NANO-ENCAPSULATION OF GASTRIC SENSITIVE COMPOUNDS

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by

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NANO-ENCAPSULATION OF GASTRIC SENSITIVE COMPOUNDS

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First and foremost I would like to thank my immediate family for their unconditional love, support, and encouragement. To my parents, I could not hope for more gracious, selfless, principled, generous, and intelligent people to have served the most important roles in my life. To my sister Sarah, as far back as I can remember you were always destined for greatness. With all of your career endeavors, and solely raising the most amazingly bright and happy little boy in the world, you certainly are the role model every younger sibling should have.

I must also say thank you to my aunt Peggy for support and helping me start this incredible new life in Texas. My grandparents, aunts, uncles, and cousins have all helped shape me into the person I am today as well, so I sincerely thank each and every one of you too.

Many thanks to Texas State University-San Marcos chemistry department for accepting me into the graduate program, in addition to my thesis committee chair, Dr. Gary Beall for sharing his guidance and knowledge during my projects. A special thanks to Dr. Chang Ji and Drew Brown for sharing lab space and allowing me to use the GC, and for their considerate and prompt assistance with my research whenever sought. Thanks are also due to the other committee members, Dr. Clois "Bert" Powell and Dr. Kevin Lewis, and to the brilliant, blossoming, and entertaining minds in Dr. Beall's research group for their assistance and chuckles as well.

I could not publish this treatise, embodying the greatest achievement of my life thus far, without expressing my appreciation to Marilyn for the inspiration to go back to school when my future was uncertain in 2008. I will also give a shout out to Melody and Matt for the good times we shared. Unexpected friendships grew out of our collective endeavor, and I hope we can continue to be friends as we all move on in our lives, with the best of luck, to find great success through many more achievements and discoveries in our respective careers.

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1.0 INTRODUCTION AND BACKGROUND

1.1 Smectite Clay: Composite Capability and Scientific Significance

Smectites are a variety of hydrous aluminosilicate clays that swell when introduced to water. Smectites have an octahedral layer of metal oxides sandwiched between two tetrahedral layers of silicon dioxide, forming paper-like sheets, or platelets (Figure 1), and they stack atop one another into what are called tactoids.[37] Smectite clay plates are one nanometer thick but on average 200 nm in length and width.[32]

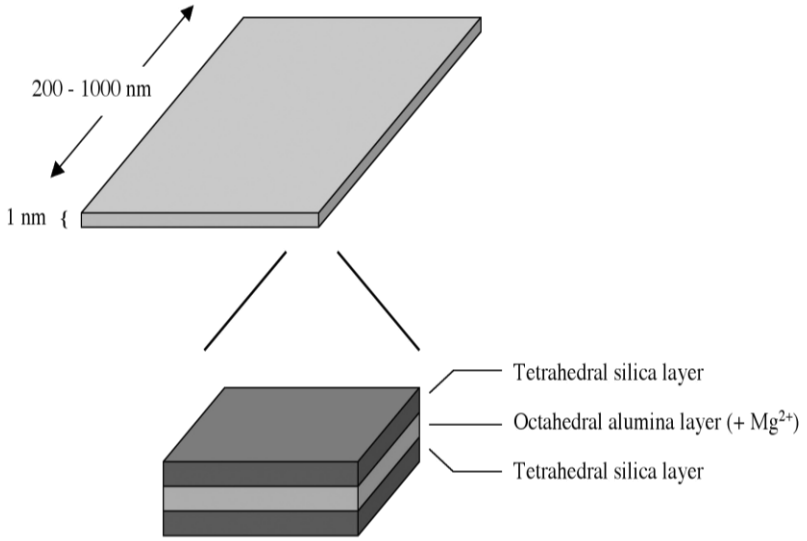

Figure 1. Smectite platelet and cross-section. Typical dimensions of a smectite clay platelet and cross-section.

The most common smectite clay, montmorillonite, is the primary mineral constituent of bentonite ore, which is formed from the weathering of volcanic ash and

mined at various locations around the world. Figure 2 exhibits the skeletal cross section of the chemical and structural arrangement of each platelet layer and the gallery; the space between them.

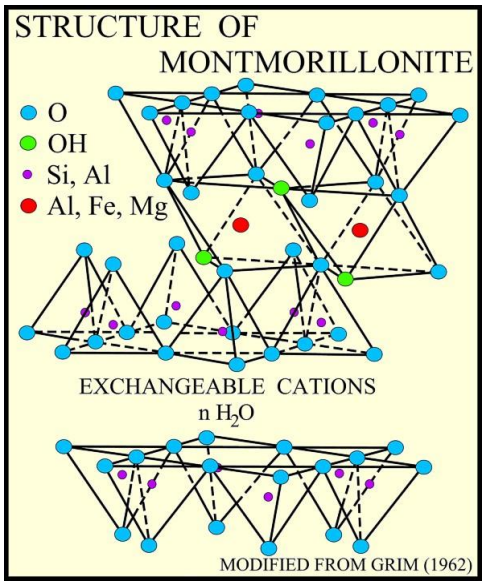

Figure 2. Skeletal arrangement of montmorillonite.

