

**New Synthetic Route  
to Acylated Ferrocene Derivatives  
for Controlling the CisPlat Drug**

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In fulfillment of their research project requirements

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# Introduction

## A. Goal

The ultimate goal of this project is to develop a synthetic route to what we hope will be a stable organometallic complex of the acyl-ferrocene compound. The derivative in sight is that of the CISPLAT compound. CISPLAT is actually cis-Amino Platinum (II) Chloride. This compound is the only NIH approved organometallic complex with anticancer activity.

## B. The History of CISPLAT

This drug has been approved for aggressive cancers. It was very aggressive for carcinoma (skin cancer) but fell out of favor in the 1970's due to its nonspecific nature through which it killed all cells. Its mechanism is that of a polar complex acting as molecular scotch tape to the advanced DNA replication process. DNA in cancer cells replicate at tremendous rates. The thought behind the use of the complex is the space between nitrogen bases in the double helix. The spacing, according to Watson and Crick, is about 3 angstroms. The "d" lobes of the cis-platinum complex are about 2.5 angstroms apart. This bridge is just large enough to let the platinum complex insert itself at several points and act as chemical scotch tape. The platinum holds the amines of the phosphate sugar backbone together with a bond strength far greater than the enzymes can use to split the DNA. The drug became a success overnight when applied directly to the site of the cancer.

The initial talk in the 1970's was to reduce the aggressiveness of this complex to allow the drug to be used in other cancers. Since that point the drug was found useful in the 80's and 90's against other aggressive cancers such as uterine cancers. The drug in the late 90's was found to lose its effectiveness in an immunity type reaction. The enzymes in the nucleus which are used by the cell for DNA repair were found to be splicing out the platinum sections. The research goes on today in several countries looking at CISPLAT and trying derivatives that might get around this enzyme behavior.

### **C. Theory behind the Current Complex under Investigation**

The aggression behind the CISPLAT molecule is tied to the acidic “d” electron lobes of the platinum group. The ability of a molecule to accept electrons (particularly metal complexes) is tied to the acidity of the metal, as well as the ligands of the complex and the geometry of the overall molecule.

In the cis-Platinum (II) complex the platinum will easily substitute the ammonia ligands for more Lewis basic adenine and guanine base pairs of the DNA backbone. The basicity of ammonia is noted to be close to a pH of 9.4 while the basicity of guanine and adenine are close to 10.2-10.9 pH units. The clear preference for the ligands is well established in the journals of medicine during cancer trials.

The aggression of the CISPLAT can be reduced by simply attaching electron withdrawing groups to the complex. The electron withdrawing groups chosen to place on the platinum should be benign to the body. One such group that would allow us to fine tune the electron density at the metal would be ferrocene. Ferrocene is a complex that is stable in the human body and is easily excreted if it should break apart from the platinum complex.

In order to attach the platinum group onto the ferrocene, the acetyl group allows an attachment point at one or possibly both ferrocene rings. By adding acetic anhydride to the ferrocene the synthesis can produce both the single acyl derivative and the double derivative. The double derivative adds the acetyl groups to opposite rings.

The thrust of using the ferrocene as the electronic conduit comes from several studies on the molecule in the 60's and 70's. These studies demonstrated that the p-pi electrons of cyclopentadiene overlap well with the iron d orbitals. This allows electronic flow of density through the iron from one ring to the other. It is this electronic flow that the molecular design is based upon.

Electronic groups will be added to the bottom ring after we add the acyl group followed by the platinum complex. The result we predict will be to tune the platinum complex and allow custom aggression of the CISPLAT derivative toward nuclear DNA.

## **D. The Acyl Platinum Derivative -- a Possible Path to Overcome Resistance**

One of the discussions going on concerning the immunity build up of the CISPLAT is that the size of the CISPLAT is small enough to allow the nuclear enzymes room enough to cut out section of the DNA and allow the cancer growth to proceed. The enzymes are generally very sensitive to the chemistry as well as the physical environment of the DNA area.

It is hoped that this larger complex will allow for more electronic tuning of the complex and perhaps extend the use of the complex to other types of cancer which do not require as aggressive an agent. In addition, the size of the complex with the ferrocene molecule bulking out its size could indeed prevent the enzymes the room needed to cut these sections out of the DNA strands and stop the immunity that has been recently observed in several patients.

## **Materials and Methods**

All procedures were done in open air flasks and require no Schlenk glassware or other air sensitive equipment. No special preparation was done to solvents used in the synthesis.

### **Past History of Attempts**

Several pathways were looked at by this supervisor in the past. Some met with promise but most resulted in air sensitive compounds. Several pathways tried over the last few years involved the production of methyl derivatives of ferrocene. Lithium was involved in the production of methyl derivatives of ferrocene and then making lithium derivatives of the methyl groups to further react those compounds. Many of the compounds produced were found to be air sensitive and in most cases thermally unstable.

## Current Research Synthesis

### 1. Acyl Ferrocene

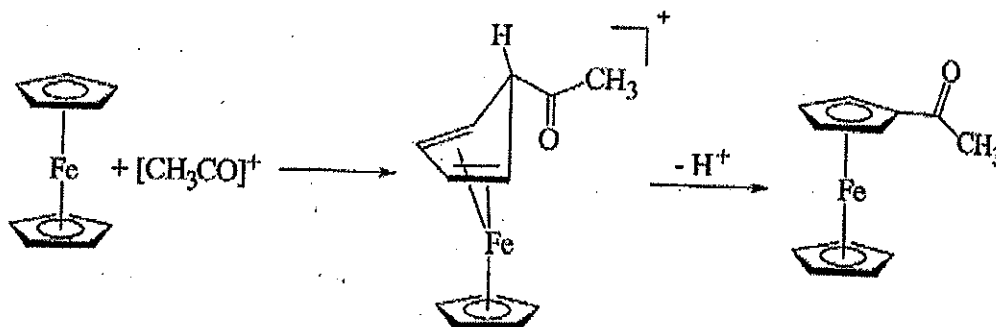
0.5 grams of powder ferrocene (Aldrich) 99.9% was placed in a round bottom. 5 mls of acetic anhydride was added to the solid with 0.1 g of 85% phosphoric acid. The mixture was heated in a boiling water bath for 30 minutes. The color of the mixture would start as a reddish mixture and proceed to an off green-brown.

The resulting products were dissolved in a hexane/ether mixture after being cooled for several hours. The liquid mixture was added to an alumina column (alumina oxide) of about 15 centimeters by 0.5 cm in radius. The material was added to the column and slowly eluted first with dry hexane solvent and then a hexane/ethylene chloride mixture.

A green brown material, probably oxidized iron complex, was stuck at the column top. Two bands were routinely seen. Both of these bands are red in color. The first is the single acyl substituted complex and the second is the di-substituted complex. The ratio of the single substituted product to the double substituted product is usually 80:20.

The product was compared to purchased acyl ferrocene from Aldrich.

The acyl ferrocene material has a red color has an Rf value in ethylene chloride of 0.20, and a classic IR stretching frequency of the acyl carbonyl of 1700  $\text{cm}^{-1}$ .



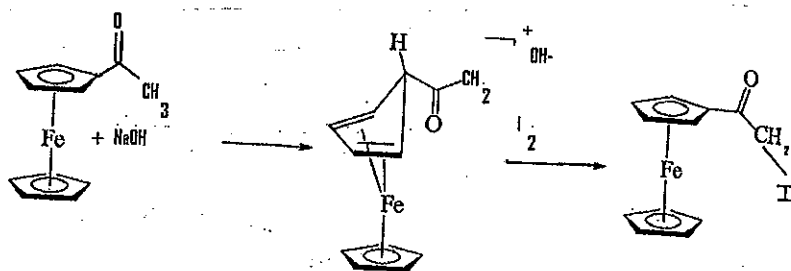
## 2. (Alpha) Acyl Iodo Ferrocene (first attempt)

Fresh acyl ferrocene (0.6grams) made by the method above was added to 30 mls of 10% sodium hydroxide. A sludge of solid was separated from the soluble materials in the solution.

Three milliliters of toluene solvent was added at room temperature with one gram of iodine solid. The mixture was stirred at room temperature for 5 hours. The product was separated from the solution by alumina chromatography (alumina oxide was added to a glass pipet with glass wool in the tip and solid alumina oxide in toluene).

The resulting material was not moisture sensitive but after several tries proved to be thermally sensitive. The materials produced were separated on the alumina column by acetone and kept at ice water temperatures. The isolated products were stored in a sub zero freezer.

When some of the material would decompose it would turn the typical iron +3 green color. The product was a deep red stable material while kept cold.

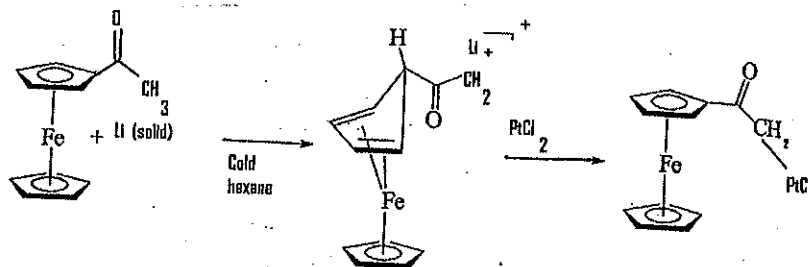


### 3. (Alpha) Acyl Iodo Ferrocene (second attempt)

0.6 grams of purified acyl ferrocene was added to 25 mls of cold toluene. To the solution 1.0 gram of iodine solid was added at 0C. The material was first stirred cold then warmed to 25C room temperature over 6 hours. The result was a mixture of red product and green oxidized material. The results of the reaction were an oil that was separated on a very short alumina column. The green material stayed at the top and approximately 0.3 grams of red oil was isolated and kept cold by vacuum evaporation.

