MODULATING THE AQUEOUS SOLUBILITY AND DISSOLUTION RATE OF HUPERZINE A THROUGH PHARMACEUTICAL CO-CRYSTALS

by

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Chemistry Research Project Report

in Partial Fulfillment of the

Requirements for the Course

BIOL4992 Independent Research

at

The University of Belize, Belmopan Campus

May, 2012

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2 Introduction

2.1 Literature Review of Huperzine A

Huperzine A is an alkaloid compound extracted from the firmoss, *Huperzia serrata*, found in Zhejiang, China. It is known as Qian Ceng Ta in Chinese and has been used as Traditional Chinese Medicine to treat swelling, hemorrhoids, vaginal yeast infections, and physical injuries (Raves et al., 1997; Chu, 2012). Being an organic compound, it is soluble in organic solvents such as chloroform, methanol and ethanol, but only sparingly or not at all soluble in water. Figure 1 shows the molecular structure of Huperzine A. The primary focus of this research is on the 2-pyridone group on the aromatic ring to the right of the figure; however, the free amino group in the lower left plays a significant role in the findings of this research, as will be shown later in this paper.

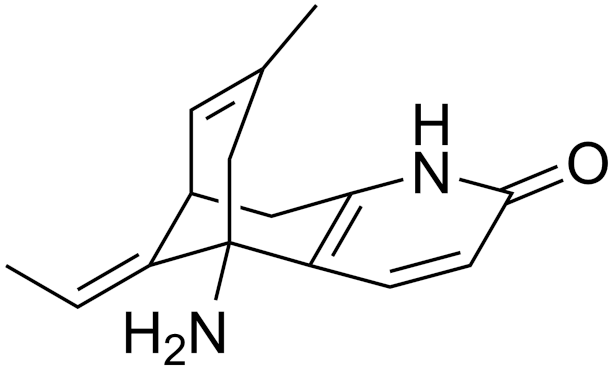

FIGURE 2.1.1 MOLECULAR STRUCTURE OF HUPERZINE A

Huperzine A is sold as a white crystalline powder or brown fine powder in 1%, 5%, 98% and 99% purity from suppliers mainly in Mainland China and at prices ranging from US$1 to $2,000 per bottle or package. As a herbal medicine, neutraceutical, and dietary supplement, it is marketed to have high efficacy in combating dementias or neurodegenerative disorders. It works by strongly and very specifically inhibiting acetylcholinesterase, an enzyme that catalyzes the hydrolytic breakdown of the neurotransmitter acetylcholine and interferes with neural transmissions in the brain (Raves et al., 1997). Other common acetylcholinesterase inhibitors that are clinically used include those under the brand names Tacrine, Donepezil, Galanthamine, and Rivastigmine, but Huperzine A is said to produce fewer side effects while being even more effective (Chu, 2012; Li, 2012). Its antioxidant and neuroprotective properties as revealed by several controlled clinical trials led to it being approved as the treatment drug for Alzheimer's disease (AD) in China (Jiang, Luo, & Bai, 2003). Huperzine A is often prescribed to enhance cerebral functions, particularly for patients of Alzheimer's disease to improve memory, recognition, and behavior while producing no severe side effects; it is regarded as a promising drug for symptomatic treatment of Alzheimer's disease (Xu, et al., 1995). Clinical trials are undergoing in the U.S., where clinicians have used it for AD, to test for its efficacy on cognitive function and toxicity to human body (National Institute on Aging, 2008).

2.2 Scientific Problem

Solubility is an important parameter in measuring efficacy of active pharmaceutical ingredients (APIs) and a key issue in oral administration of drugs (Aakero¨ y, Forbes, & Desper, 2009). Many APIs have poor solubility in aqueous medium, which inhibits them from producing desired effects by oral ingestion, the most common method by which pharmaceutical drug is administered. Such water-insoluble drugs, for example, Huperzine A, are usually given in high

doses for therapeutic effects (Hickey, et al., 2007). Strategies that have been implemented to deal with these problems of poor solubility and thus low bioavailability include salt formation, physical stabilization of amorphous solids, complexation, encapsulation of organic solutions, and supramolecular synthesis (Remenar, 2003). In the pharmaceutical industry, a subfield of supramolecular synthesis known as crystal engineering of co-crystals, presents a relatively unexplored area and is becoming increasingly valuable as a method of producing crystalline solids that can offer altered/improved physical properties of an active pharmaceutical ingredient (API) without changing its chemical identity or biological activity (Aakero¨y, Grommet, & Desper, 2011).

2.3 Crystal Engineering

A co-crystal is defined in Shan & Zaworotko (2008) as "a multiple component crystal in which all components are solid under ambient conditions when in their pure form." Co-crystal synthesis is distinctly different from organic synthesis, which is based on the formation or cleavage of covalent bonds (Boese, Kirchner, Gehrke, Bendele, & Sustmann, 2003). Instead, the technique is based on the formation of non-covalent intermolecular interactions, namely hydrogen bond, to construct new structural blocks for the formation of new molecules using two complementary hydrogen-bonding moieties (Aakero¨ y, et al., 2000). Crystal engineering of active pharmaceutical ingredients (APIs) is an emerging research field that has received considerable attention and is widely recognized as the best approach to form solid crystals of a marketed drug with improved performance characteristics, including solubility, dissolution rate, thermal stability, and low physical and chemical instability of the final modified product (Aakero¨ y, et al., 2009; Aakero¨ y, Beatty, Nieuwenhuyzen, & Zou, 2000; & Remenar, et al., 2003). Co-crystals offer unique opportunities to develop new solid forms, as opposed to traditional crystalline forms such as salts, polymorphs, and hydrates/solvates, of pharmaceutical drugs whose physico-chemical properties can be fine-tuned to offer optimal bioavailability on the final product. Examples of pharmaceutical co-crystals that have been studied include saccharin cocrystal of carbamazepine, an anti-epileptic agent; glutaric acid cocrystal of 2-[4-(4-chloro-2-fluorophenoxy)phenyl]pyrimidine-4-carboxamide, a drug candidate for treating or preventing surgical, chronic, and neuropathic pain; and itraconazole, an anti-fungal drug, (Hickey, et al., 2007; NcNamara, 2006; Shan & Zarowotko, 2008).

2.4 Design of Co-crystals - Strategy

Some structural complementarities considered in modern supramolecular syntheses are

carboxylic acid/2-aminopyridine, carboxylic acid/urea, amides/acids, carboxylic acid/oxime, and hydroxyl/amino (Aakero¨y, et al., 2000). In these pairs, one of the molecule acts as the hydrogen bond donor, and the other the acceptor. 2-Pyridone group of Huperzine A and carboxyl group of organic acids are two self-complementary hydrogen-bonding entities that can also form hydrogen bonds with each other. A scheme of intramolecular hydrogen bonding of the two groups is shown in Figure 2.4.1 below:

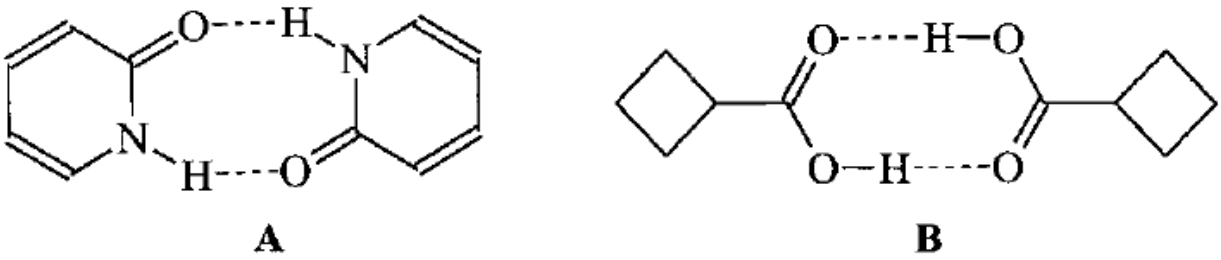

Figure 2.4.1 Hydrogen-Bonded Dimer of Two 2-Pyridone Moieties (**A**) and Dimer of Two Carboxylic Acid Moieties (**B**)[7.1]

The 2-pyridone dimers can form rigid structures; they are also flexible as they are capable of forming infinite chains whose intermolecular arrangements can be altered (Aakero¨ y, et al., 2000). Carboxylic acid head-to-head dimer as shown in B of Figure 1 is the most well-known and widely used hydrogen-bond motif in co-crystal synthesis as it also engages in a variety of intermolecular interactions. Figure 2.4.2 (adapted from Aakero¨ y, et al., 2000) shows two possible hydrogen-bonding motifs for 2-pyridone dimers and dicarboxylic acids and presents the type of intermolecular interaction that will be focused in this research: that the 2-pyridone group in Huperzine A will be forming hydrogen bonds with the carboxyl group from several selected carboxylic acids.

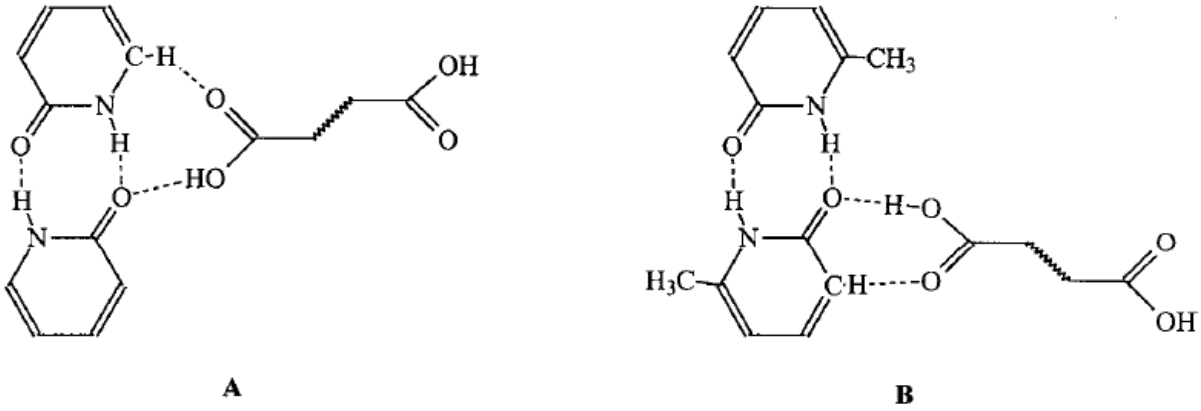

Figure 2.4.2 Two Possible Hydrogen-Bonding Motifs for 2-Pyridone Dimers and Dicarboxylic Acids **A** and **B** [7.1]

An earlier study of five co-crystals of 2-pyridone and some aliphatic dicarboxylic acids cited in Aakero¨ y, et al., 2000 showed that each crystal structure contained planar dimers of 2-pyridone bridged by a dicarboxylic acid via a combination of O-H---O and C-H---O hydrogen bonds, resulting in infinite 1-D ribbons. Furthermore, the study concluded that substituents on either the acid or the pyridone group would not affect formation of the pyridone dimer. An example of such formation is shown with fumaric acid in Figure 2.4.3; the 2-pyridone dimers are alternatingly bridged by the aliphatic fumaric acid by hydrogen-bonds (shown by dotted lines).

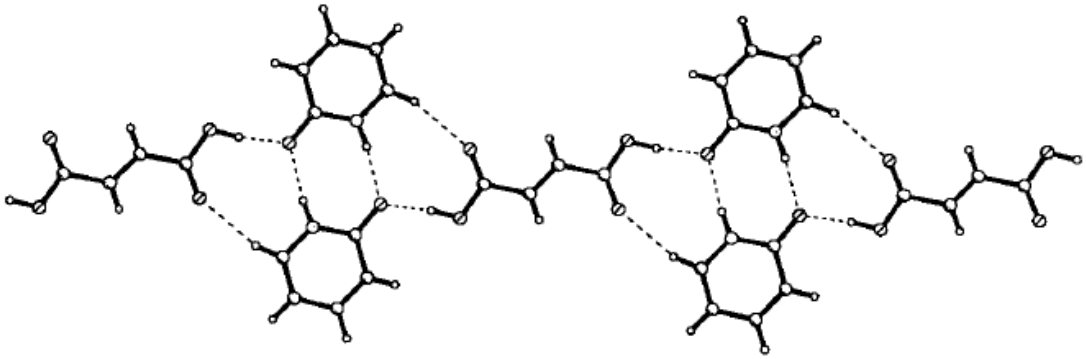

Figure 2.4.3 Hydrogen-Bonded Ribbon of Fumaric Acid and 2-Pyridone[7.1]

As previous studies of Huperzine A have primarily focused on clinical trials for potential treatment for Alzheimer's disease and other neuroprotective functions, this study will focus on increasing Huperzine A's aqueous solubility and dissolution rate through the formation of co-crystals as a way to enhance its bioavailability to humans in consumption for its potent benefits.

2.5 Objectives

The study aims to synthesize cocrystals of Huperzine A with pharmaceutically acceptable carboxylic acids to form new compounds that have greater aqueous solubility and dissolution rate in order to enhance Huperzine A's bioavailability. The specific objectives to be achieved through this research are:

1. To perform co-crystal experiments between Huperzine A and carboxylic acids using various techniques, including slow evaporation, dry/pure grinding, solvent-drop grinding, and melt experiments.
2. To analyze prepared samples through melting point determination and infra-red spectrometry as a means of screening potential co-crystals that may have formed from the co-crystal experiments.
3. To perform aqueous solubility and dissolution rate experiments of successfully synthesized co-crystals of Huperzine A.

3 Experimental Procedure

3.1 Preparation of Co-crystals

3.1.1 Slow Evaporation Experiments

3.1.1.1 Reacting 10mg scale Huperzine A with Carboxylic Acids in a 1:1 Ratio

Huperzine A (10mg, 0.04127mmol) was dissolved in 1 mL of methanol. To this solution was added an ethanolic solution of a carboxylic acid (0.04127mmol) in a test tube. The mouth of the test tube was covered with perforated parafilm. The mixture was left to stand for slow evaporation of the solvent at ambient temperature. Observations were made daily. The physical appearance of the product formed (sample) was observed. The melting point was tested using a melting point apparatus for those products that had a crystalline consistency

3.1.1.2 Reacting 10mg scale Huperzine A with Carboxylic Acids in a 1:2 Ratio

Huperzine A (10mg, 0.04127mmol) was dissolved in 1 mL of methanol. To this solution was added an ethanolic solution of a carboxylic acid (0.08254mmol) in a test tube. The mouth of the test tube was covered with perforated parafilm. The mixture was left to stand for slow evaporation of the solvent at ambient temperature. Observations were made daily. The physical appearance of the product formed (sample) was observed. The melting point was tested using a melting point apparatus for those products that had a crystalline consistency

3.1.1.3 Reacting 50mg scale Huperzine A with Carboxylic Acids in a 1:1 Ratio

Huperzine A (50mg, 0.20634mmol) was dissolved in 1 mL of methanol. To this solution was added an ethanolic solution of a carboxylic acid (0.20634mmol) in a test tube. The mouth of the test tube was covered with perforated parafilm. The mixture was left to stand for slow evaporation of the solvent at ambient temperature. Observations were made daily. The physical appearance of the product formed (sample) was observed. The melting point was tested using a melting point apparatus for those products that had a crystalline consistency.

After the melting points were determined for those samples forming crystalline solids at 50mg scale, all mixtures were re-dissolved in isopropanol (2-propanol) in an attempt to form crystalline solids. The mixtures were re-covered with perforated parafilm, and left to stand. Observations were made daily. Solid formations were scraped off the side and bottom of beaker to provide samples for testing melting points.

3.1.1.4 Reacting 250mg scale Huperzine A with Fumaric Acid in a 1:1 Ratio

Huperzine A (250mg, 1.0317mmol) was dissolved in methanol, using a total of 3mL with heating. Fumaric acid (119.7mg, 1.0317mmol) was dissolved in isopropanol, using a total of 3mL with heating. The two solutions were combined and swirled to mix well. The mouth of the test tube was covered with perforated parafilm. The mixture was left to stand for slow evaporation of the solvent at ambient temperature. Observations were made daily. The physical appearance of the product formed (sample) was observed.