The base formula for montmorillonite is $(Si_8)(Al_{3.33},Mg_{0.67} / Na_{0.67})(O_{20})(OH)_4$.[39] Iron is another common constituent found in the octahedral layer. For every divalent (Mg^{2+}) substituted for a trivalent (Al^{3+}) in the octahedral layer, a single negative charge exists within the central "bulk" layer. For every trivalent aluminum (Al^{3+}) substituted for quadrivalent silicon (Si^{4+}) in the tetrahedral layer, a single negative charge is imparted on the surface, thereby giving the surface of each platelet a net negative charge density. This allows for exchangeable counter-cations to adsorb, most commonly Mg^{2+}, Ca^{2+}, Na^{+}, and K^{+}. These adsorbed cations are primarily what adheres the platelets to one another. The d-spacing between platelets, (distance between top surface of one platelet and the top surface of the one below), varies depending on the size of adsorbed cations and water

content. Clays have cation-exchange capability and are also ubiquitous and inexpensive, thus they have become a significant interest to the scientific and industrial community.

The United States has the largest deposits of relatively pure sodium montmorillonite.[36] Montmorillonite is refined further into a variety of Cloisite® Nanoclays.[38] Cloisite® Na^+ is highly refined sodium montmorillonite. Accessory minerals are removed by high speed centrifugation. All the counter-cations are replaced with Na^+, which can be readily exchanged with various other onium ions. Quantifying the surface charge density, or cation exchange capacity (CEC) is done by designating milliequivalents per unit mass, typically (*n* meq/100 g) calculated from the formula unit cell weight based upon the cation constituents.[39] For this research project Cloisite® Na^+ was used which has a 95 meq/100 g exchange capacity.

In aqueous solution, the Cloisite® Na^+ acts like a salt. The sodium ions dissociate into the water, the tactoids exfoliate, albeit with considerable shear to reach complete exfoliation. The free flowing platelets provide extensive surface area in excess of 750 m^2/g for interaction with cationic compounds.[39] Upon centrifugation and drying, the tactoids are reassembled with the compounds intercalated within the gallery. The composited molecules are normally referred to as "intercalates" and they are sandwiched between the clay plates. Due to the expansive surface area, these composites create a very special environment that could provide protection for the intercalates. Pure Cloisite® Na^+ has a d-spacing of approximately 11.9 Å. However, once a compound is intercalated within the gallery, the d-spacing is forced to expand. This process is confirmed utilizing X-ray diffraction (XRD) (Figure 3). Other possible morphologies are illustrated in Figure 4.

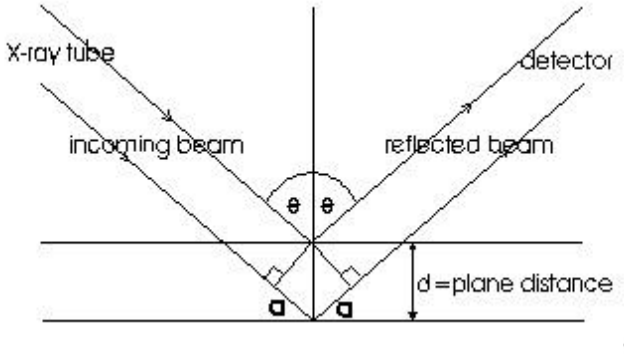

Figure 3. XRD diagram. XRD determines the d-space distance between two consecutive layers by following simple trigonometry defined in Bragg's Law: $n\lambda=2d \sin\theta$. [40]

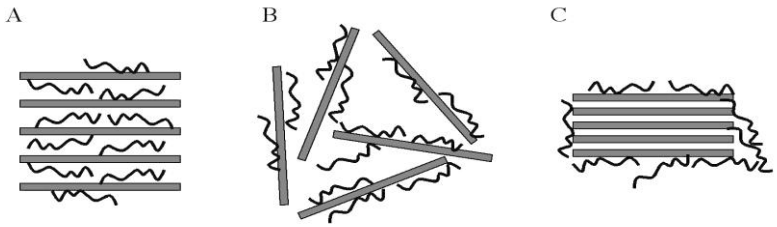

Figure 4. Adsorption arrangements. (A) Intercalated into gallery. (B) Exfoliated. (C) Peripherally adsorbed. Ideal arrangement for this study is intercalation as shown in A.

Smectites possess the ability to adsorb and form complex components with many organic compounds including pharmaceuticals, synthetic polymers, as well as biomolecular species, such as DNA and other nucleic acids.[1,27,30,31,33] The nano-scale particle size[34] allows for transport across a cell membrane without lysing the cell.[35]

The protective environment of the clay could be of significant use to the pharmaceutical industry. The Food and Drug Administration (FDA) has designated bentonite, (and therefore Cloisite® Na^+), a Generally Recognized as Safe (GRAS) food

additive.[2] There is a myriad of drugs available for treatment of ailments, with a variety of methods for administration. Many drugs are administered by invasive means which carry the risk of infection, and some are simply frightening or unpleasant. Unfortunately in medicine, convenience is expendable when lives are at stake. There are several factors which must be considered to determine the most appropriate method of administration; most importantly the urgency for onset of action by the drug. For instance, if someone is suffering from an asthma attack, a pill which takes 20 minutes to digest and reach its peak efficacy will not suffice. An inhalant with immediate results is required. Another factor is the drug must be able to reach its intended target in the potent form of the drug. