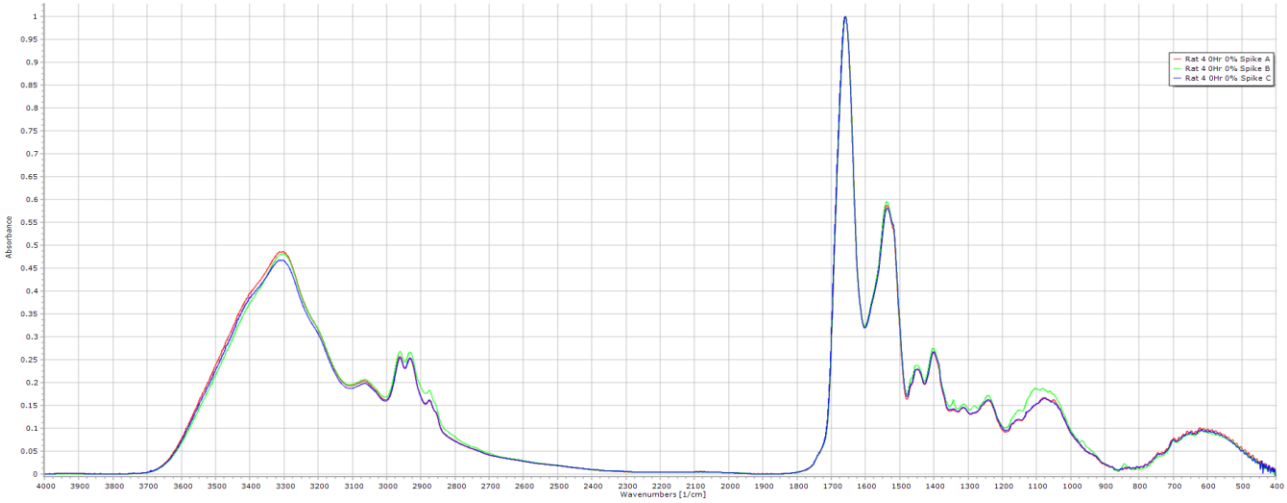


*Figure 30: Rat 4—0 Hour 0% Spike*

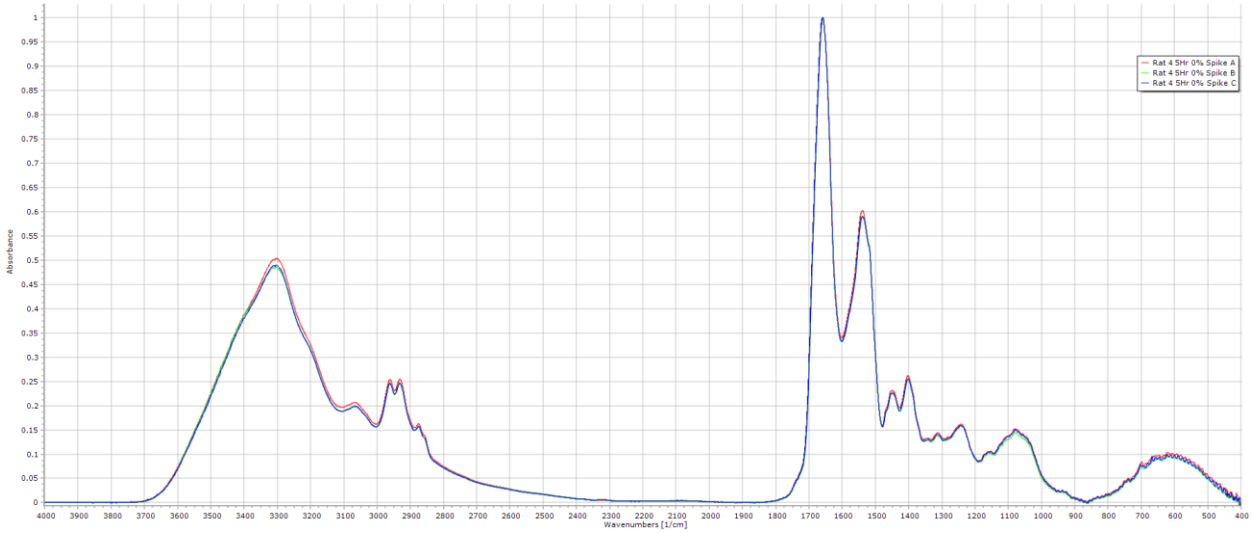


*Figure 31: Rat 4—5 Hour 0% Spike*

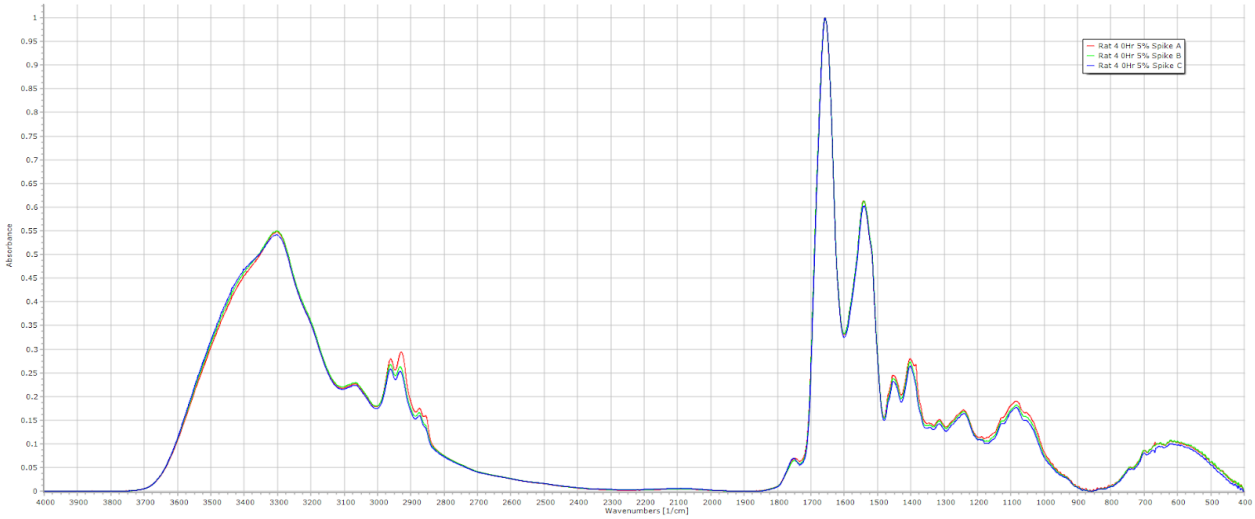


*Figure 32: Rat 4—0 Hour 5% Spike*

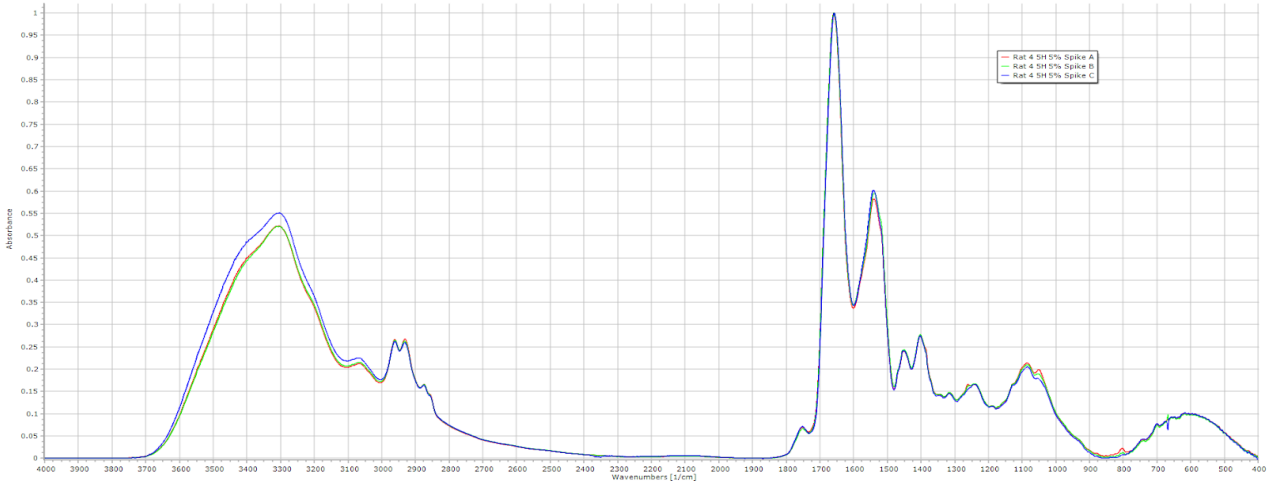


*Figure 33: Rat 4—5 Hour 5% Spike*