The results for slow evaporation experiments were recorded in a table like the following:

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample	Description of Sample
1	Benzoic acid	122.38		
2	Trans-3,4-dihydroxycinnamic acid	194-198		
3	Sorbic acid	135		
4	DL-Tartaric acid	206		
5	Gallic acid	250		
6	Glycolic acid	75		
7	DL-Malic acid	130		
8	Maleic acid	135		
9	Ibuprofen	76		
10	Isophthalic acid	347		
11	Acetylsalicylic acid	135		
12	Citric acid	153		
13	Adipic acid	152.1		
14	Succinic acid	184		
15	Hippuric acid	187-188		

16	Salicylic acid	159		
17	Fumaric acid	287		
18	Malonic acid	135-136		
19	Terephthalic acid	300		
20	Orotic acid	345		
*melting point of Huperzine A is 217^{o}C				

TABLE 3.1.1.1 DATA TABLE FOR SLOW EVAPORATION EXPERIMENTS

3.1.2 Grinding Experiments

Reacting Huperzine A (0.20634mmol) with Carboxylic Acids (0.20634mmol) in a 1:1 Ratio

3.1.2.1 Pure Grinding

Huperzine A (50mg, 0.20634mmol) was measured on a weighing paper using a balance and then transferred to a mortar. A carboxylic acid (0.20634mmol) was measured on a weighing paper using a balance and then added to the Huperzine A. The two substances were ground using a pestle, aided with spatula until a uniform crystalline mixture was formed. A small amount of the mixture (sample) was loaded into a capillary tube and tested for melting point using a melting point apparatus.

3.1.2.2 Solvent-Drop Grinding – One Drop Ethanol

Huperzine A (50mg, 0.20634mmol) was measured on a weighing paper using a balance and then transferred to a mortar. A carboxylic acid (0.20634mmol) was measured on a weighing paper using a balance and then added to the Huperzine A. The two substances were ground using a pestle, aided with spatula until a uniform crystalline mixture was formed. One drop of ethanol was dispensed unto the mixture using a plastic dropper. The mixture was ground until it formed a uniform crystalline mixture again. A small amount of the mixture (sample) was loaded into a capillary tube and tested for melting point using a melting point apparatus.

3.1.2.3 Solvent-Drop Grinding – Two Drops Ethanol

Huperzine A (50mg, 0.20634mmol) was measured on a weighing paper using a balance and then transferred to a mortar. A carboxylic acid (0.20634mmol) was measured on a weighing paper using a balance and then added to the Huperzine A. The two substances were ground using a pestle, aided with spatula until a uniform crystalline mixture was formed. One drop of ethanol was dispensed unto the mixture using a plastic dropper. The mixture was ground until it formed a

uniform crystalline mixture again. To the mixture was dispensed a second drop of ethanol, it was then ground to form a uniform crystalline mixture again. A small amount of the mixture (sample) was loaded into a capillary tube and tested for melting point using a melting point apparatus.

The results for grinding experiments were recorded in a table like the following:

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample
1	Benzoic acid	122.38		
2	Trans-3,4-dihydroxycinnamic acid	194-198		
3	Sorbic acid	135		
4	DL-Tartaric acid	206		
5	Gallic acid	250		
6	Glycolic acid	75		
7	DL-Malic acid	130		
8	Maleic acid	135		
9	Ibuprofen	76		
10	Isophthalic acid	347		
11	Acetylsalicylic acid	135		
12	Citric acid	153		
13	Adipic acid	152.1		
14	Succinic acid	184		
15	Hippuric acid	187-188		
16	Salicylic acid	159		
17	Fumaric acid	287		
18	Malonic acid	135-136		
19	Terephthalic acid	300		
20	Orotic acid	345		
*melting point of Huperzine A is 217^{0}C				

TABLE 3.1.2.1 DATA TABLE FOR GRINDING EXPERIMENTS

3.1.3 Melt Experiments

3.1.3.1 Reacting Huperzine A with Ditopic Bases in a 1:1 Ratio

Melt samples of Huperzine A with six selected ditopic bases were prepared. A one-to-one molar ratio of Huperzine A and a nitrogen base were placed together in a glass vial. The vial was heated directly using a heat gun until both components have been melted into a uniform molten sample which quickly formed a solid product. The procedure was repeated with all ditopic

nitrogen bases. A small amount of each solid product (a melt sample) formed was used for IR spectroscopy.

3.2 Analytical Screening of Crystal Formations

3.2.1 Infra-Red (IR) Spectrometry

3.2.1.1 IR Screening for Grinded Samples

A small quantity of each grinded sample of Huperzine A with carboxylic acid was used to obtain the infrared spectrum. The IR spectrum was recorded by passing IR radiation through the sample placed in the IR spectrometer. The spectrum was recorded in percent transmittance in the frequency range 500-4000 cm^{-1} at a resolution of 4 cm^{-1} and with a scanning speed of 32 mm/s. The recorded IR spectrum was compared with the standard spectra of the different functional groups to decipher the molecular structure of the sample.

IR spectrometry was done on grind samples of all carboxylic acids with Huperzine A, including the pure grinding and solvent-drop grinding with one drop and two drops of ethanol.

3.2.1.2 IR Screening for Melt Samples

A small quantity of each melt sample of Huperzine A with ditopic nitrogen base was used to obtain the infrared spectrum. The IR spectrum was recorded by passing IR radiation through the sample placed in the IR spectrometer. The spectrum was recorded in percent transmittance in the frequency range 500-4000 cm^{-1} at a resolution of 4 cm^{-1} and with a scanning speed of 32 mm/s. The recorded IR spectrum was compared with the standard spectra of the different functional groups to decipher the molecular structure of the sample

4 Results

4.1 Slow Evaporation Results

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample
1	Benzoic acid	122.38	--	White, filamentous crystals, translucent gel
2	Trans-3,4-dihydroxycinnamic acid	194-198	--	Small orange brown membranous flakes
3	Sorbic acid	135	--	Deep brown gel
4	DL-Tartaric acid	206	--	Clear gel*[2]
5	Gallic acid	250	130 (decomposed)	Thin, brown flakes
6	Glycolic acid	75	219	Chunk of clear crystal
7	DL-Malic acid	130	--	Yellowish gel*[2]
8	Maleic acid	135	--	Brownish gel*[2]
9	Ibuprofen	76	--	Brownish gel*[2]
10	Isophthalic acid	347	250 (decomposed)	White, snow-flake crystals
11	Acetylsalicylic acid	135	--	Clear gel*[2]
12	Citric acid	153	--	Clear gel*[2]
13	Adipic acid	152.1	--	Brownish gel *[2]
14	Succinic acid	184	--	Brownish gel*[2]
15	Hippuric acid	187-188	--	Clear gel*[2]
16	Salicylic acid	159	--	Translucent gel interspersed with brownish murky oily bubbles
17	Fumaric acid	287	--	Filamentous crystal
18	Malonic acid	135-136	--	Clear gel*[2]
19	Terephthalic acid	300	295	Cream yellow solid deposits
20	Orotic acid	345	--	N/A*[3]

*Melting point of Huperzine A is 217^{0}C

*[2]The gel that formed was simply the Huperzine A and carboxylic acid mixture when all solvent has evaporated, leaving “gel-like” formations of generally homogeneous texture at the bottom of the test tube

*[3]Orotic acid has not arrived in lab the time the experiment was conducted

TABLE 4.1.1 MELTING POINT DATA FOR SAMPLES OF SLOW EVARPOATION 10MG SCALE 1:1 RATIO

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample	Description of Sample
1	Benzoic acid	122.38	--	Translucent gel interspersed with small oily bubbles
2	Trans-3,4-dihydroxycinnamic acid	194-198	--	Yellowish milky gel
3	Sorbic acid	135	149	Rod-like brown crystal
4	DL-Tartaric acid	206	--	Yellowish gel*[2]
5	Gallic acid	250	--	Yellowish brown solution
6	Glycolic acid	75	--	Yellowish gel*[2]
7	DL-Malic acid	130	--	Yellowish gel*[2]
8	Maleic acid	135	--	Yellowish gel*[2]
9	Ibuprofen	76	--	Translucent gel with oily bubbles
10	Isophthalic acid	347	248-285	Transparent, filamentous to stellar-shaped crystals, translucent gel
11	Acetylsalicylic acid	135	--	Translucent gel interspersed with murky oily spots
12	Citric acid	153	--	Milky cream flakes*[2]
13	Adipic acid	152.1	--	Yellowish gel*[2]
14	Succinic acid	184	--	Yellowish gel*[2]
15	Hippuric acid	187-188	205	Yellowish gel*[2]
16	Salicylic acid	159	--	Translucent gel with one big oily bubble
17	Fumaric acid	287	285	Transparent, filamentous to stellar-shaped crystals
18	Malonic acid	135-136	--	Clear gel*[2]
19	Terephthalic acid	300	--	Could not dissolve in solvent
20	Orotic acid	345	--	N/A*[3]

*melting point of Huperzine A is 217^{0}C

*[2]The gel that formed was simply the Huperzine A and carboxylic acid mixture when all solvent has evaporated, leaving "gel-like" formations of generally homogeneous texture at the bottom of the test tube

*[3]Orotic acid has not arrived in lab the time the experiment was conducted

TABLE 4.1.2 MELTING POINT DATA FOR SAMPLES OF SLOW EVARPOATION

10MG SCALE 1:2 RATIO

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample (after solvent dried)	Description of Sample (after being scraped off)
1	Benzoic acid	122.38	186-190	Clear gel with tiny bubble	White powder
2	Trans-3,4-dihydroxycinnamic acid	194-198	179	Bright yellow oily gel	--
3	Sorbic acid	135	131	Red orange gel	Deep orange powder
4	DL-Tartaric acid	206	--	Nothing formed	--
5	Gallic acid	250	124	Bright cream yellow gel	Bright cream yellow flakes
6	Glycolic acid	75	--	Nothing formed	--
7	DL-Malic acid	130	--	Nothing formed	--
8	Maleic acid	135	--	Nothing formed	--
9	Ibuprofen	76	110	Clear gel	White powder
10	Isophthalic acid	347	199	Clear gel	White powder
11	Acetylsalicylic acid	135	174	Clear oily gel	White crystals
12	Citric acid	153	--	Nothing formed	--
13	Adipic acid	152.1	--	Nothing formed	--
14	Succinic acid	184	--	Nothing formed	--
15	Hippuric acid	187-188	--	Nothing formed	--
16	Salicylic acid	159	219	Clear oily gel	White powder
17	Fumaric acid	287	220	A chunk of white crystals	--
18	Malonic acid	135-136	--	Nothing formed	--
19	Terephthalic acid	300	--	Could not dissolve in solvent	--
20	Orotic acid*[2]	345	--	--	--
*melting point of Huperzine A is 217^{0}C *[2]Orotic acid has not arrived in lab the time the experiment was conducted; when it did, it was dissolved in isopropanol directly					

TABLE 4.1.3 MELTING POINT DATA FOR SAMPLES OF SLOW EVARPOATION 50MG SCALE 1:1 RATIO IN ETHANOL

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample (after solvent dried)	Description of Sample (after being scraped off)
1	Benzoic acid	122.38	193	Cottony-like	White powder

				white crystals	
2	Trans-3,4-dihydroxycinnamic acid	194-198	188	Bright yellow green gel	Yellow powder
3	Sorbic acid	135	140	Deep brown gel	Yellow crystals
4	DL-Tartaric acid	206	215	Clear gel*[2]	White powder
5	Gallic acid	250	196	Peach yellow gel	Peach yellow powder
6	Glycolic acid	75	145	Clear gel*[2]	White powder
7	DL-Malic acid	130	175	Clear gel*[2]	White flakes
8	Maleic acid	135	--	Clear gel*[2]	--
9	Ibuprofen	76	113	Clear gel	White powder
10	Isophthalic acid	347	197	Clear gel	White crystals
11	Acetylsalicylic acid	135	226	Clear gel	White powder
12	Citric acid	153	206	Clear gel*[2]	White powder
13	Adipic acid	152.1	181	Chunks of translucent crystals	--
14	Succinic acid	184	126	Clear gel*[2]	Yellowish white powder
15	Hippuric acid	187-188	154	Clear gel*[2]	White powder
16	Salicylic acid	159	221	Clear gel	White powder
17	Fumaric acid	287	234	Clusters of white, pasty formations	--
18	Malonic acid	135-136	217	--	--
19	Terephthalic acid*[3]	300	--	Could not dissolve in solvent	--
20	Orotic acid*[3]	345	--	Could not dissolve in solvent	--

*melting point of Huperzine A is 217^{0}C

*[2]The gel that formed was simply the Huperzine A and carboxylic acid mixture when all solvent has evaporated, leaving “gel-like” formations of generally homogeneous texture at the bottom of the test tube

*[3]Terephthalic acid could not be dissolve in any solvent attempted, even the 6M sodium hydroxide solution which dissolved it in Experiment IA; orotic acid did not dissolve in isopropanol

TABLE 4.1.4 MELTING POINT DATA FOR SAMPLES OF SLOW EVARPOATION 50MG SCALE 1:1 RATIO IN ISOPROPANOL

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample (after solvent dried)
1	Fumaric acid	287	158	Pale yellow gel
*melting point of Huperzine A is 217^{0}C				

TABLE 4.1.5 MELTING POINT DATA FOR SAMPLES OF SLOW EVARPOATION 250MG SCALE 1:1 RATIO IN ISOPROPANOL

4.2 Grinding Results

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample	Description of Sample
1	Benzoic acid	122.38	116-8	White powder
2	Trans-3,4-dihydroxycinnamic acid	194-198	203	Milky yellow to greenish powder
3	Sorbic acid	135	95-97	Pale orange powder
4	DL-Tartaric acid	206	195	White powder
5	Gallic acid	250	191	Greyish powder
6	Glycolic acid	75	136	Grey granules
7	DL-Malic acid	130	130	Grey, hygroscopic granules
8	Maleic acid	135	119	Grey, hygroscopic granules
9	Ibuprofen	76	98-99	Greyish white powder
10	Isophthalic acid	347	201	White powder
11	Acetylsalicylic acid	135	126-127	Greyish white powder
12	Citric acid	153	230	White grey chunks
13	Adipic acid	152.1	129-130	Hygroscopic granules
14	Succinic acid	184	149-150	White powder
15	Hippuric acid	187-188	154-157	Grey, hygroscopic granules
16	Salicylic acid	159	218	White powder
17	Fumaric acid	287	188-190	White powder
18	Malonic acid	135-136	178	Hygroscopic
19	Terephthalic acid	300	212	White powder
20	Orotic acid	345	273	White powder
*melting point of Huperzine A is 217^{0}C				

TABLE 4.2.1 MELTING POINT DATA FOR SAMPLES OF PURE GRINDING

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample
1	Benzoic acid	122.38	116	White powder
2	Trans-3,4-dihydroxycinnamic acid	194-198	203	Milky yellow to greenish powder
3	Sorbic acid	135	74-78	Pale orange powder
4	DL-Tartaric acid	206	120	White powder
5	Gallic acid	250	182-183	Greyish powder
6	Glycolic acid	75	131	Greyish white granules
7	DL-Malic acid	130	116-118	Grey, hygroscopic granules
8	Maleic acid	135	190	Grey, hygroscopic granules
9	Ibuprofen	76	91	Greyish white powder
10	Isophthalic acid	347	196-200	White powder
11	Acetylsalicylic acid	135	114	White powder
12	Citric acid	153	225-230	Grey chunks
13	Adipic acid	152.1	182	Hygroscopic granules
14	Succinic acid	184	108-110	Grey hygroscopic granules
15	Hippuric acid	187-188	110-111	Grey, hygroscopic granules
16	Salicylic acid	159	138	White powder
17	Fumaric acid	287	108-110	White powder
18	Malonic acid	135-136	178	Hygroscopic
19	Terephthalic acid	300	196	White powder
20	Orotic acid	345	266	White powder
*melting point of Huperzine A is 217^{0}C				

TABLE 4.2.2 MELTING POINT DATA FOR SAMPLES OF SOLVENT-DROP GRINDING – ONE DROP ETHANOL

Experiment	Carboxylic Acid	Melting Point of Carboxylic Acid (^{0}C)	Melting Point of Sample (^{0}C)	Description of Sample
1	Benzoic acid	122.38	191	Grey powder
2	Trans-3,4-dihydroxycinnamic acid	194-198	175	Milky yellow to greenish powder
3	Sorbic acid	135	112	Orange powder
4	DL-Tartaric acid	206	115	Grey powder
5	Gallic acid	250	186	White powder
6	Glycolic acid	75	--*[2]	--
7	DL-Malic acid	130	107	Grey puddle
8	Maleic acid	135	180	No more sample left
9	Ibuprofen	76	109	Greyish white powder
10	Isophthalic acid	347	207	White powder
11	Acetylsalicylic acid	135	100	Grey, hygroscopic granules
12	Citric acid	153	230	Grey powder
13	Adipic acid	152.1	179-183	Grey powder
14	Succinic acid	184	120-121	White powder
15	Hippuric acid	187-188	111	Greyish white powder
16	Salicylic acid	159	129	White powder
17	Fumaric acid	287	117	Fine grey powder
18	Malonic acid	135-136	--*[2]	--
19	Terephthalic acid	300	196-200	White powder
20	Orotic acid	345	263	White powder