### 4. (Alpha) Acyl Lead Chloride Ferrocene Derivative

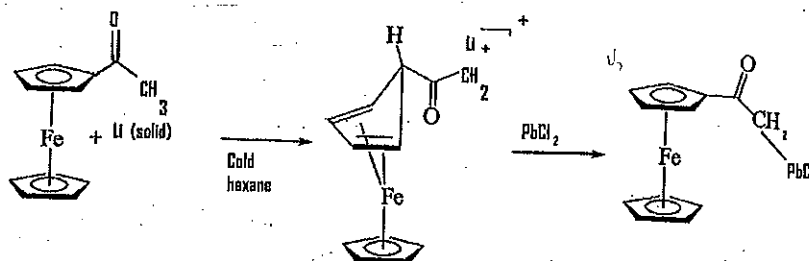
0.1 grams of acyl ferrocene was added to 0.25 grams of lithium in toluene and stirred at 0C for one hour. The deep red solution was kept at 0C while 0.5 grams of lead chloride (PbCl<sub>2</sub>) was added and stirred for an additional hour at 0C. The solution at 0C was slowly raised to room temperature where the color went from a deep red to a yellow red within 30 minutes. A short alumina column with a small sample revealed a stable red-orange compound. Some decomposition material was seen at the top of the column but all soluble material was the product.





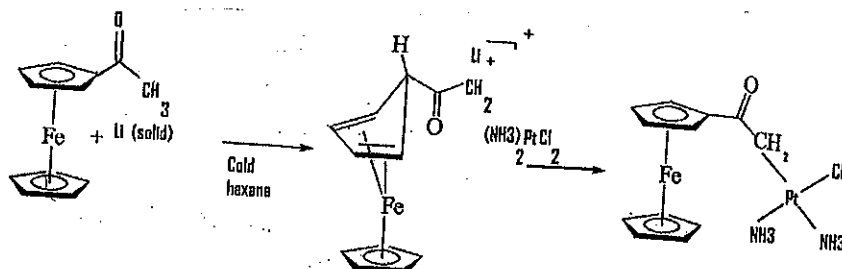
## 5. (Alpha) Acyl Ferrocene Platinum (II) Chloride Derivative

This reaction is similar to the reaction in #4 above. 0.1 grams of acyl ferrocene was added to 0.25 grams of lithium in toluene and stirred at 0C for one hour. The deep red solution was kept at 0C while 0.2 grams of platinum chloride ( $\text{PtCl}_2$ ) was added and stirred for an additional hour at 0C. The solution at 0C was slowly raised to room temperature where the color went from a deep red to a yellow red within 30 minutes. A short alumina column with a small sample revealed a stable red-orange compound. Some decomposition material was seen at the top of the column but all soluble material was the product.



## 6. (Alpha) Acyl Ferrocene Platinum (II) Diamino Chloride or (Alpha) Acyl CISPLAT Ferrocene

This reaction is similar to the reaction in #4 above. 0.1 grams of acyl ferrocene was added to 0.25 grams of lithium in toluene and stirred at 0C for one hour. The deep red solution was kept at 0C while 0.2 grams of di-amino platinum chloride  $[(\text{NH}_3)_2\text{PtCl}_2]$  was added and stirred for an additional hour at 0C. The solution at 0C was slowly raised to room temperature where the color went from a deep red to a yellow red within 30 minutes. A short alumina column with a small sample revealed a stable red-orange compound. Some decomposition material was seen at the top of the column but all soluble material was the product



# Results

The proof that the acyl CISPLAT was made appears to be strong. There are several steps in characterization that still need to be completed. Those steps include elemental analysis, NMR etc. The purification of the product seems to be relatively simple since the product appears to be very stable and is not solvent sensitive or moisture sensitive as previously feared by our attempts from other routes.

## Discussion of Rf values

The following Rf values were obtained in methylene chloride on alumina plates. The container was a 400 ML beaker with Parafilm™ as a cover. One can see the increase polarity of the metal complexes.

| <b>Compound</b>                       | <b>Chromatography Rf Value<br/>(methylene chloride)</b> |
|---------------------------------------|---|
| Ferrocene                             | 0.77  |
| Acyl Ferrocene                        | 0.20  |
| Acyl Lead Chloride Ferrocene          | 0.33  |
| Acyl Iodo Ferrocene                   | 0.33  |
| Acyl Platinum (II) Chloride Ferrocene | 0.42  |

## Discussion of IR Values

The following Infrared analyses were done on a Nicolet 510 FTIR machine. The IRs were done through a generous donation of machine time by Glendale Community College.

When examining the IR spectra of the compounds of interest it was obvious that the carbonyl stretch was the most sensitive marker of the compounds. The following were the observed stretches of the products we believe to be made at our labs:

| <b>Compound</b>                               | <b>IR C=O Stretch (cm-1)</b> |
|---|------------------------------|
| Acyl Ferrocene                                | 1700                         |
| Acyl Iodo Ferrocene                           | 1630                         |
| Acyl Lead Chloride Ferrocene                  | 1617                         |
| Acyl Platinum (II) Chloride Ferrocene         | 1712                         |
| Acyl Platinum (II) Diamine Chloride Ferrocene | 1731                         |

## **Conclusion**

1. Air stable platinum acyl-ferrocene derivatives are possible.
2. Stable complex compounds are possible that allow for fine electronic tuning of the CISPLAT complex.
3. New complexes of ferrocene are now being discovered by this group.

## Next Steps in this Research

1. Purification of the complex.
2. Elemental analysis.

The stable complexes will be run in a series of solutions with mixtures of toluene and methylene chloride. These solutions, placed over long periods of time, should produce crystals for elemental analysis.

3. Complete characterization of the complexes.

The two platinum complexes and the lead complex will be sent in for elemental analysis, proton H1 – NMR and if possible X ray diffraction.

4. Derivatization of the bottom ring.

The next step in the research will be to create a derivative with the di-acyl ferrocene complex. These complexes will be engaged to make a complex with the platinum group at one end and a strong electron withdrawing group at the other end of the ferrocene complex. These complexes will complete the long term goal of making a tunable platinum derivative that will tame the anticancer reactivity.

5. Submit samples for culture tests.

Contacts with local hospitals will be used to enter purified complexes for further bio-medical studies.