*melting point of Huperzine A is 217^{0}C

*[2]Too little an amount would be available for melting point testing and storage, as the solids diminished in volume after addition of solvent, so no grinding was done

TABLE 4.2.3 MELTING POINT DATA FOR SAMPLES OF SOLVENT-DROP GRINDING – TWO DROPS ETHANOL

4.3 IR Screening Results

Experiment	Carboxylic Acid	Frequencies (cm^{-1})	
		1650-1550	1400
1	Benzoic acid	1535.90	1372.63
2	3,4-dihydroxycinnamic acid	1547.31	1375.93
3	Sorbic acid	1547.08	1384.45
5	Gallic acid*	1540.60	1383.92
6	Glycolic acid*	1548.36	1377.43
7	Malic acid	1547.25	1359.93
9	Ibuprofen	1546.37	1381.86
10	Isophthalic acid	1545.69	1360.93
11	Acetylsalicylic acid	1551.96	1380.47
12	Citric acid	1545.33	1358.96
14	Succinic acid	1538.54	1359.83
15	Hippuric acid	1544.83	1388.44
17	Fumaric acid	1536.95	1360.46
18	Malonic acid*	1544.48	1359.48
19	Terephthalic acid	1545.86	1364.15
*The frequencies are for one drop ethanol because IR spectra for two drops ethanol were not available			

TABLE 4.3.1 INFRA-RED STRETCHING FREQUENCIES OF CARBOXYLATES FORMED IN SAMPLES OF SOLVENT-DROP GRINDING – TWO DROPS ETHANOL, COMPARED WITH TYPICAL CARBOXYLATE STRETCHES AT 1650-1550 AND 1400 CM^{-1}

Melt Sample	Frequencies (cm^{-1})		
	Free NH_2 Group		C=O of 2-Pyridone Group
Huperzine A only	3471.93	3338.22	1643.37
1,2-bis(4-pyridyl)ethane	3362.87	3273.34	1650.18
1,4-bis(5,6-dimethylbenzimidazol-1-yl)methylbenzene	3354.17	3276.87	1650.98
4,4'-dipyridyl	3370.68	3284.69	1663.89
4,4-trimethylenedipyridine	3355.05	3268.78	1650.01
4-phenylpyridine	3358.96	3270.69	1649.96
Trans-1,2-bis(4-pyridyl)ethylene	3421.66	3351.14	1644.44

TABLE 4.3.2 INFRA-RED STRETCHING FREQUENCIES OF FREE NH_2 GROUP AND C=O GROUP OF 2-PYRIDONE GROUP OF HUPERZINE A AND THE MELT SAMPLES

5 Discussion

5.1 Analysis of Grind Samples

It would seem that co-crystals may have been formed when melting points of the grind samples were found to be between the range of temperatures bounded by the melting point of Huperzine A and that of the carboxylic acid. However, infra-red spectra indicated otherwise. Fifteen of the twenty IR spectra of the grind samples showed two peaks at frequencies typical of the carboxylate (RCO-) group, 1650-1550 and 1400 cm^{-1} (refer to Table 4.3.1). This is an indication that the reaction between Huperzine A and the carboxylic acids resulted in the formation of salts, not co-crystals. The IR spectrum of Huperzine A itself shows two peaks at high frequencies of 3471.93 and 3338.22 cm^{-1} (refer to Figure 4.3.2), indicating the presence of the free amino ($-NH_2$) group in the molecular structure of pure Huperzine A. The free amino group was lost in the IR spectra of the grind samples, denoting that the amino group had reacted, forming NH_3^+ cation. The formation of co-crystals would reveal an IR spectrum that shows shifts in the major functional peaks, not the loss of peaks. The free nitrogen of the amino group ($-NH_2$) of Huperzine A competed against the oxygen of the 2-pyridone group of Huperzine A, using its one pairs to form a covalent bond with the acidic hydrogen of the carboxyl group (-COOH) of the carboxylic acids, turning it into a carboxylate moiety ($-COO^-$), with itself becoming protonated (NH_3^+) and forming salts. All these confirmed that the grinding experiments of Huperzine A with the carboxylic acids have formed salts instead of co-crystals as expected. It was not certain whether the rest five of the twenty grind samples (adipic acid, maleic acid, salicylic acid, tartaric acid, and orotic acid) had formed salts or co-crystals and other screening techniques such as mass spectrometry may be performed to confirm the structure of the products formed.

An example of salt formation between the carboxylic acid and Huperzine A is shown in Figure 4. The yellow highlighted numbers mark the peaks that indicate the stretches of the functional groups named below the numbers.

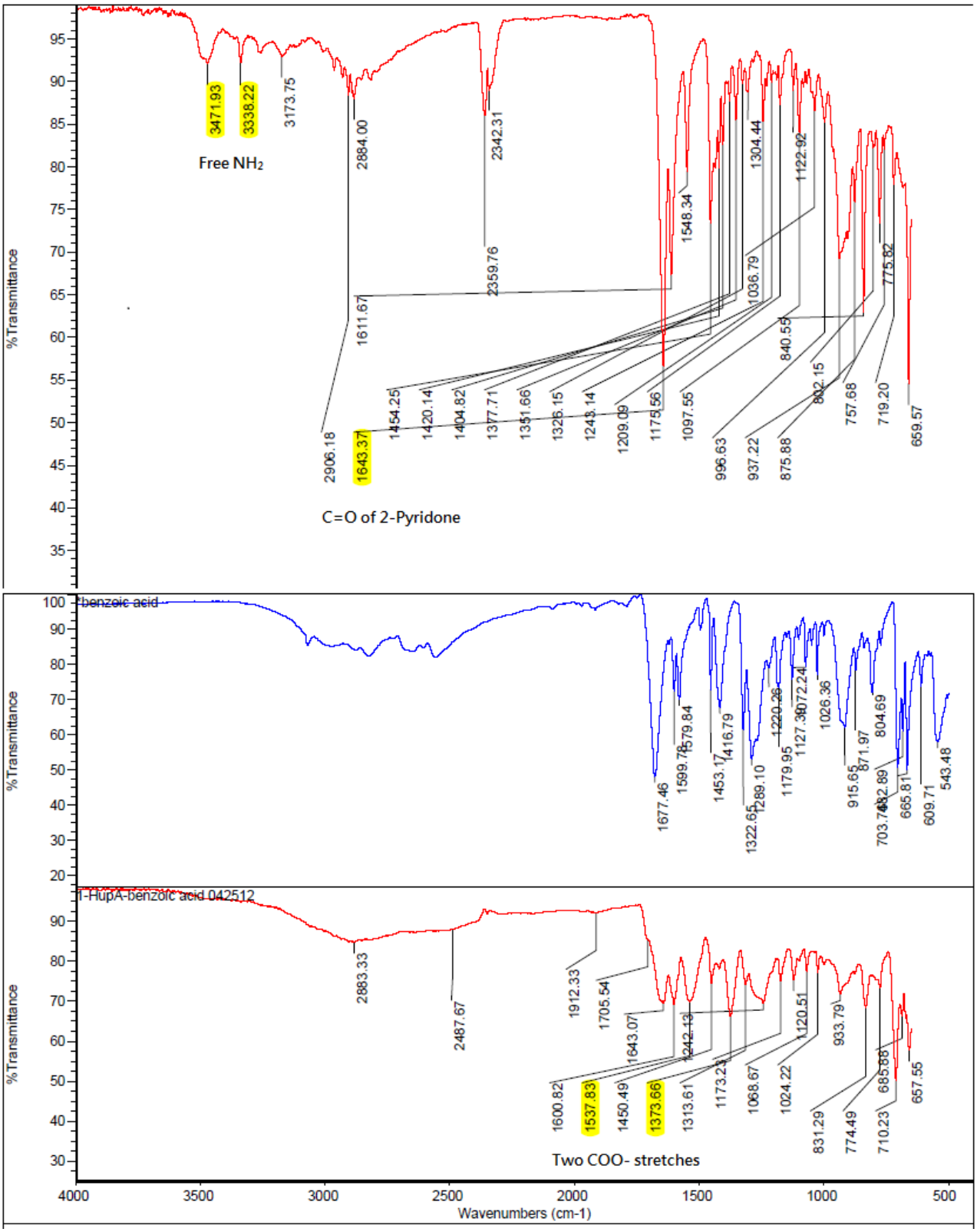

FIGURE 5.1.1 INFRA-RED SPECTRA SHOWING SALT FORMATION BETWEEN HUPERZINE A AND BENZOIC ACID

A segment of the possible structural pattern of the crystal formations from the fifteen grind

samples that formed salts is shown in Figure 5, with pairs of Huperzine A molecules forming a dimer by hydrogen-bonding between the 2-pyridone groups (refer to Figure 1 A), and the ionic bond forming between the hydrogen of the carboxyl group of carboxylic acid and the nitrogen of the free amino group of Huperzine A. The structures of Huperzine A and carboxylic acid are simplified to highlight the bonding occurring between the functional groups.

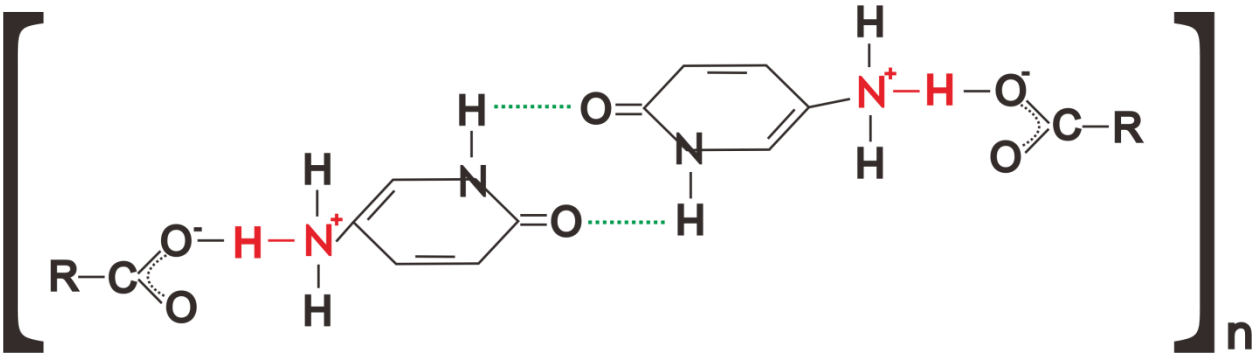

FIGURE 5.1.2 POSSIBLE STRUCTURAL PATTERN OF SALT FORMATIONS IN GRIND SAMPLES

The differences among the melting points of samples from pure grinding and solvent-drop grinding with one drop ethanol and two drops ethanol must have been due to changes in the composition of the samples. Observation of the infra-red spectra of the grind samples revealed a general trend of more complete conversion from two individual reactants, Huperzine A and carboxylic acid, to one uniform product, the Huperzine A-carboxylate salt, as more solvent was added, shown by peaks of frequencies closer and closer to the typical carboxylate stretches from dry grinding to solvent-drop grinding methods. Ethanol as solvent served to facilitate the coming together of the two reactants in the right orientation and energy to react, in favor of forming salts instead of co-crystals as was expected. The change in melting temperature from pure grind to solvent-grind with two drops of solvent was not the evidence of the formation of a different product (co-crystal), but was a result of the change in the chemical composition of the mixture as the ratio of product over reactants increased as more salt was formed.

Salt formations are not desired. They easily attained by reacting a base and an acid, such as Huperzine A and a carboxylic acid, respectively, as were obtained in the grinding experiments, although they were not desired results for the purpose of this research. Because salt formations are easily attained, the chemical interactions resulting in the formation of ionic bonds in salts are less predictable than the hydrogen-bonds that occur in the formation of co-crystals. This research is targeting hydrogen-bonds that would form specifically between the hydrogen of the carboxyl group of carboxylic acids and the oxygen of the 2-pyridone group of Huperzine A. This specificity would permit modulating the physico-chemical properties of the modified product, while maintaining its structural consistency and stability and not compromising its biological activity (Shan & Zaworotko, 2008).

5.2 Analysis of Melt Samples

The IR stretching frequencies of the free amino group (NH_2) at 3471.93 and 3338.22 cm^{-1} and the carbonyl group (C=O) of the 2-pyridone group at 1643.37 cm^{-1} of pure Huperzine A have shifted in the melt samples (refer to Table 10). The IR spectra of the melt samples showed a shift to lower wavenumbers in the first NH_2 peak at 3471.93 cm^{-1}, and shifts to higher wavenumbers in the second NH_2 at 3338.22 cm^{-1} and in the C=O peak at 1643.37 cm^{-1}. Also, NH_2 peaks have become broadened in the melt samples compared to the pure Huperzine A. In the absence of solvent effects of ethanol which promoted the formation of salts as seen in the grind samples, this may indicate possible co-crystal formations because the NH_2 peaks have only shifted and not lost. Figure 5.2.1 shows possible molecular structure for the crystal formation of the melt samples. A hydrogen-bond (represented by dotted line) is expected to occur between the partially negative nitrogen on one pyridine group of the nitrogen base, 4,4-dipyridyl in this case, and the partially positive hydrogen of the free amino group on the Huperzine A.

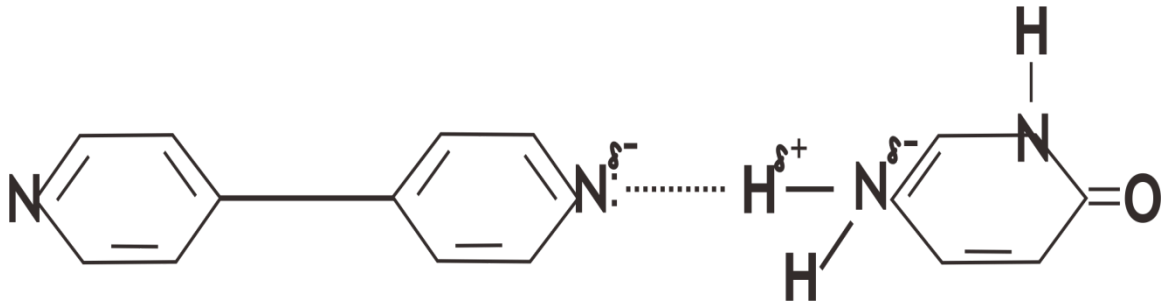

FIGURE 5.2.1 POSSIBLE STRUCTURAL PATTERN OF CRYSTAL FORMATIONS IN MELT SAMPLES

6 Future Work

6.1 Single Crystal Experiments with the Fifteen Salts

Single crystals, also known as monocrystalline solids, are crystals of uniform chemical composition throughout the crystalline lattice. As the grinding experiments have yielded salts of differing melting points, the ratio of product (salt) formation is variable, and further work will be targeting at complete reaction from two component solids to single crystals of salts. Once formed, solubility and dissolution rate experiments may be conducted to investigate the possibility of modulating these physico-chemical properties of the salts based on variation in the lengths of carbon-hydrogen backbone of carboxylic acids. Research into this aspect may also produce variation of Huperzine A, that is, Huperzine A-carboxylate salts, which exhibit better pharmaceutical performance than the pure form of the drug.

6.2 Co-crystallization Experiments of Huperzine A with ditopic nitrogen bases

Slow evaporation experiments of the ditopic nitrogen bases with Huperzine A are ongoing in continuation of this research in an attempt to successfully form co-crystals so that further investigations on solubility and dissolution rate can be conducted to meet the objectives.

6.3 Aqueous solubility and dissolution rate experiments of Huperzine A and Huperzine A co-crystals

These experiments will compare the solubility and dissolution rate in water for Huperzine A and Huperzine A co-crystals. The results will determine which co-crystals exhibit better aqueous solubility and dissolution rate than pure Huperzine A in terms of more quantity of the substance can be dissolved in a particular quantity of water and these co-crystals will present promising candidates for new formulations of the drug Huperzine A with improved pharmaceutical performance, which in turn will benefit patients suffering from cognitive defects and neurodegenerative disorders such as Alzheimer's Disease and dementia.

7 References

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8 Appendices

The IR spectra of the grind samples and melt samples are provided here in the Appendices in the following pages as a reference for the results and discussion sections of this paper. The IR

spectra in Figures 8.1 to 8.15 correspond to the IR frequency values that approximate typical carboxylate stretches shown in Table 4.3.1 for grind samples, and Figures 1.16 to 8.21 are comparable to the IR values showing shifts in amino (NH_2) group and 2-pyridone group in Table 4.3.2 for melt samples.

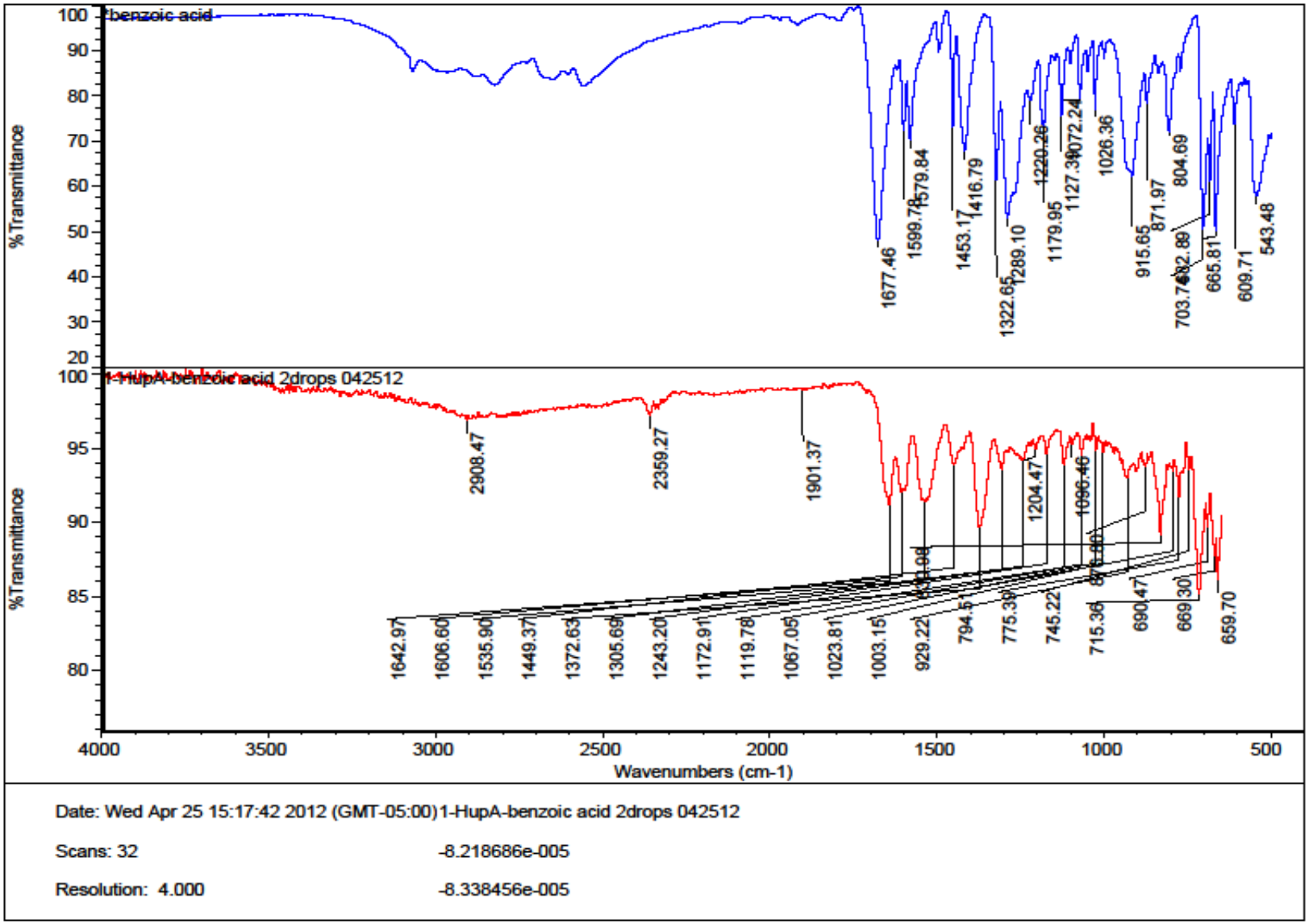


FIGURE 8.1 IR SPECTRUM OF BENZOIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

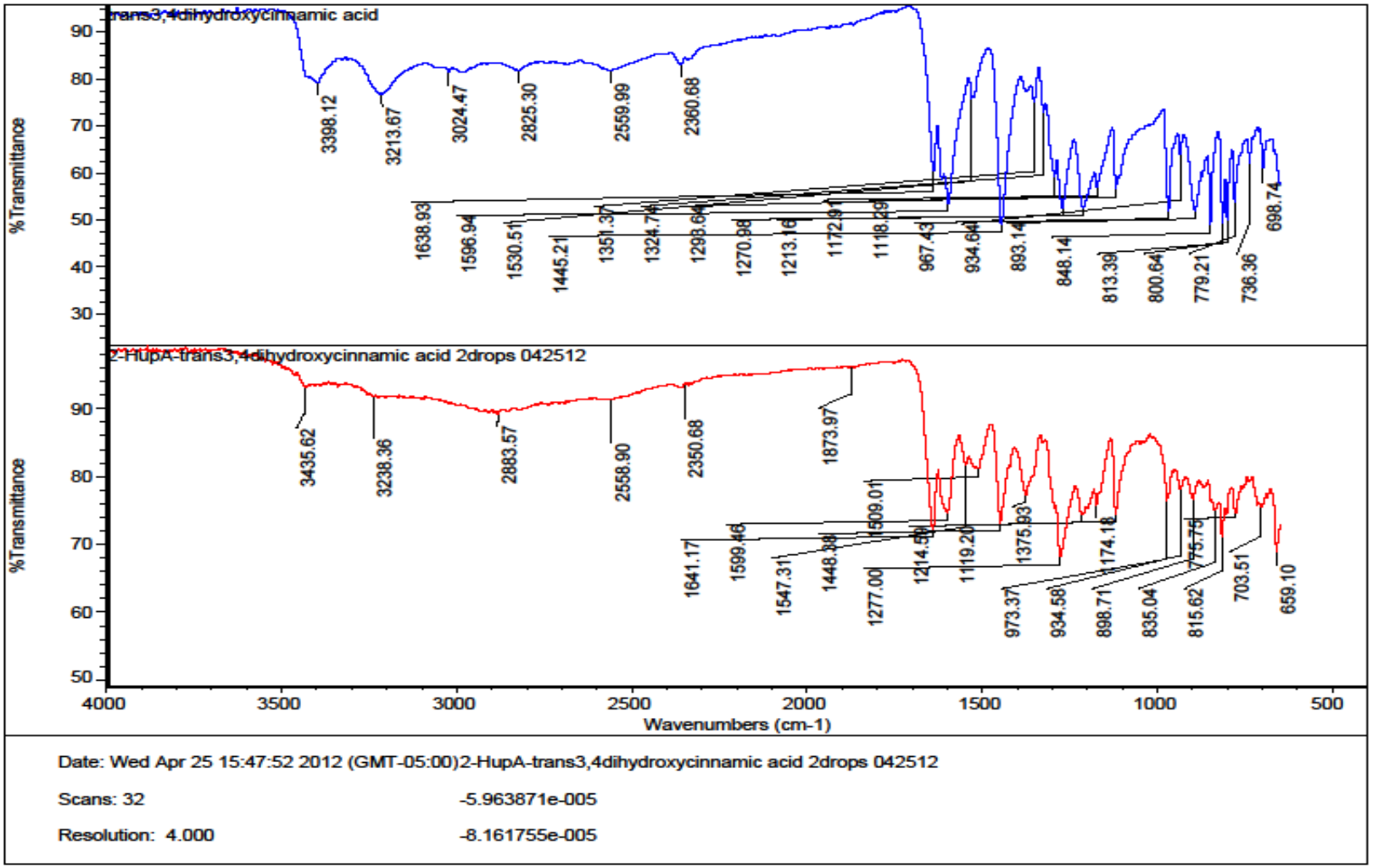
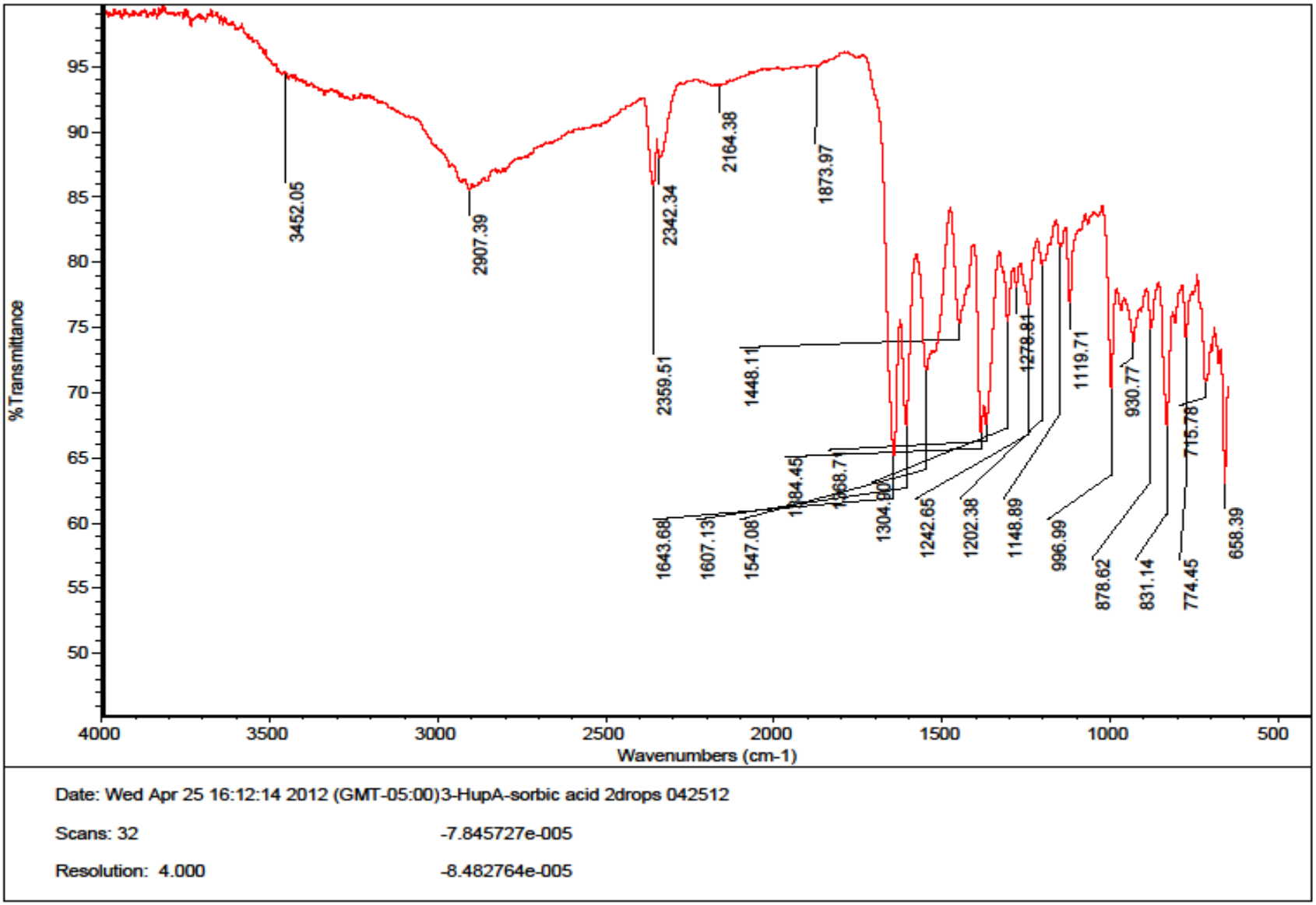
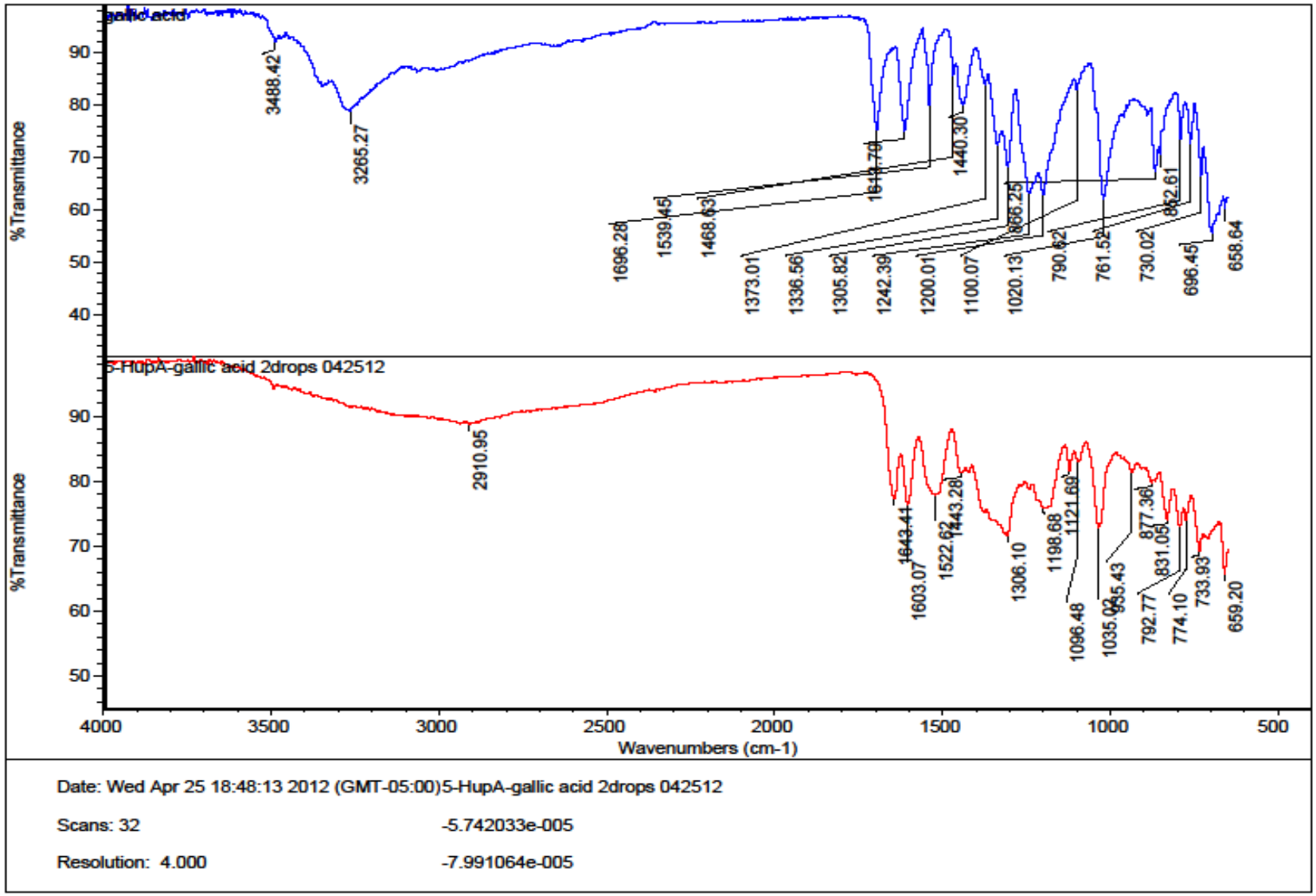


FIGURE 8.2 IR SPECTRUM OF TRANS-3,4-DIHYDROXYCINNAMIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL


% Transmittance
95
90
85
80
75
70
65
60
55
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
3452.05
2907.39
2342.34
2164.38
1873.97
2359.51
1448.11
1278.81
1119.71
930.77
715.78
1643.68
1607.13
1547.08
1484.45
1468.71
1304.90
1242.65
1202.38
1148.89
996.99
878.62
831.14
774.45
658.39
Date: Wed Apr 25 16:12:14 2012 (GMT-05:00)3-HupA-sorbic acid 2drops 042512
Scans: 32
Resolution: 4.000
-7.845727e-005
-8.482764e-005


gallic acid
3488.42
3265.27
1696.28
1539.45
1468.63
1373.01
1336.56
1305.82
1242.39
1200.01
1100.07
1020.13
790.62
761.52
730.02
696.45
658.64
5-HupA-gallic acid 2drops 042512
2910.95
1643.41
1603.07
1522.62
1443.28
1306.10
1198.68
1121.69
1096.48
1035.02
877.36
831.05
792.77
774.10
733.93
659.20
%Transmittance
Wavenumbers (cm-1)
Date: Wed Apr 25 18:48:13 2012 (GMT-05:00)5-HupA-gallic acid 2drops 042512
Scans: 32
Resolution: 4.000
-5.742033e-005
-7.991064e-005