# Pictures and Spectra

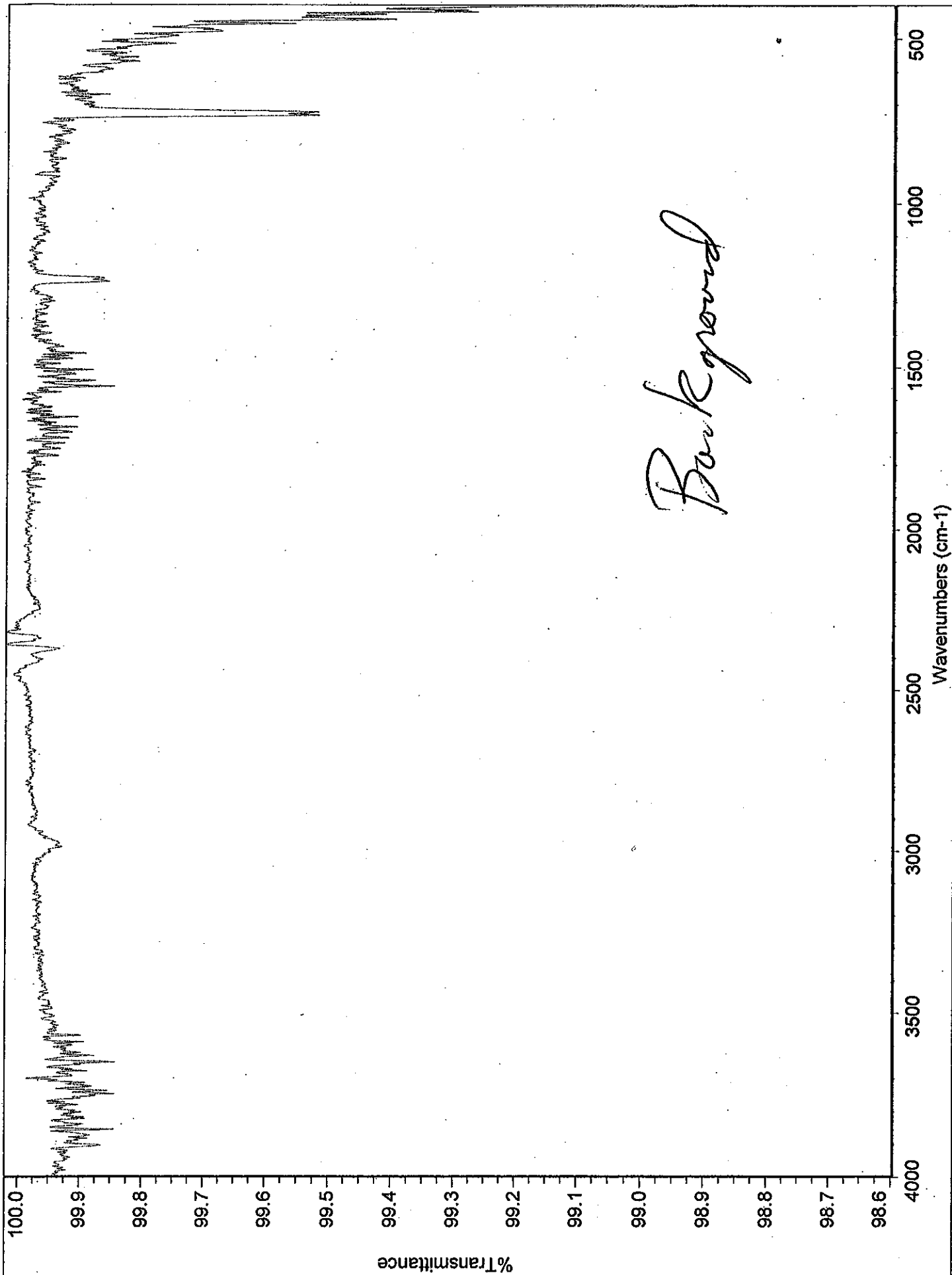
**Background**

**Polystyrene**

**and**

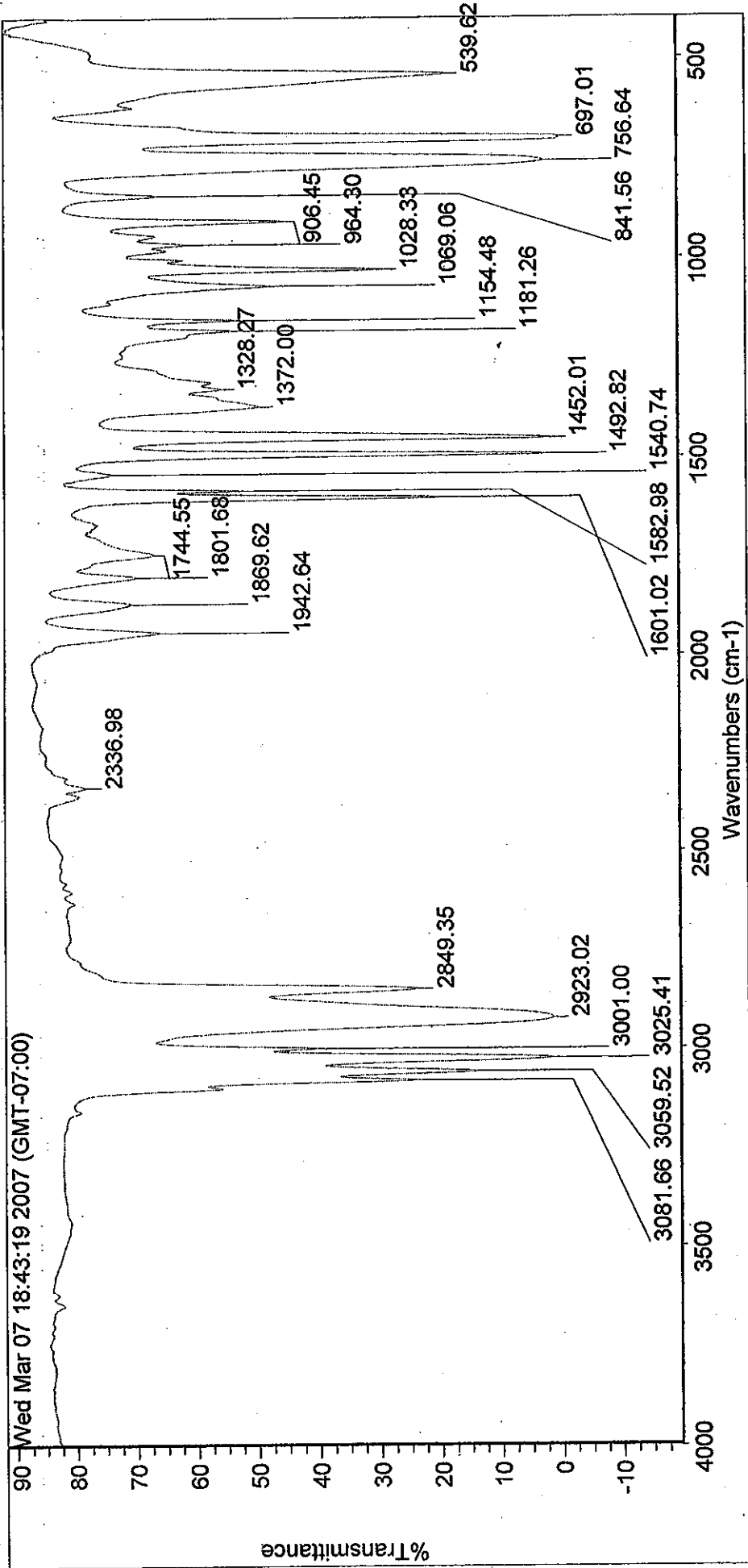
**Solvent**

**Spectra**



*Background*

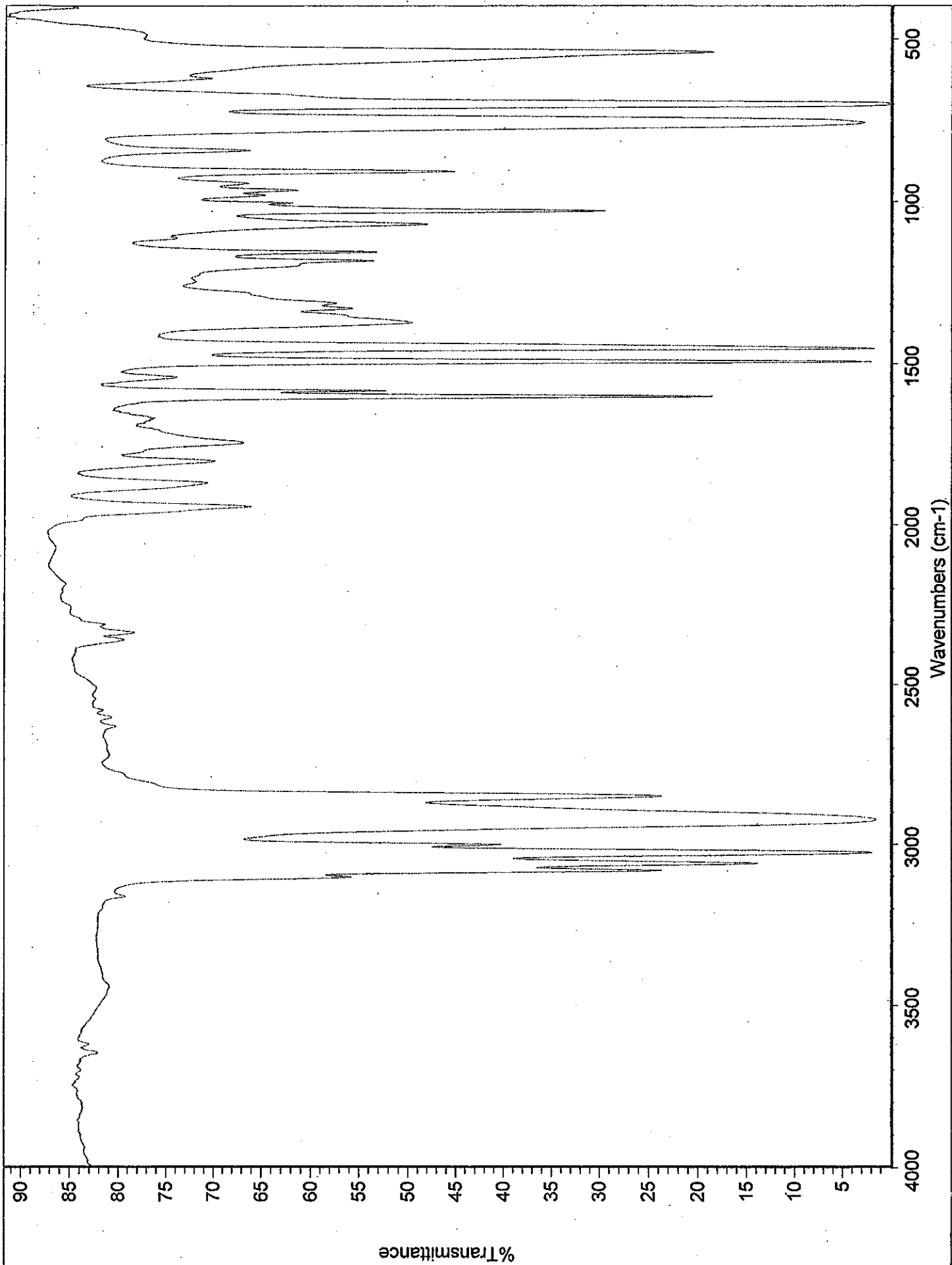




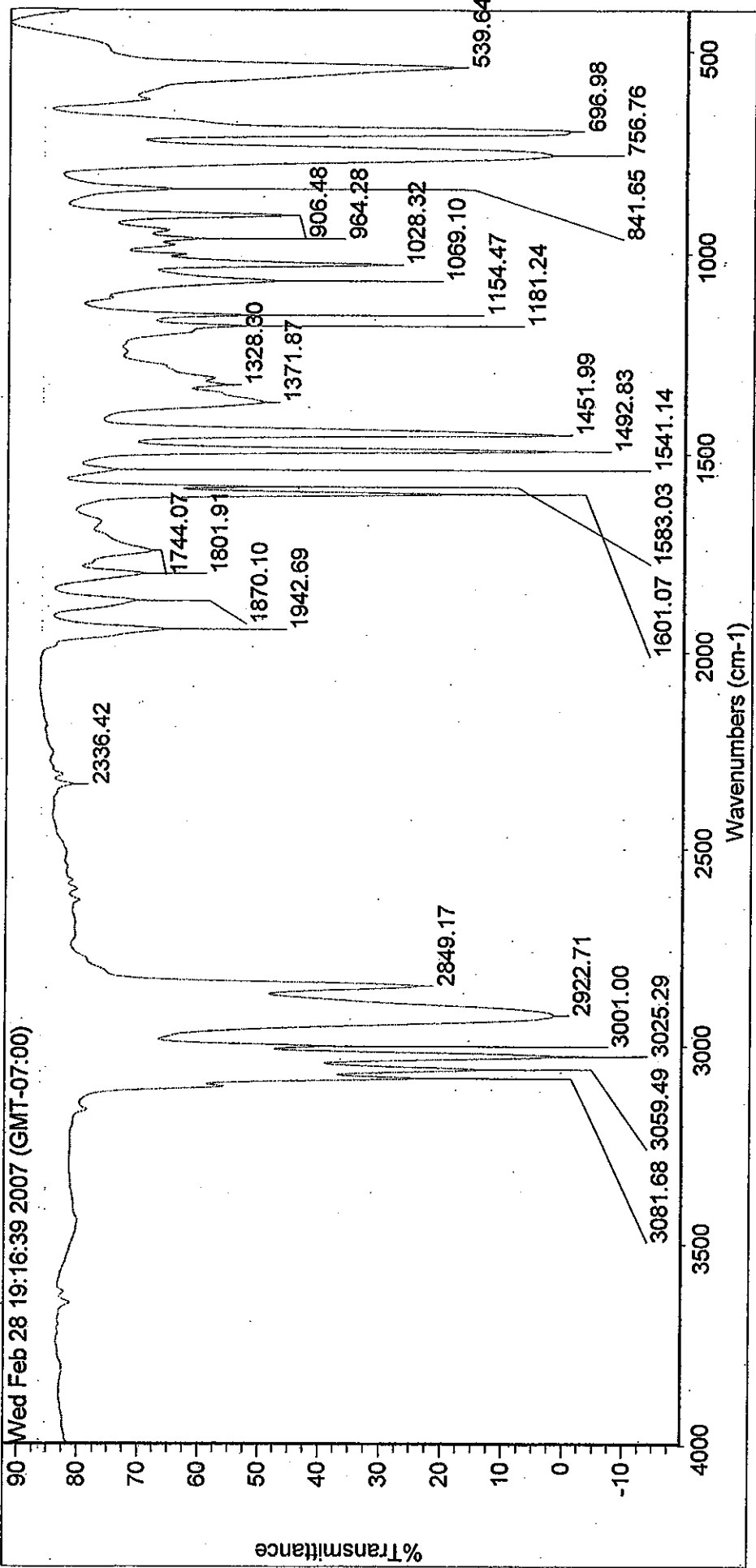
*Polystyrene*  
3/7/07

Wed Mar 07 18:46:58 2007 (GMT-07:00)  
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 Region: 4000.00 400.00  
 Absolute threshold: 88.771  
 Sensitivity: 50  
 Peak list:

| Position: | Intensity: |
|-----------|------------|
| 539.62    | 18.367     |
| 697.01    | -0.0779    |
| 756.64    | 2.639      |
| 841.56    | 66.047     |
| 906.45    | 45.005     |
| 964.30    | 61.225     |
| 1028.33   | 29.084     |
| 1069.06   | 47.831     |



|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1154.48 | Intensity: | 52.898 |
| Position: | 1181.26 | Intensity: | 53.241 |
| Position: | 1328.27 | Intensity: | 55.575 |
| Position: | 1372.00 | Intensity: | 49.502 |
| Position: | 1452.01 | Intensity: | 1.372  |
| Position: | 1492.82 | Intensity: | 1.411  |
| Position: | 1540.74 | Intensity: | 73.806 |