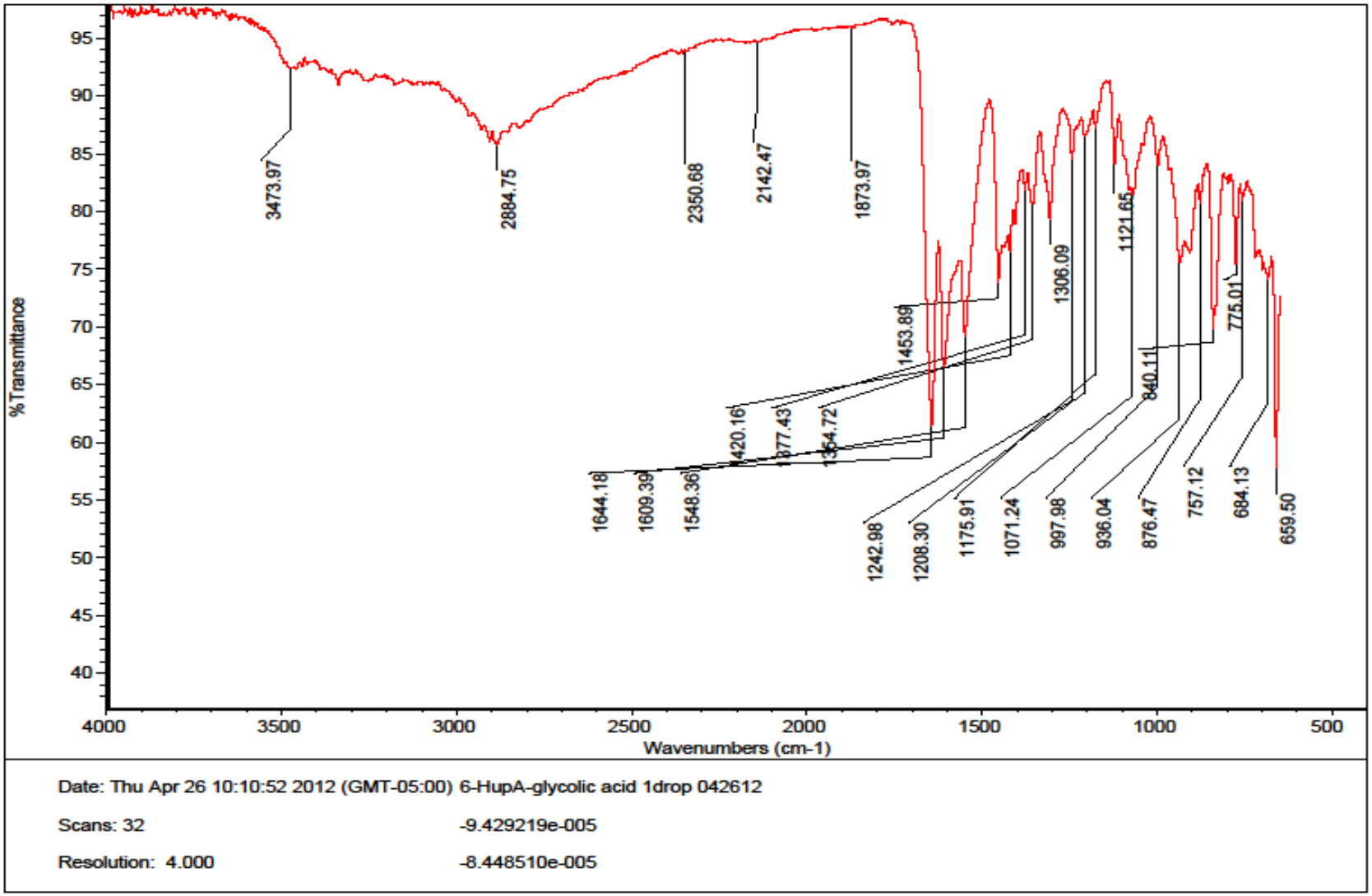


FIGURE 8.5 IR SPECTRUM OF GLYCOLIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH ONE DROP ETHANOL

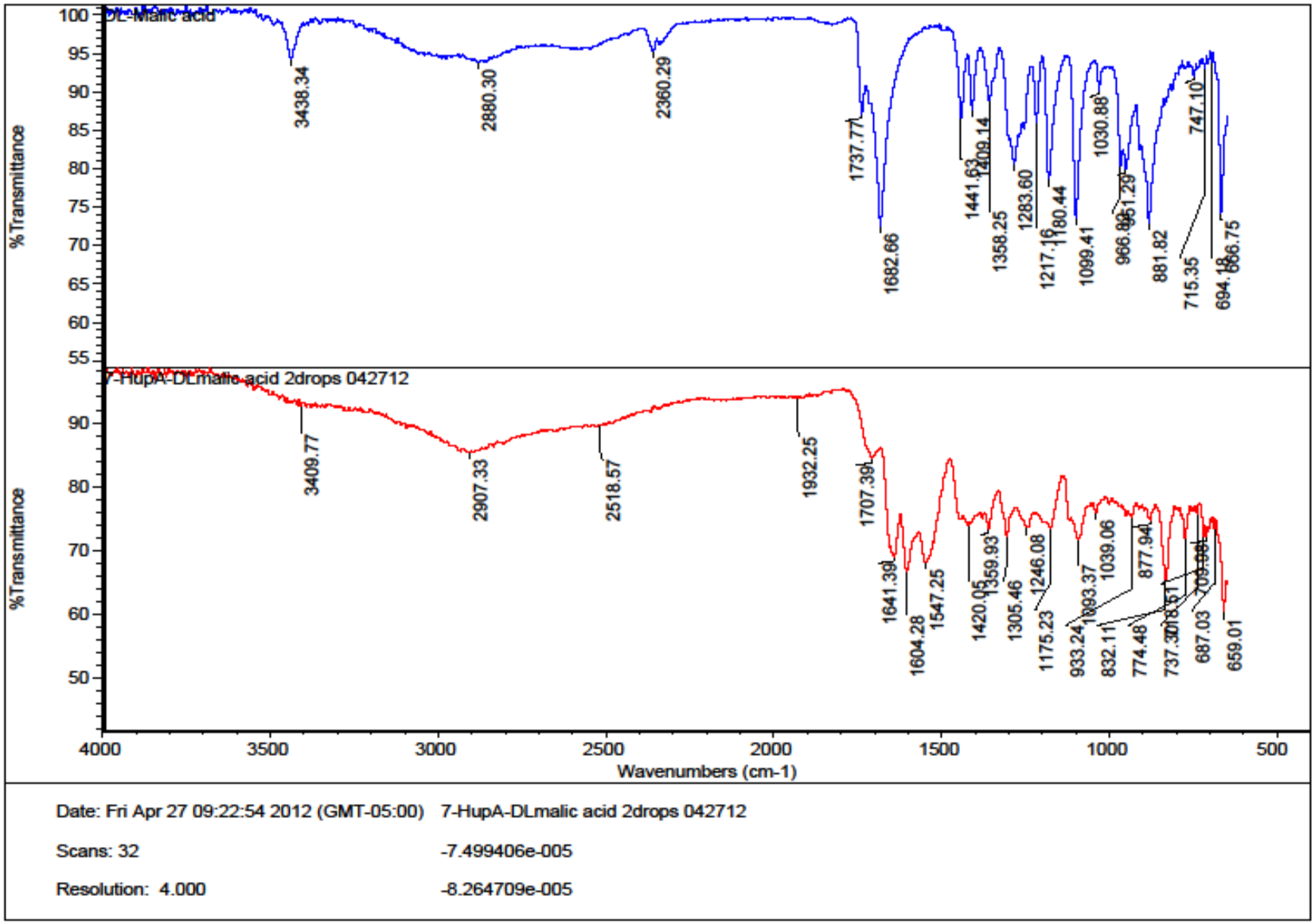
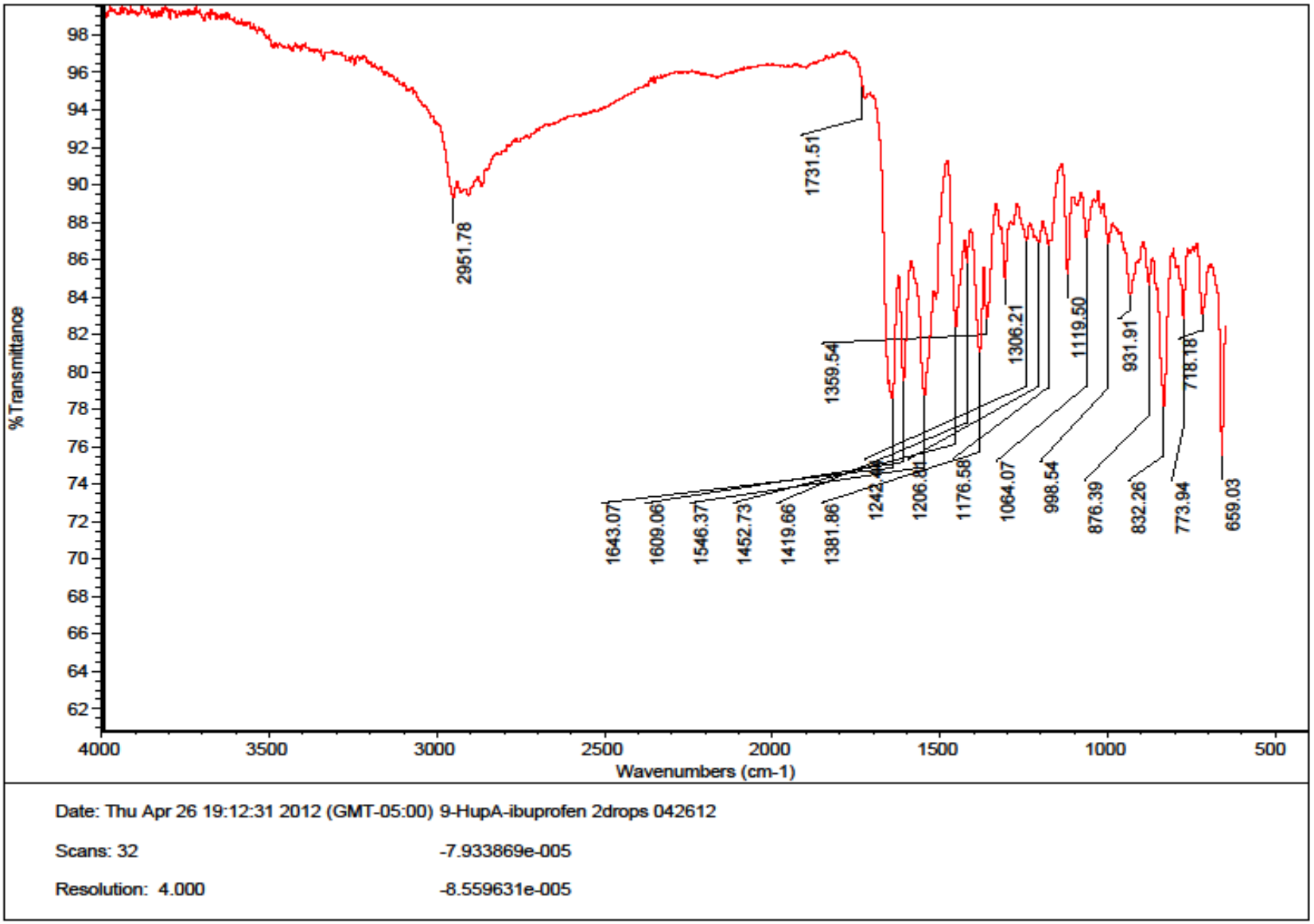


FIGURE 8.6 IR SPECTRUM OF MALIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL


%Transmittance
Wavenumbers (cm-1)
2951.78
1731.51
1359.54
1306.21
1119.50
931.91
718.18
1643.07
1609.06
1546.37
1452.73
1419.66
1381.86
1242.40
1206.80
1176.58
1064.07
998.54
876.39
832.26
773.94
659.03
Date: Thu Apr 26 19:12:31 2012 (GMT-05:00) 9-HupA-ibuprofen 2drops 042612
Scans: 32
-7.933869e-005
Resolution: 4.000
-8.559631e-005

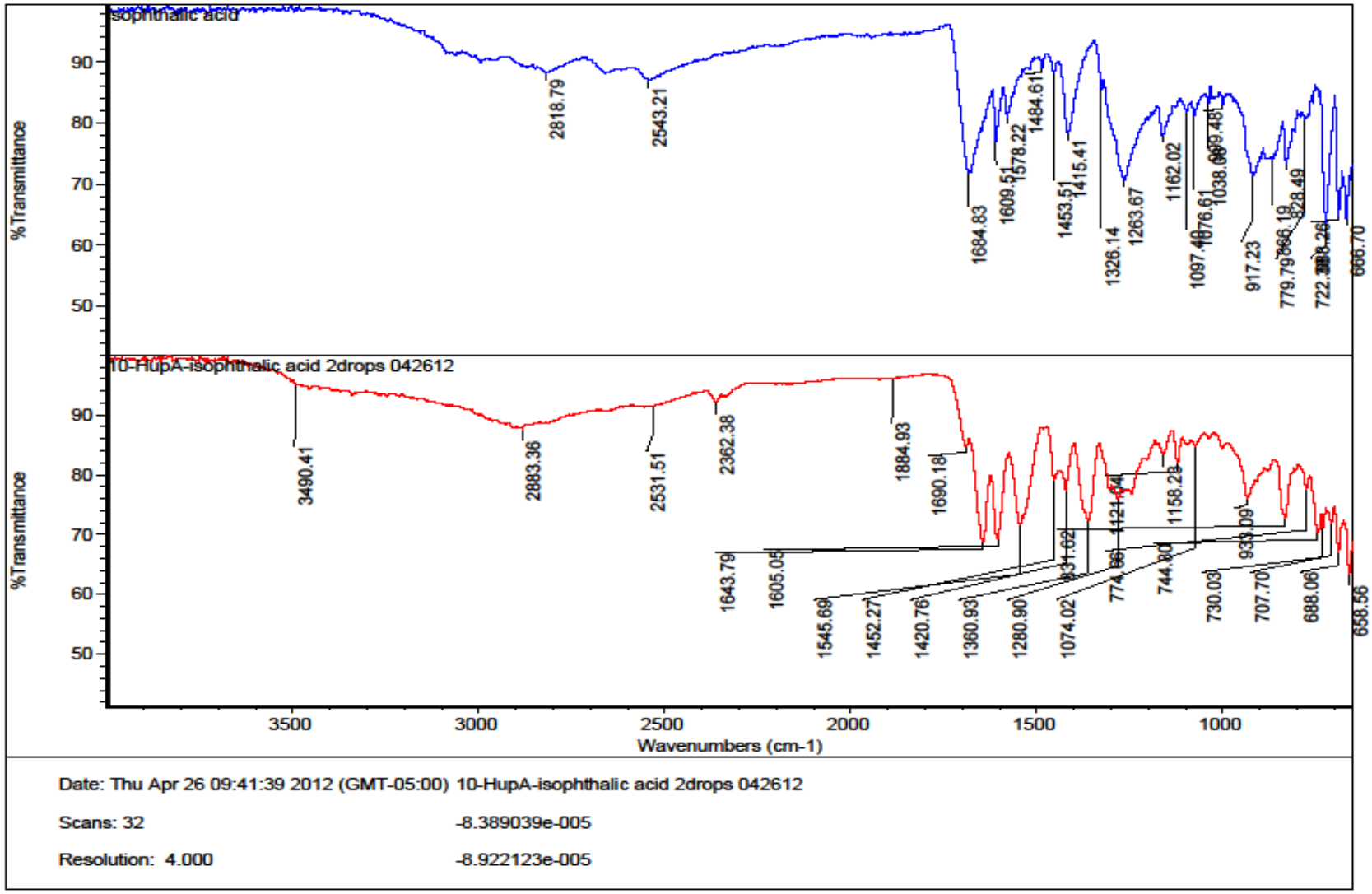
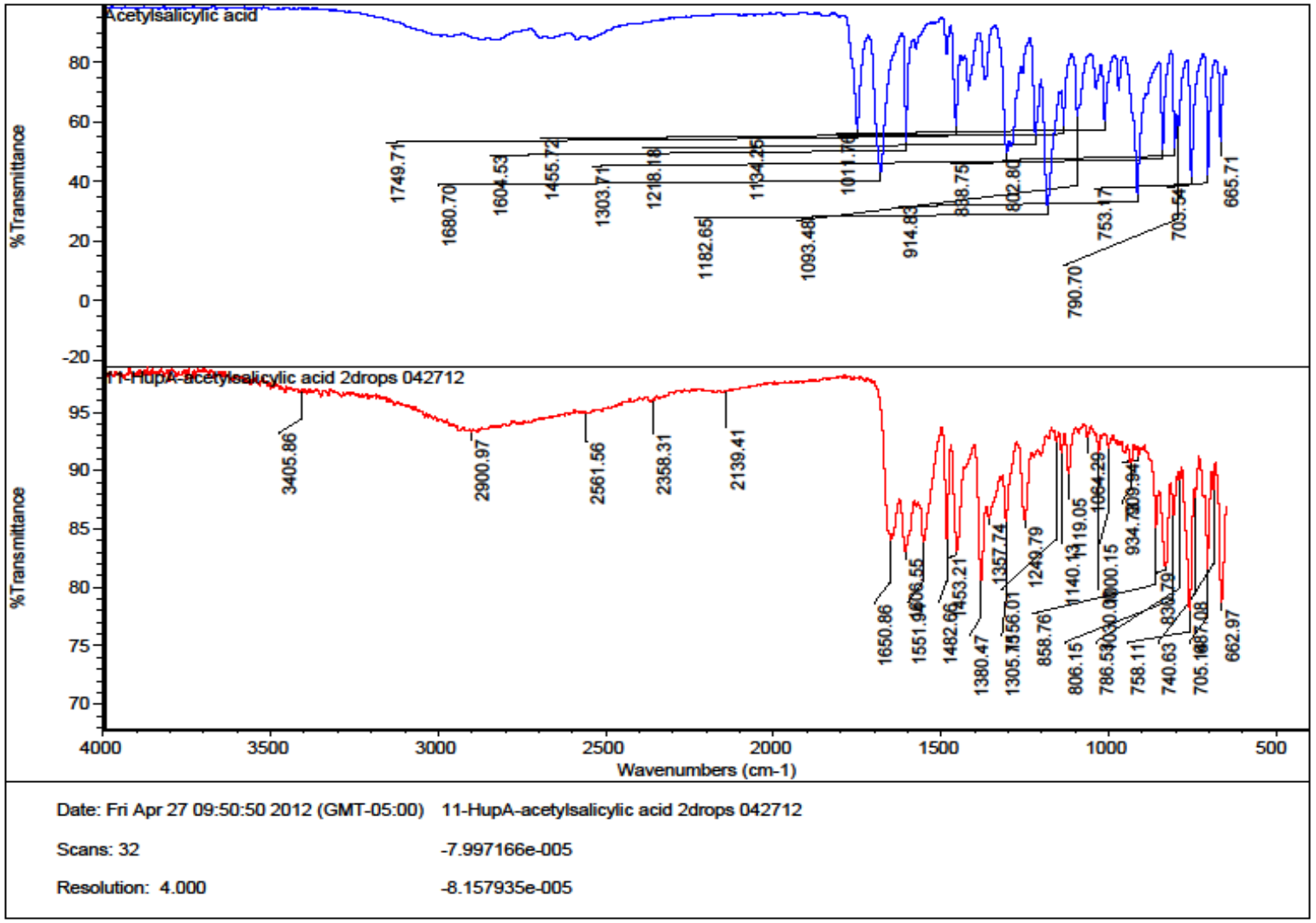


FIGURE 8.8 IR SPECTRUM OF ISOPHTHALIC ACID SAMPLE OF SOLVENT-DROP GRINDING

WITH TWO DROPS ETHANOL



FIGURE

8.9 IR SPECTRUM OF ACETYLSALICYLIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

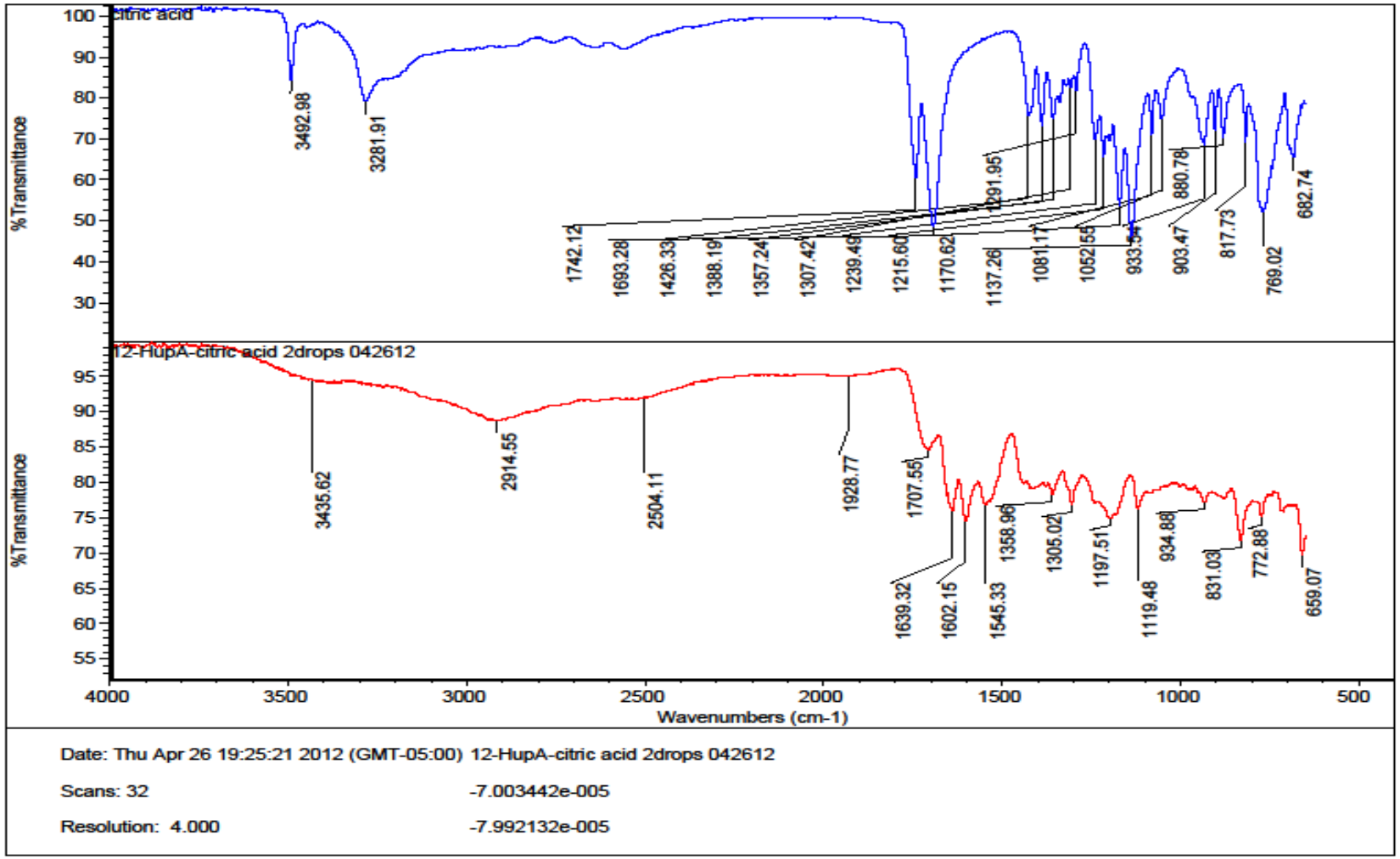


FIGURE 8.10 IR SPECTRUM OF CITRIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

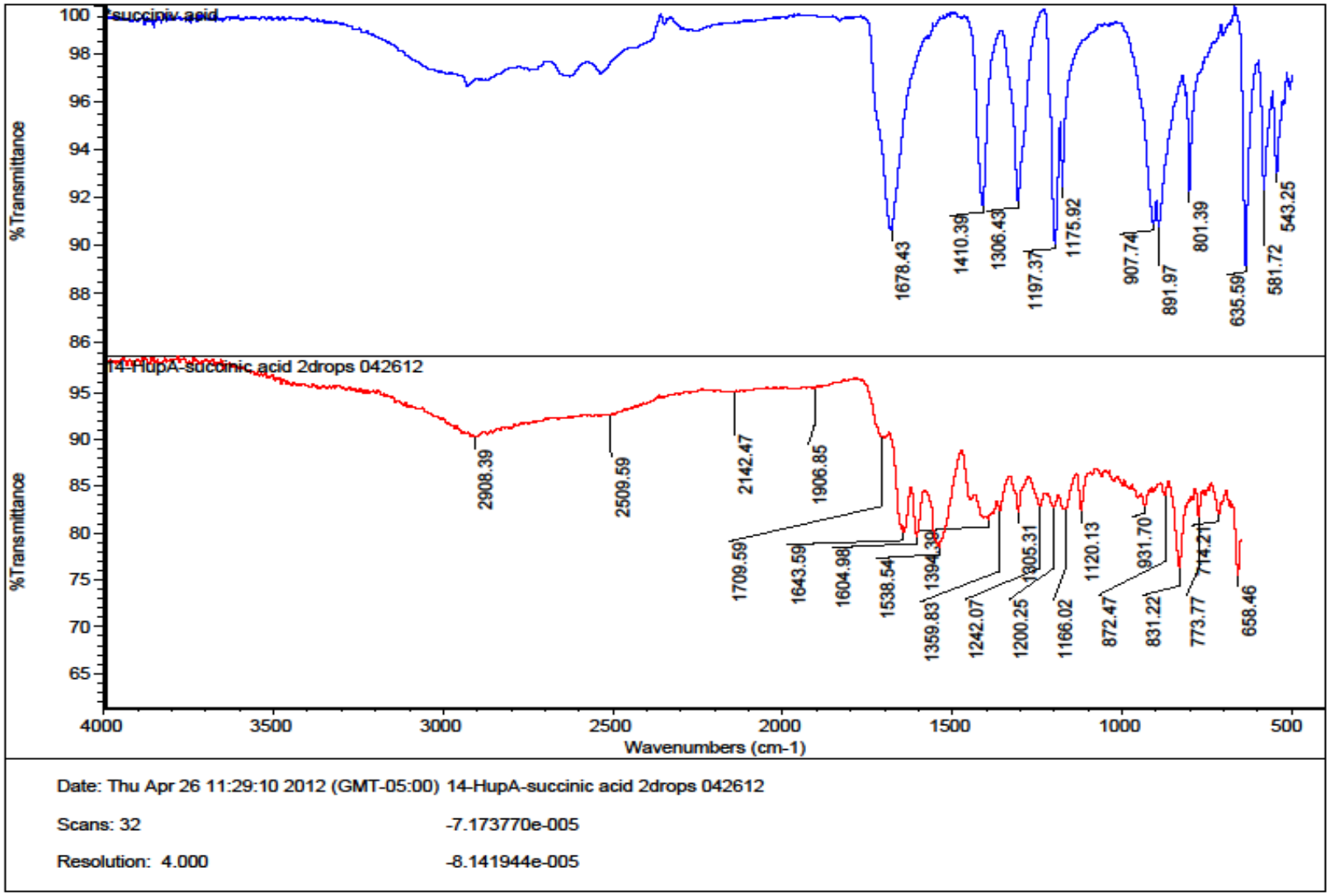


FIGURE 8.11 IR SPECTRUM OF SUCCINIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

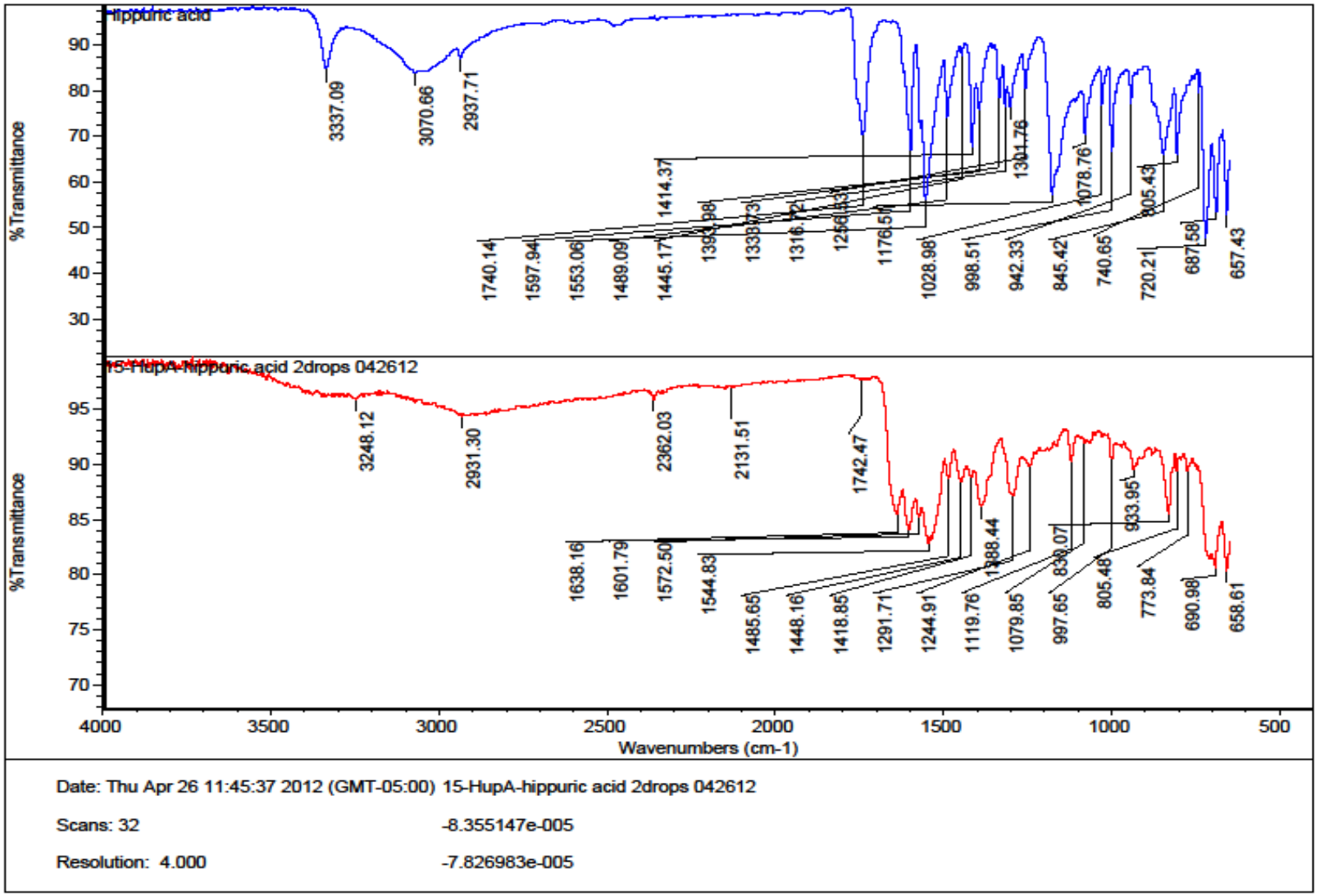


FIGURE 8.12 IR SPECTRUM OF HIPPURIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

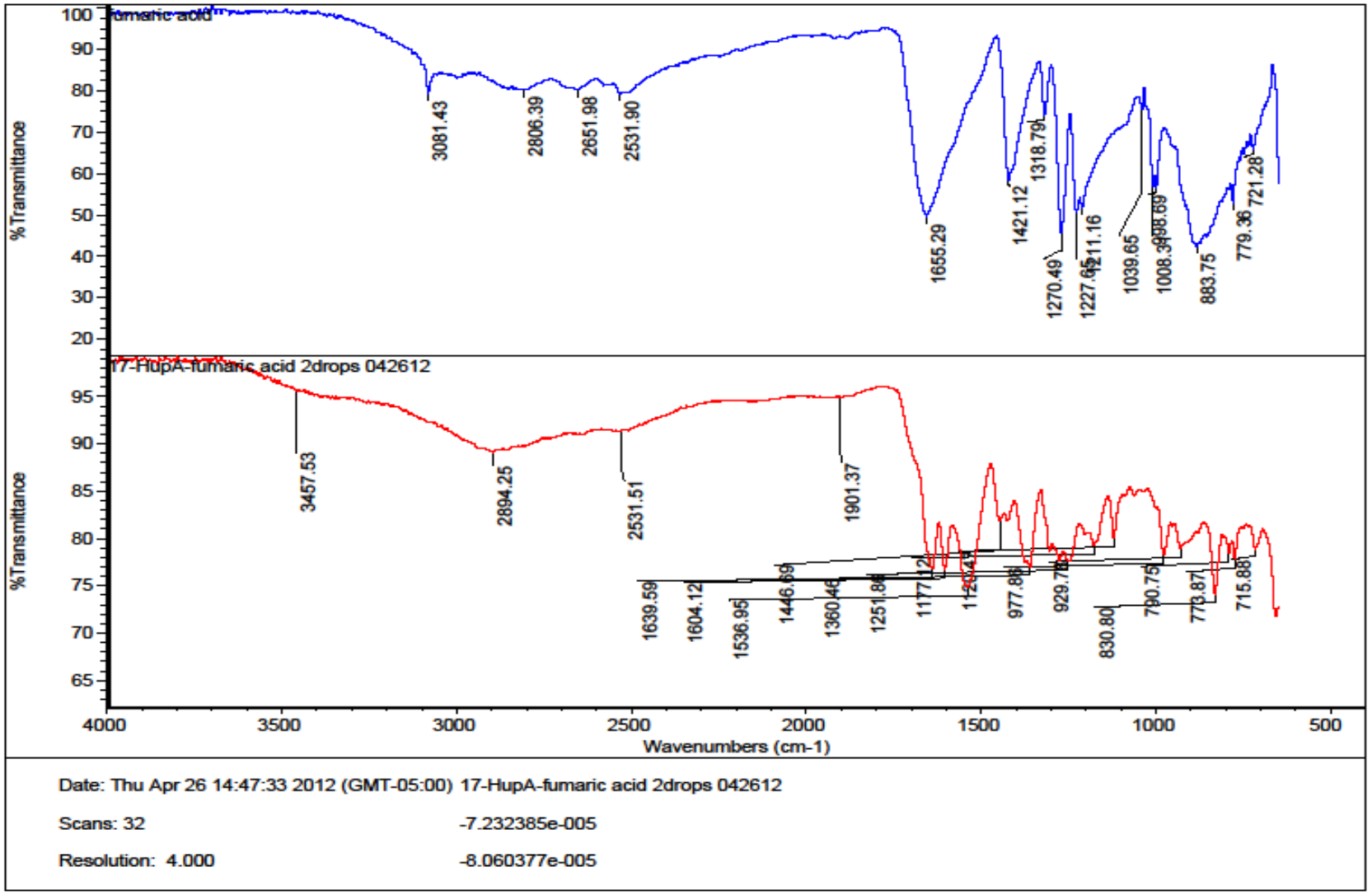


FIGURE 8.13 IR SPECTRUM OF FUMARIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