| Position: | 1582.98 | Intensity: | 51.576 |
| Position: | 1601.02 | Intensity: | 17.951 |
| Position: | 1744.55 | Intensity: | 66.766 |
| Position: | 1801.68 | Intensity: | 69.732 |
| Position: | 1869.62 | Intensity: | 70.565 |
| Position: | 1942.64 | Intensity: | 65.929 |
| Position: | 2336.98 | Intensity: | 78.233 |
| Position: | 2849.35 | Intensity: | 23.533 |
| Position: | 2923.02 | Intensity: | 1.401  |
| Position: | 3001.00 | Intensity: | 40.164 |
| Position: | 3025.41 | Intensity: | 1.778  |
| Position: | 3059.52 | Intensity: | 13.625 |
| Position: | 3081.66 | Intensity: | 23.408 |



Wed Feb 28 19:17:43 2007 (GMT-07:00)

FIND PEAKS:

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Absolute threshold: 91.681

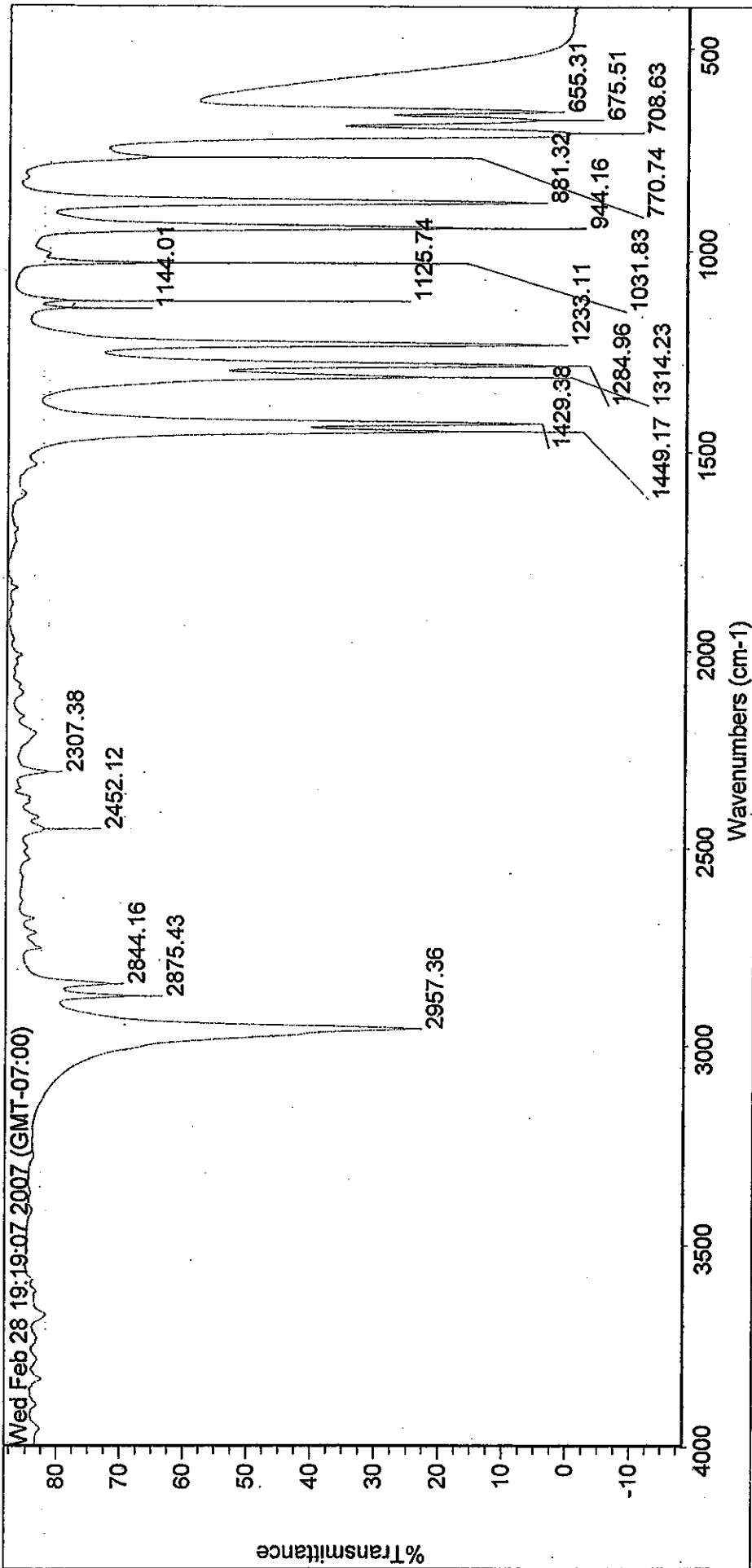
Sensitivity: 50

Peak list:

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|-----------|------------|
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| 756.76    | 2.713      |
| 841.65    | 65.183     |
| 906.48    | 45.608     |
| 964.28    | 60.791     |
| 1028.32   | 29.401     |
| 1069.10   | 47.610     |