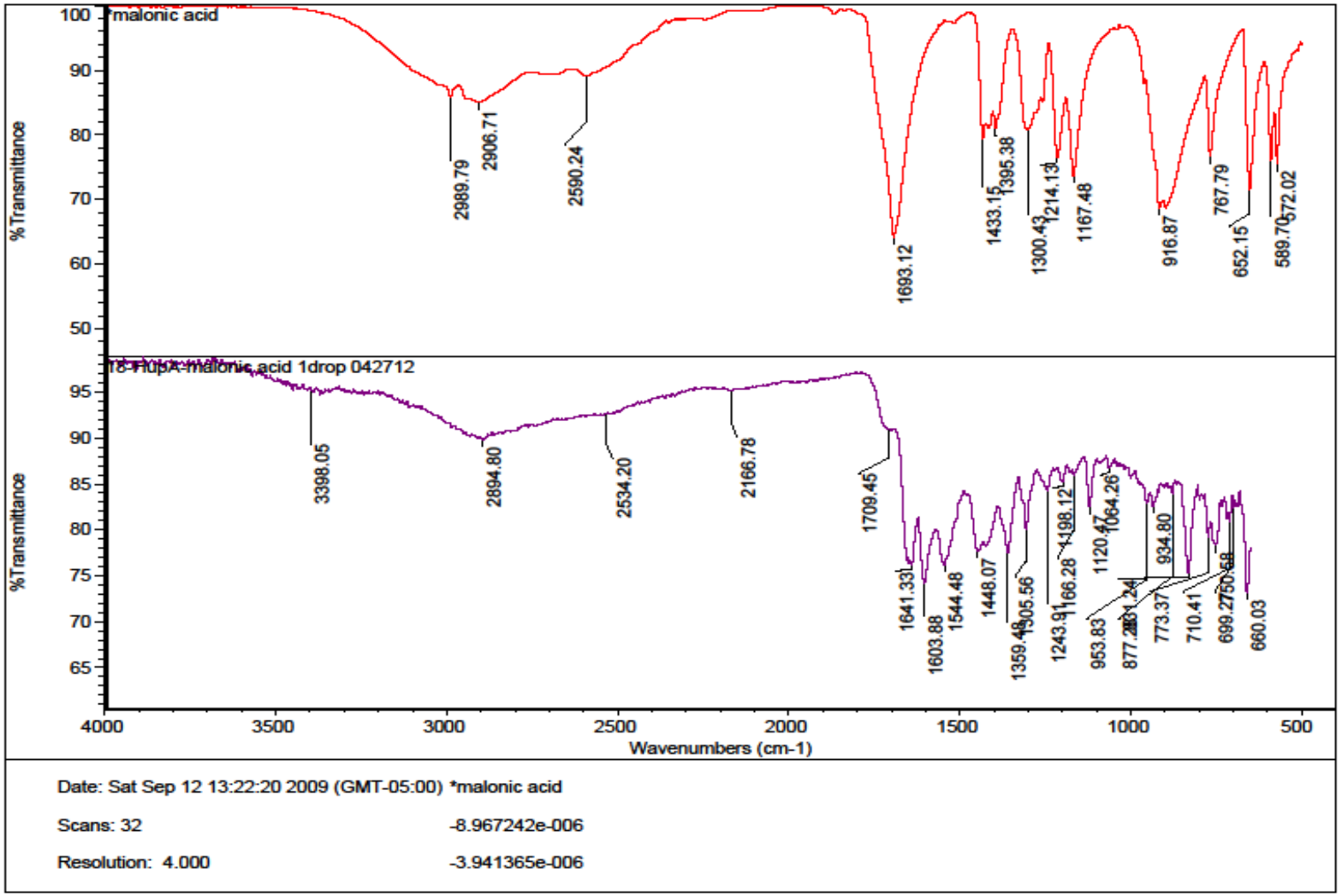


FIGURE 8.14 IR SPECTRUM OF MALONIC ACID SAMPLE OF PURE GRINDING

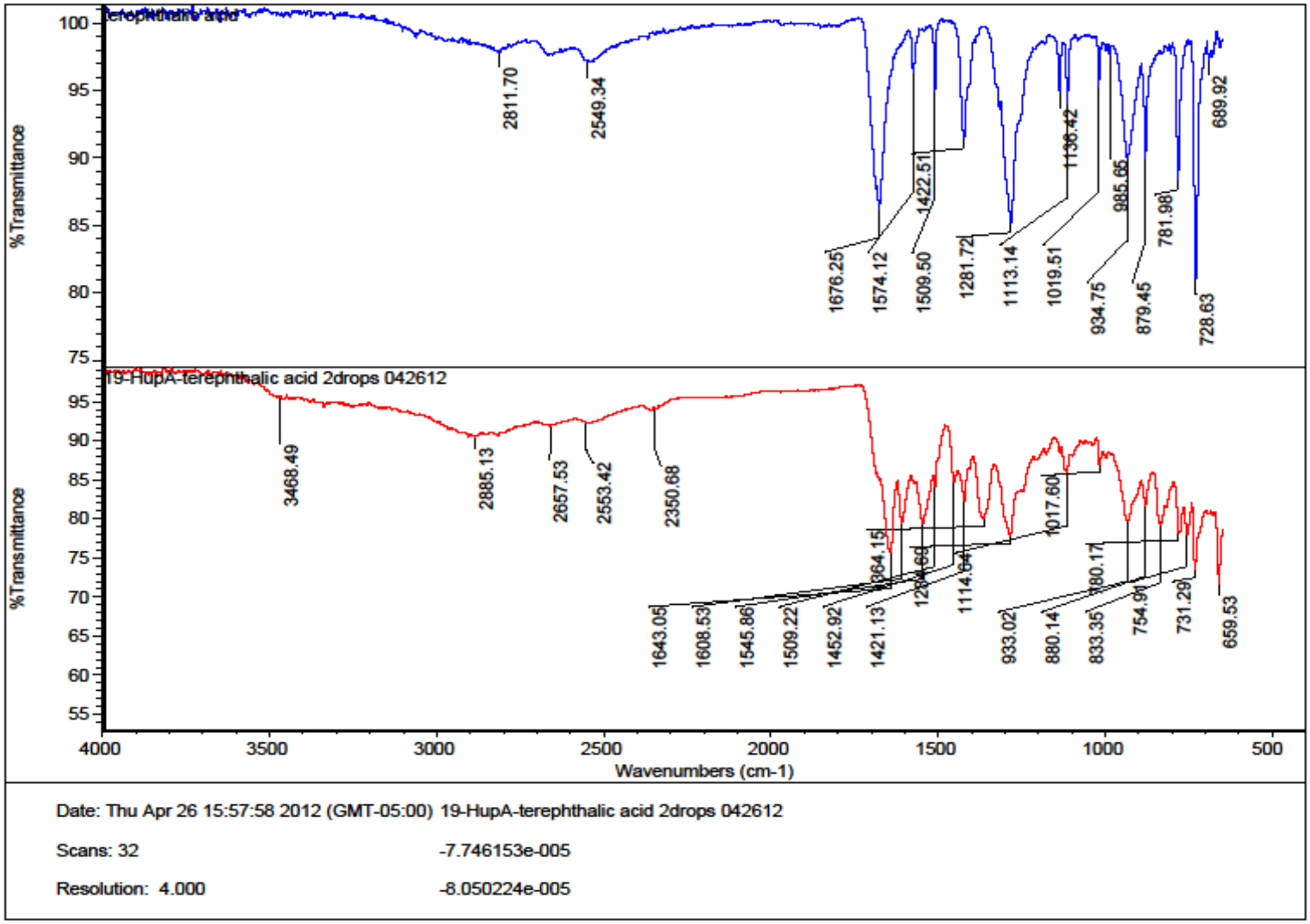


FIGURE 8.15 IR SPECTRUM OF TEREPHTHALIC ACID SAMPLE OF SOLVENT-DROP GRINDING WITH TWO DROPS ETHANOL

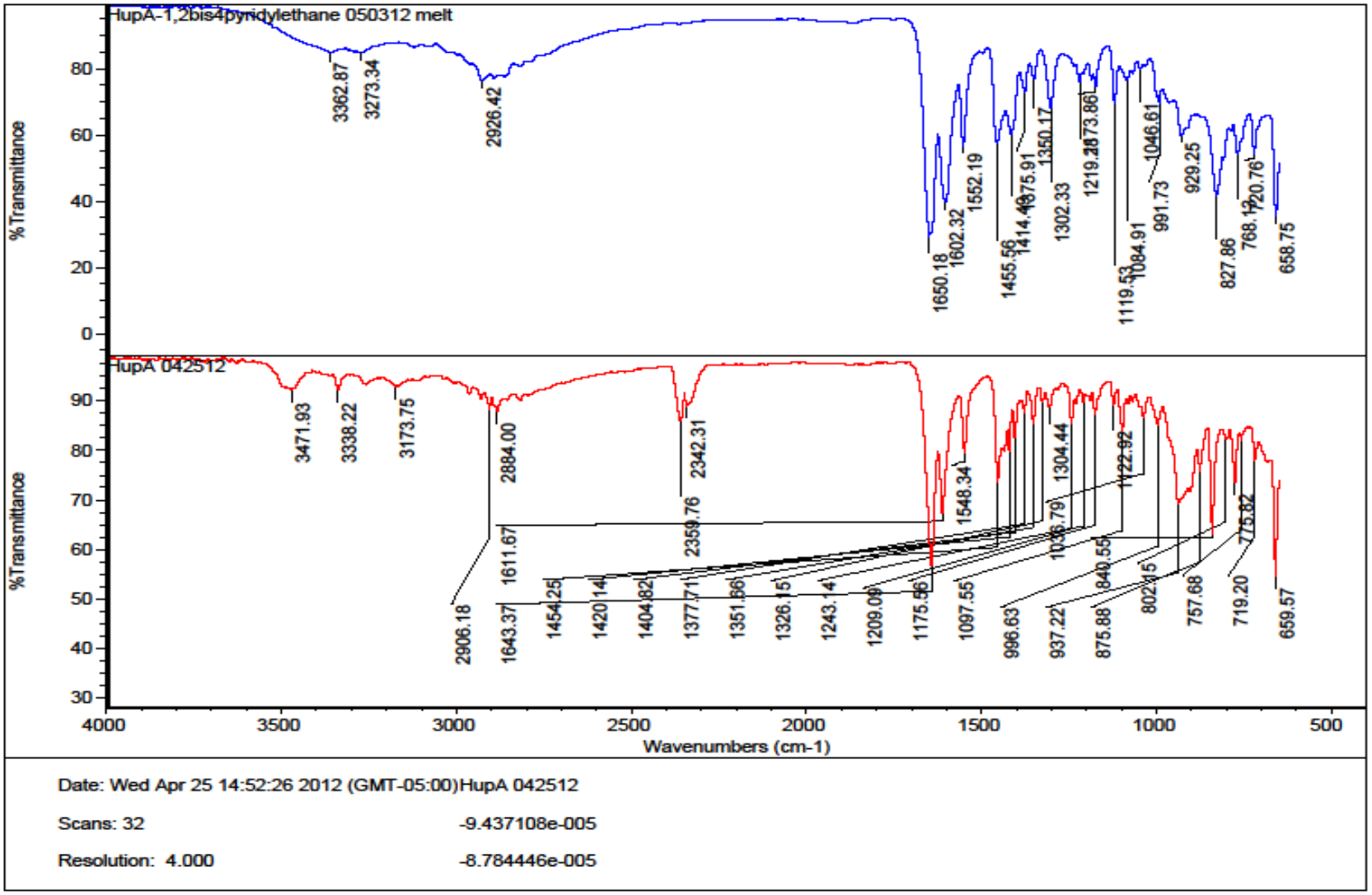


FIGURE 8.16 IR SPECTRUM OF 1,2-BIS(4-PYRIDYL)ETHANE MELT SAMPLE

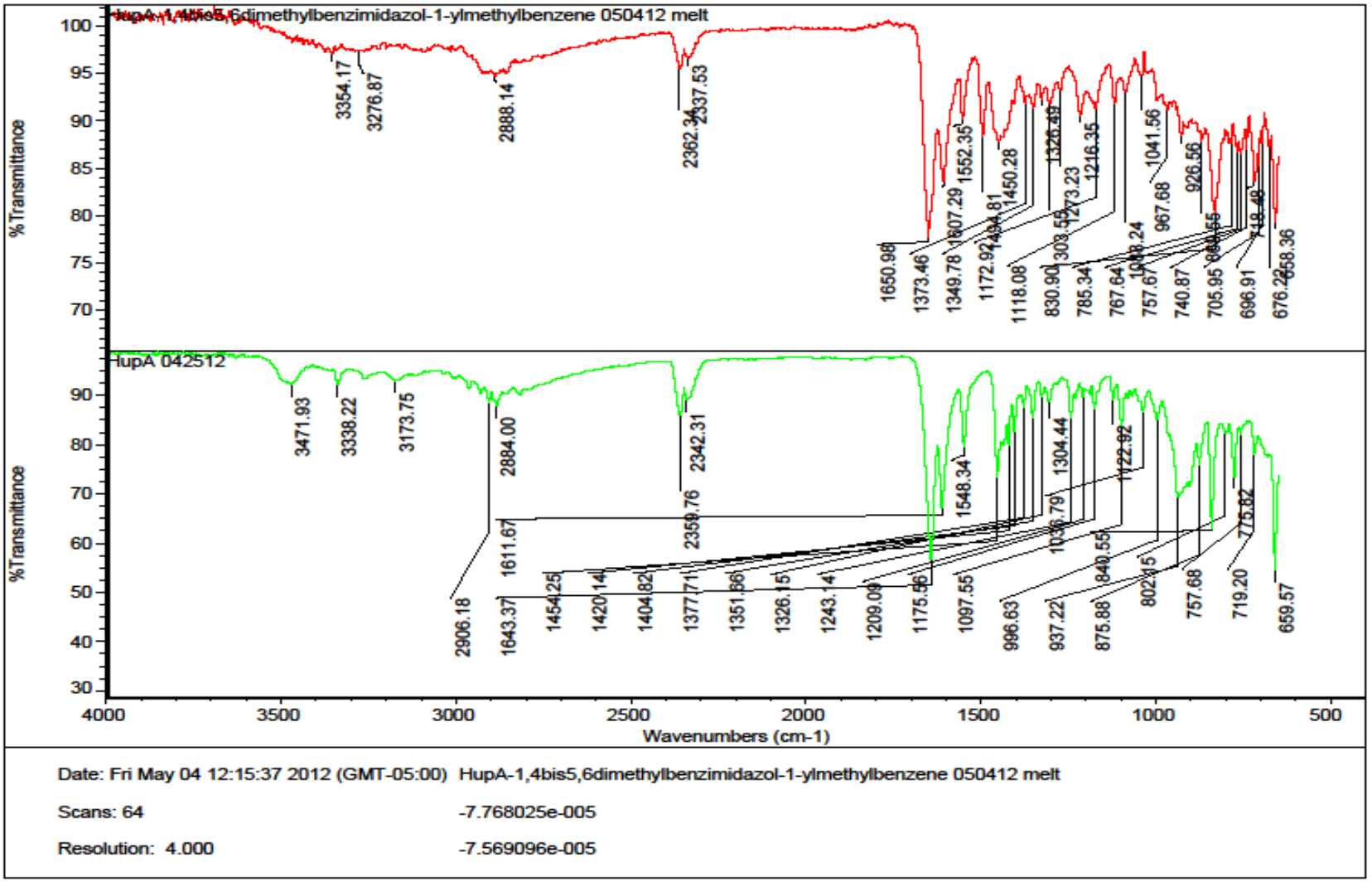


FIGURE 8.17 IR SPECTRUM OF 1,4-BIS(5,6-DIMETHYLBENZIMIDAZOL-1-YL)METHYLBENZENE MELT SAMPLE

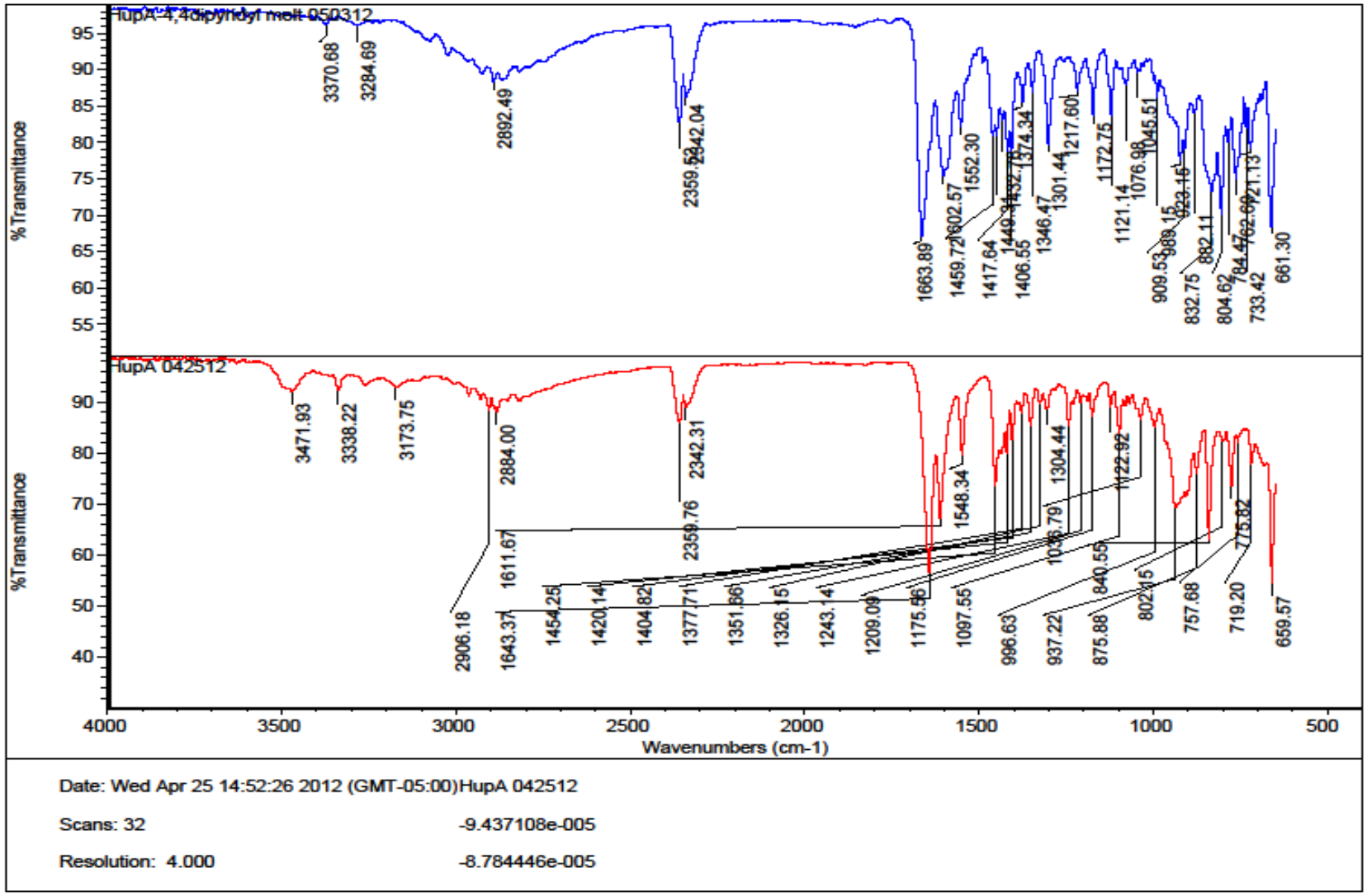


FIGURE 8.18 IR SPECTRUM OF 4,4'-DIPYRIDYL MELT SAMPLE

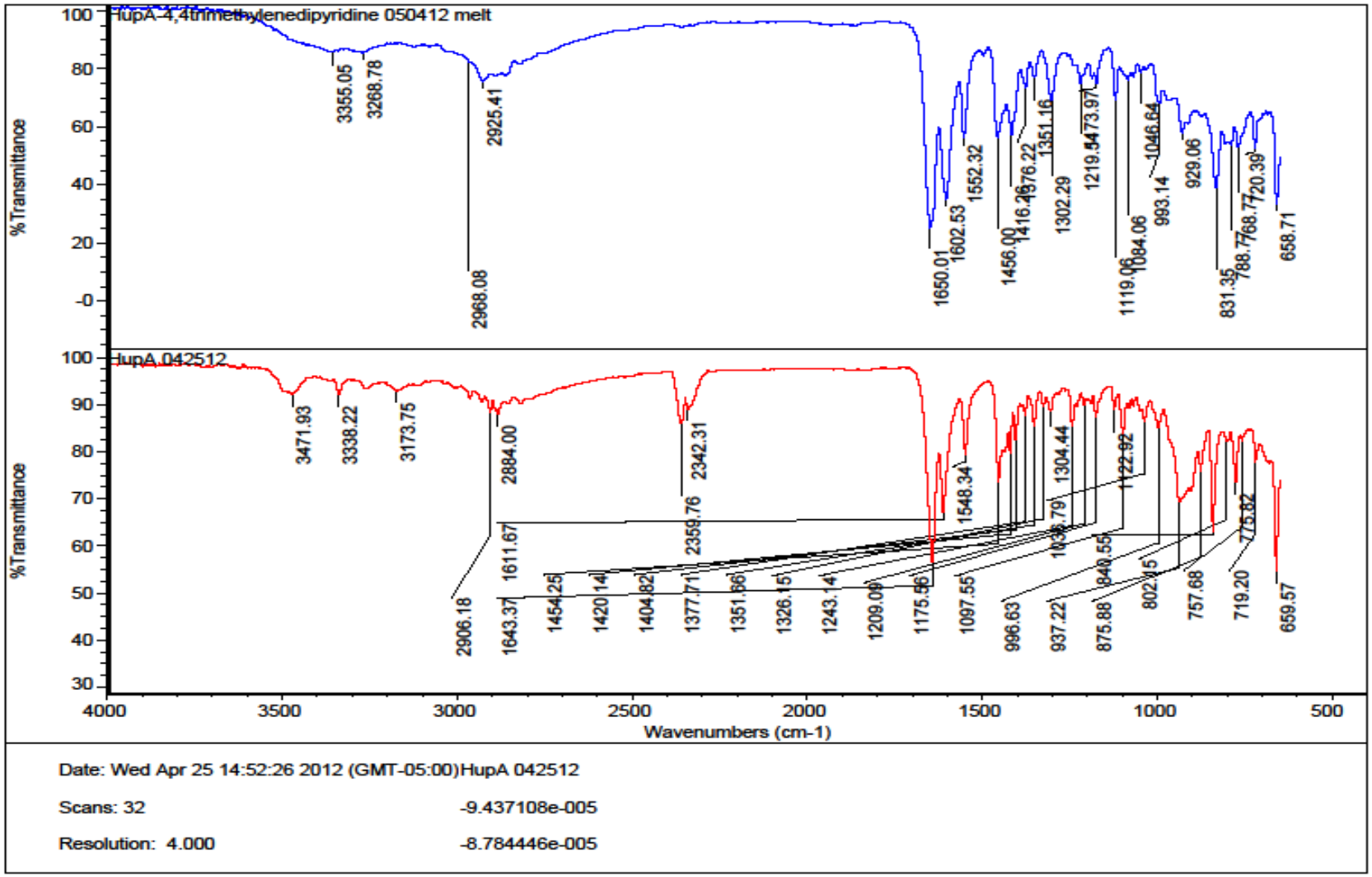


FIGURE 8.19 IR SPECTRUM OF 4,4-TRIMETHYLENEDIPYRIDINE MELT SAMPLE

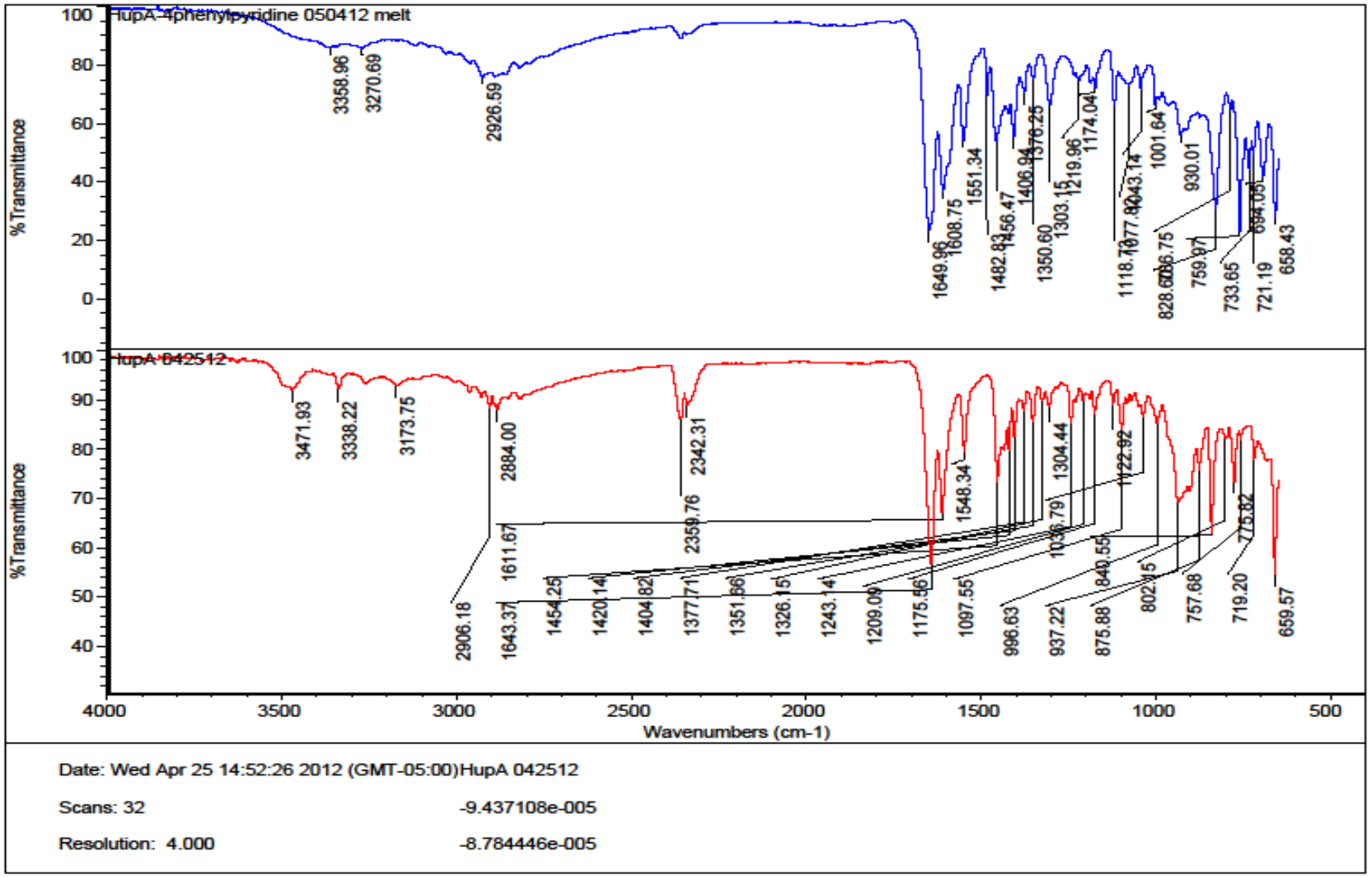


FIGURE 8.20 IR SPECTRUM OF 4-PHENYLPYRIDINE MELT SAMPLE

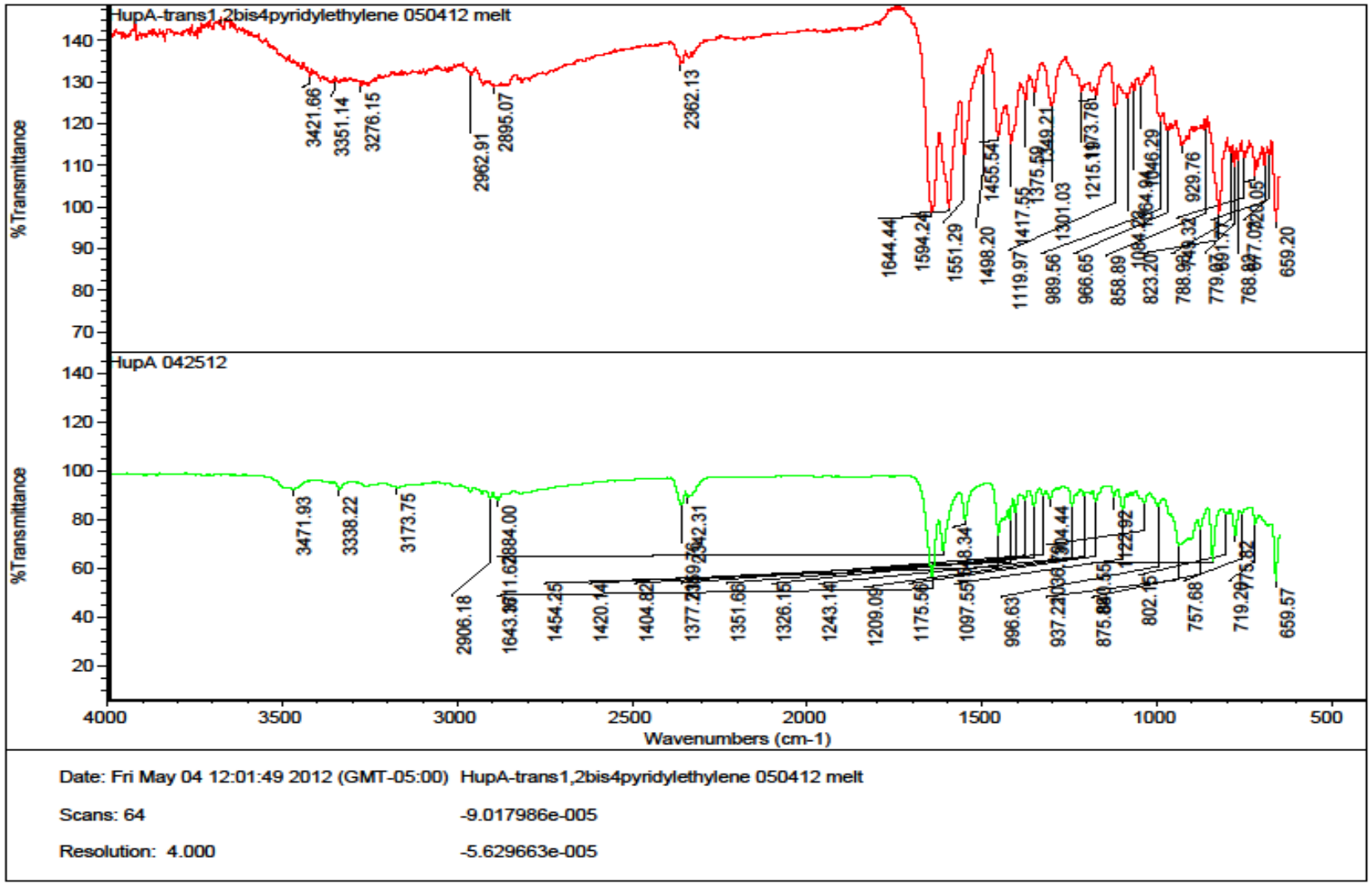


FIGURE 8.21 IR SPECTRUM OF TRANS-1,2-BIS(4-PYRIDYL)ETHYLENE MELT SAMPLE