*Poly styrene*  
2/28/07

|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1154.47 | Intensity: | 52.996 |
| Position: | 1181.24 | Intensity: | 53.249 |
| Position: | 1328.30 | Intensity: | 56.184 |
| Position: | 1371.87 | Intensity: | 49.566 |
| Position: | 1451.99 | Intensity: | 1.547  |
| Position: | 1492.83 | Intensity: | 1.560  |
| Position: | 1541.14 | Intensity: | 74.376 |
| Position: | 1583.03 | Intensity: | 51.545 |
| Position: | 1601.07 | Intensity: | 18.211 |
| Position: | 1744.07 | Intensity: | 68.268 |
| Position: | 1801.91 | Intensity: | 69.601 |
| Position: | 1870.10 | Intensity: | 70.946 |
| Position: | 1942.69 | Intensity: | 65.969 |
| Position: | 2336.42 | Intensity: | 81.006 |
| Position: | 2849.17 | Intensity: | 23.828 |
| Position: | 2922.71 | Intensity: | 1.579  |
| Position: | 3001.00 | Intensity: | 39.954 |
| Position: | 3025.29 | Intensity: | 1.640  |
| Position: | 3059.49 | Intensity: | 14.019 |
| Position: | 3081.68 | Intensity: | 23.936 |



Wed Feb 28 19:20:15 2007 (GMT-07:00)  
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 Peak list:

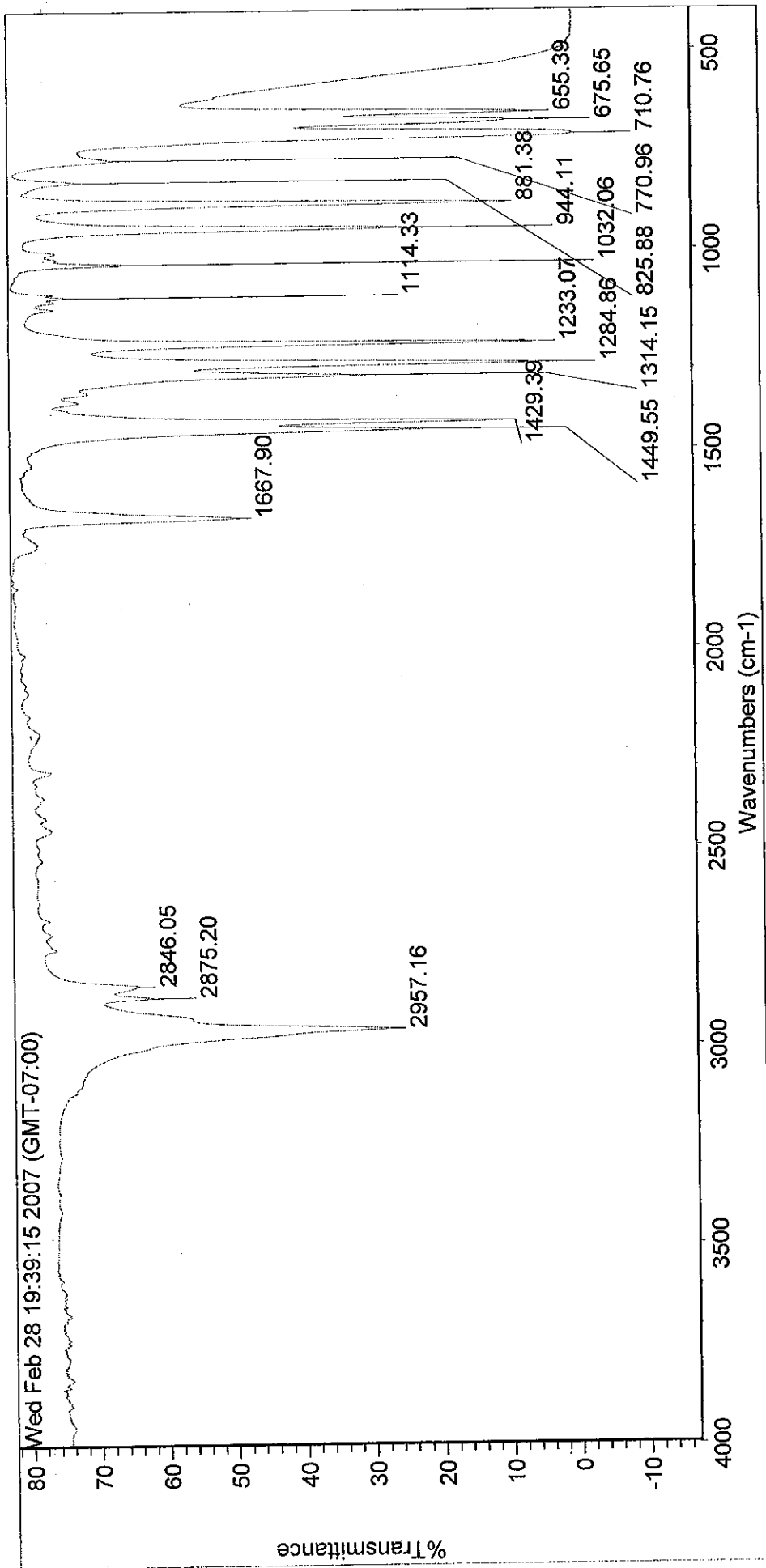
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| Position: | 708.63  | Intensity: | 0.0852 |
| Position: | 770.74  | Intensity: | 65.863 |
| Position: | 881.32  | Intensity: | 5.990  |
| Position: | 944.16  | Intensity: | 17.426 |
| Position: | 1031.83 | Intensity: | 65.345 |
| Position: | 1125.74 | Intensity: | 78.958 |

*Ethylene  
 Di-chloride  
 Cl-CH<sub>2</sub>-CH<sub>2</sub>-  
 Solvent*

|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1144.01 | Intensity: | 78.910 |
| Position: | 1233.11 | Intensity: | 1.978  |
| Position: | 1284.96 | Intensity: | 2.508  |
| Position: | 1314.23 | Intensity: | 23.375 |
| Position: | 1429.38 | Intensity: | 5.497  |
| Position: | 1449.17 | Intensity: | 18.028 |
| Position: | 2307.38 | Intensity: | 81.689 |
| Position: | 2452.12 | Intensity: | 82.315 |
| Position: | 2844.16 | Intensity: | 71.850 |
| Position: | 2875.43 | Intensity: | 68.472 |
| Position: | 2957.36 | Intensity: | 24.845 |

**Lead Chloride derivative  
Of Acyl Ferrocene**





Wed Feb 28 19:40:08 2007 (GMT-07:00)

FIND PEAKS:

Spectrum: Wed Feb 28 19:39:15 2007 (GMT-07:00)

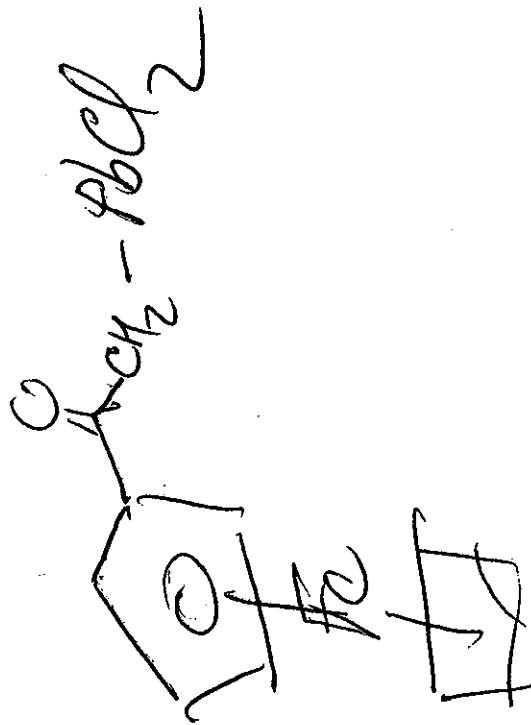
Region: 4000.00 400.00

Absolute threshold: 81.289

Sensitivity: 50

Peak list:

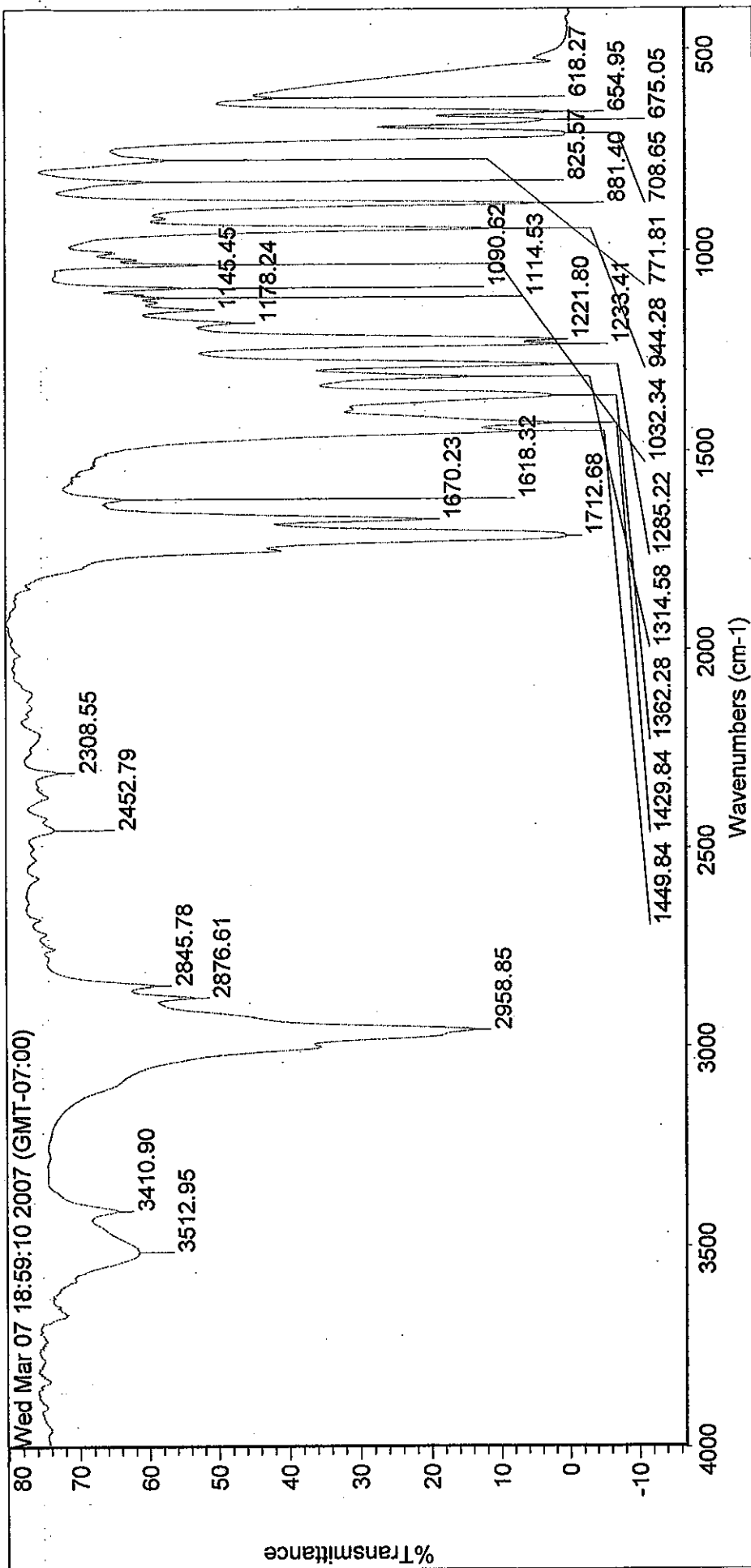
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| Position: | 825.88  | Intensity: | 72.585 |
| Position: | 881.38  | Intensity: | 11.050 |
| Position: | 944.11  | Intensity: | 24.681 |
| Position: | 1032.06 | Intensity: | 64.741 |



|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1114.33 | Intensity: | 73.972 |
| Position: | 1233.07 | Intensity: | 4.163  |
| Position: | 1284.86 | Intensity: | 5.057  |
| Position: | 1314.15 | Intensity: | 30.327 |
| Position: | 1429.39 | Intensity: | 9.680  |
| Position: | 1449.55 | Intensity: | 21.601 |
| Position: | 1667.90 | Intensity: | 49.280 |
| Position: | 2846.05 | Intensity: | 64.109 |
| Position: | 2875.20 | Intensity: | 62.110 |
| Position: | 2957.16 | Intensity: | 27.550 |

**CIS PLAT derivative**  
**Of Acyl Ferrocene**

**IR Spectra**



*More Con antitrated  
CsPlat - Acyl Ferrocene*

Wed Mar 07 19:08:42 2007 (GMT-07:00)  
 FIND PEAKS:  
 Spectrum: Wed Mar 07 18:59:10 2007 (GMT-07:00)  
 Region: 4000.00 400.00  
 Absolute threshold: 79.318  
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 Peak list:

|           |        |            |        |
|-----------|--------|------------|--------|
| Position: | 618.27 | Intensity: | 41.375 |
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| Position: | 675.05 | Intensity: | 3.524  |
| Position: | 708.65 | Intensity: | 0.386  |
| Position: | 771.81 | Intensity: | 57.324 |
| Position: | 825.57 | Intensity: | 60.042 |
| Position: | 881.40 | Intensity: | 4.241  |
| Position: | 944.28 | Intensity: | 11.542 |

|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1032.34 | Intensity: | 51.653 |
| Position: | 1090.62 | Intensity: | 54.737 |
| Position: | 1114.53 | Intensity: | 56.966 |
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| Position: | 1314.58 | Intensity: | 13.688 |
| Position: | 1362.28 | Intensity: | 2.278  |
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| Position: | 3512.95 | Intensity: | 61.541 |



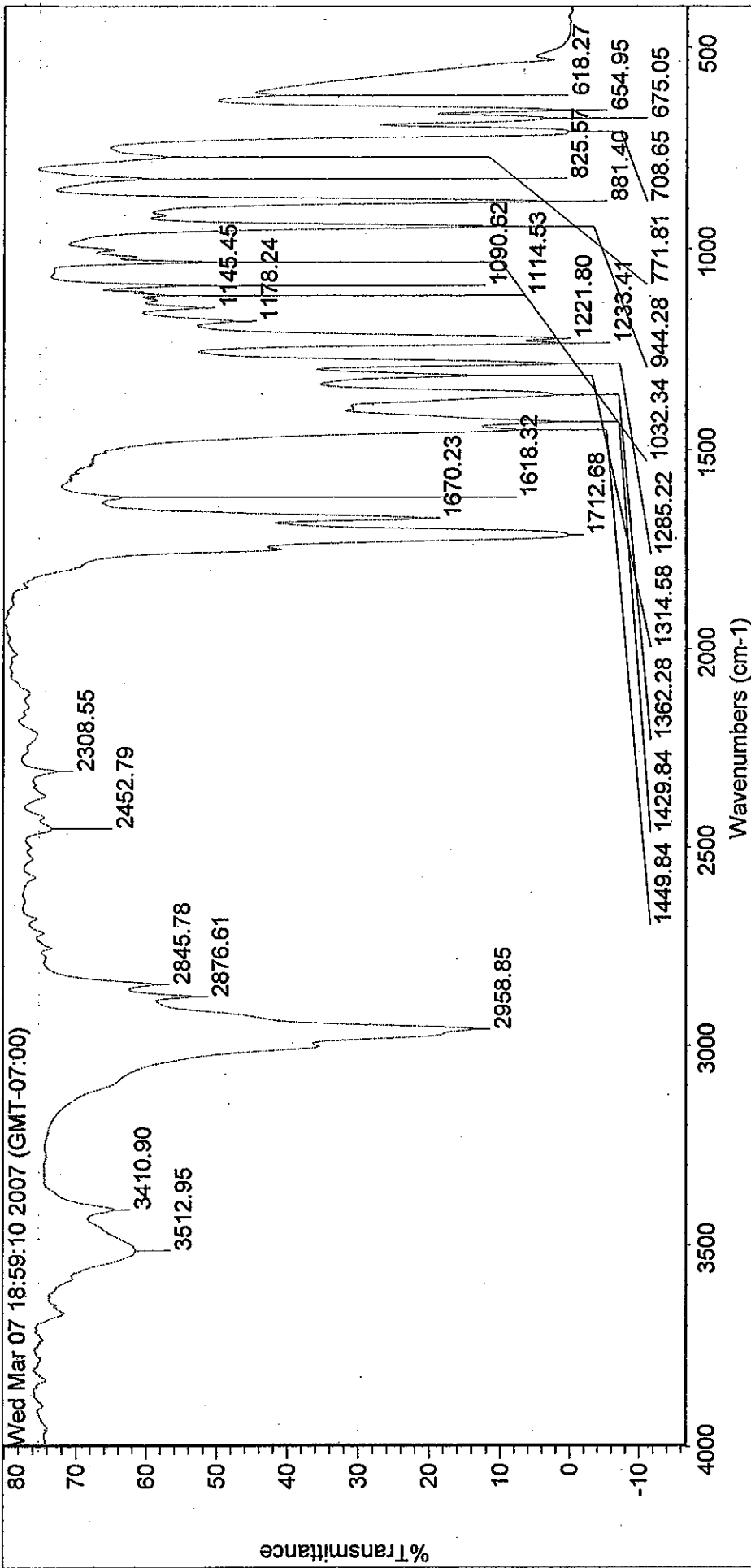
|           |         |            |        |
|-----------|---------|------------|--------|
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| Position: | 1233.00 | Intensity: | 2.491  |
| Position: | 1284.69 | Intensity: | 2.706  |
| Position: | 1314.12 | Intensity: | 17.193 |
| Position: | 1377.98 | Intensity: | 65.312 |
| Position: | 1429.35 | Intensity: | 4.257  |
| Position: | 1449.53 | Intensity: | 10.586 |
| Position: | 1668.00 | Intensity: | 33.159 |
| Position: | 1731.07 | Intensity: | 75.903 |
| Position: | 2211.18 | Intensity: | 76.713 |
| Position: | 2307.62 | Intensity: | 74.167 |
| Position: | 2452.99 | Intensity: | 74.651 |
| Position: | 2753.00 | Intensity: | 74.212 |
| Position: | 2846.18 | Intensity: | 56.075 |
| Position: | 2875.26 | Intensity: | 53.471 |
| Position: | 2957.12 | Intensity: | 15.285 |





|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1125.59 | Intensity: | 72.885 |
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| Position: | 1233.08 | Intensity: | 1.365  |
| Position: | 1284.95 | Intensity: | 1.524  |
| Position: | 1314.17 | Intensity: | 14.078 |
| Position: | 1429.46 | Intensity: | 2.581  |
| Position: | 1449.32 | Intensity: | 9.333  |
| Position: | 1668.18 | Intensity: | 63.510 |
| Position: | 2211.07 | Intensity: | 80.376 |
| Position: | 2307.58 | Intensity: | 77.572 |
| Position: | 2452.52 | Intensity: | 78.553 |
| Position: | 2752.95 | Intensity: | 78.834 |
| Position: | 2844.76 | Intensity: | 62.137 |
| Position: | 2875.41 | Intensity: | 58.205 |
| Position: | 2957.26 | Intensity: | 13.919 |

**Platinum Chloride Derivative  
Of Acyl Ferrocene  
IR Spectra**



Handwritten notes and signatures:

Q-Q-PCD

1712

Handwritten signature: 李 (Li)

Wed Mar 07 19:01:17 2007 (GMT-07:00)

FIND PEAKS:

Spectrum: Wed Mar 07 18:59:10 2007 (GMT-07:00)

Region: 4000.00 400.00

Absolute threshold: 78.706

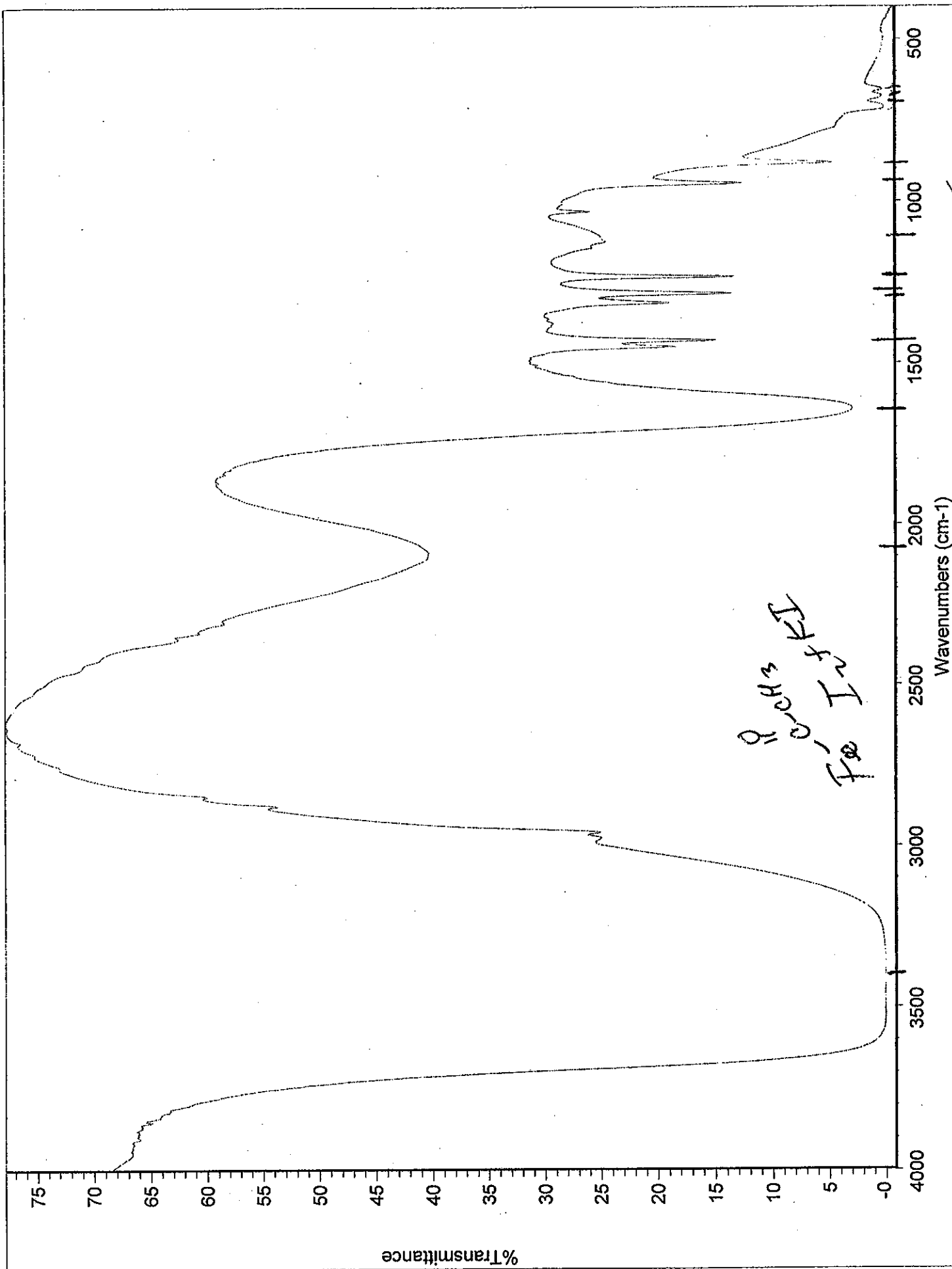
Sensitivity: 50

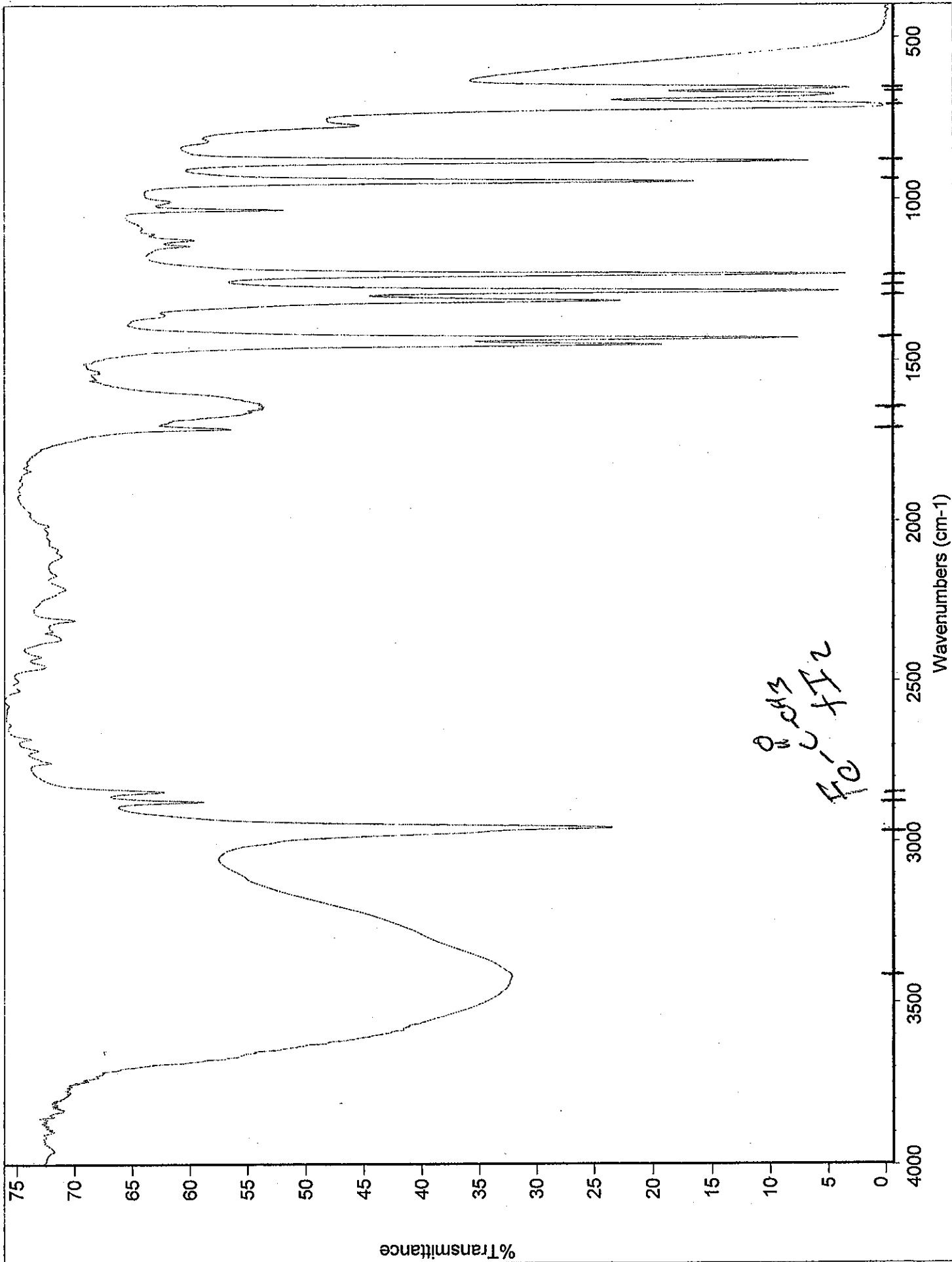
Peak list:

| Position: | Intensity: |
|-----------|------------|
| 618.27    | 41.375     |
| 654.95    | 2.416      |
| 675.05    | 3.524      |
| 708.65    | 0.386      |
| 771.81    | 57.324     |
| 825.57    | 60.042     |
| 881.40    | 4.241      |
| 944.28    | 11.542     |

|           |         |            |        |
|-----------|---------|------------|--------|
| Position: | 1032.34 | Intensity: | 51.653 |
| Position: | 1090.62 | Intensity: | 54.737 |
| Position: | 1114.53 | Intensity: | 56.966 |
| Position: | 1145.45 | Intensity: | 52.561 |
| Position: | 1178.24 | Intensity: | 47.711 |
| Position: | 1221.80 | Intensity: | 1.926  |
| Position: | 1233.41 | Intensity: | 1.147  |
| Position: | 1285.22 | Intensity: | 1.547  |
| Position: | 1314.58 | Intensity: | 13.688 |
| Position: | 1362.28 | Intensity: | 2.278  |
| Position: | 1429.84 | Intensity: | 1.900  |
| Position: | 1449.84 | Intensity: | 7.489  |
| Position: | 1618.32 | Intensity: | 63.523 |
| Position: | 1670.23 | Intensity: | 20.680 |
| Position: | 1712.68 | Intensity: | 0.193  |
| Position: | 2308.55 | Intensity: | 72.721 |
| Position: | 2452.79 | Intensity: | 73.450 |
| Position: | 2845.78 | Intensity: | 58.883 |
| Position: | 2876.61 | Intensity: | 53.284 |
| Position: | 2958.85 | Intensity: | 13.437 |
| Position: | 3410.90 | Intensity: | 64.523 |
| Position: | 3512.95 | Intensity: | 61.541 |

**Iodine derivative of**  
**Acyl Ferrocene**  
**IR Spectra**





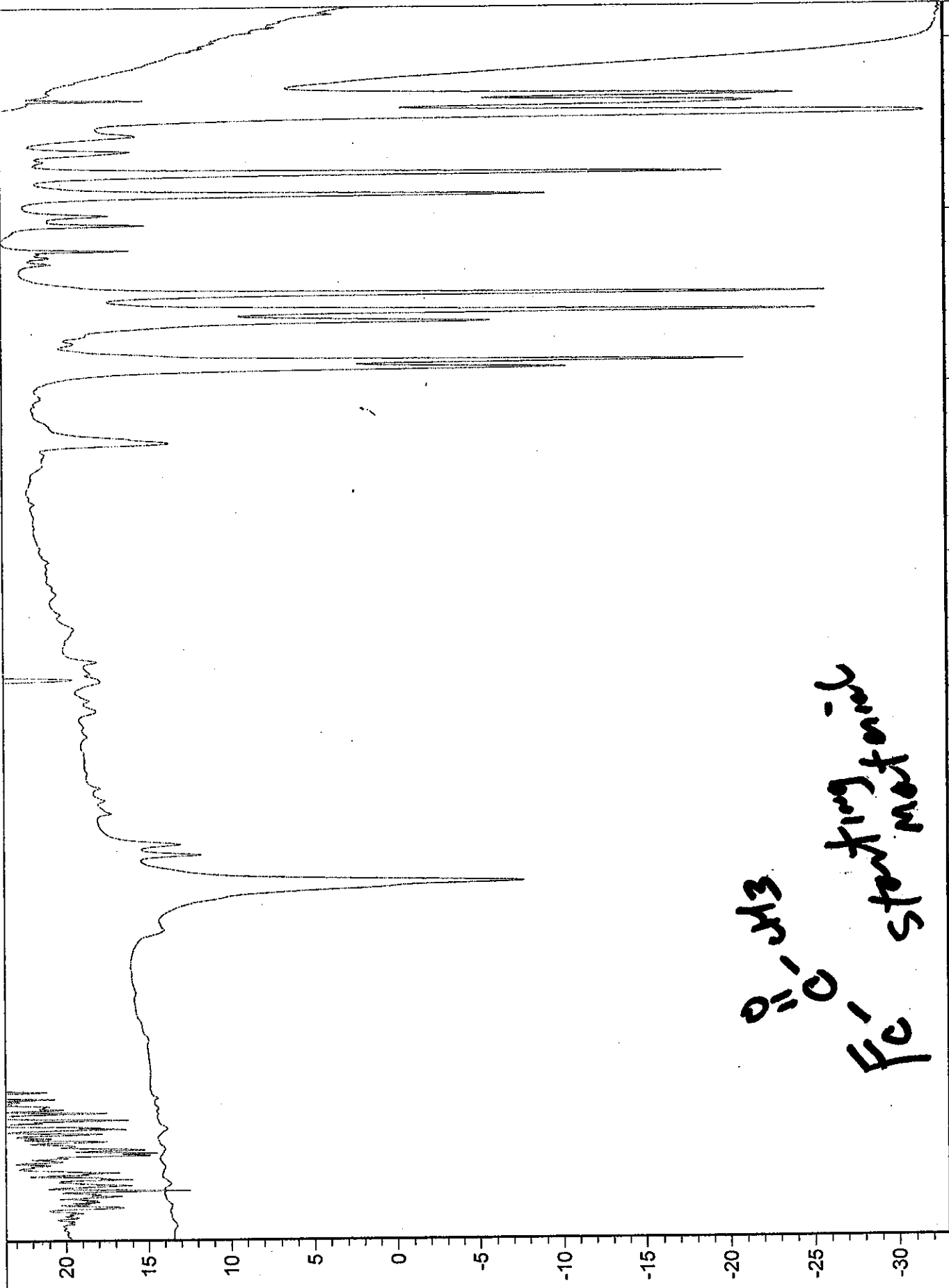
# **Acyl Ferrocene**

## **IR Spectra**



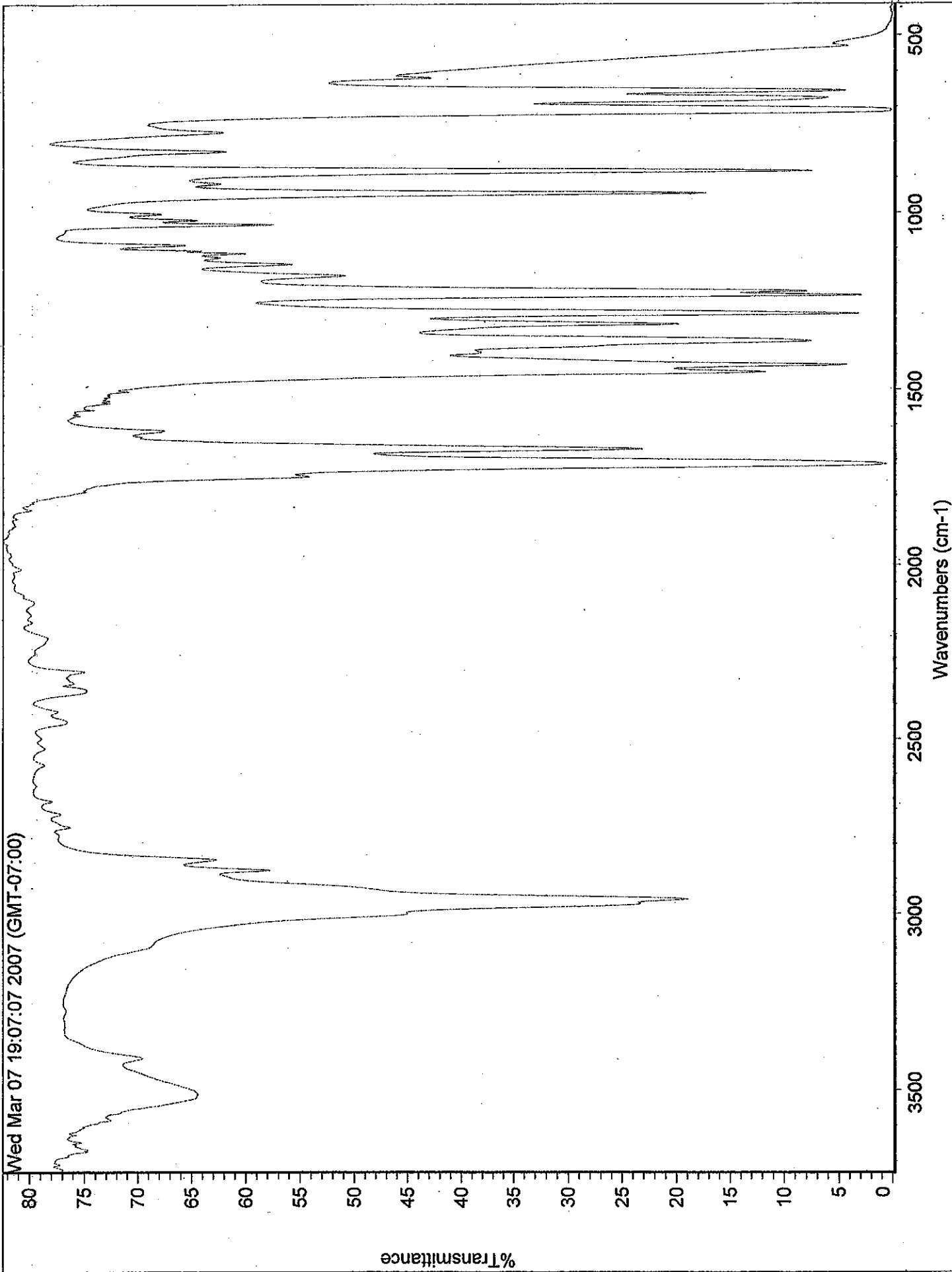
Single Beam

4000 3500 3000 2500 2000 1500 1000 500  
Wavenumbers (cm-1)



$\text{O}=\text{C}-\text{O}$   
Fe starting material

Wed Mar 07 19:07:07 2007 (GMT-07:00)



## **Acknowledgements**

**We would like to thank Glendale Community College for the use of the the FTIR machine to help us in these studies.**

# References

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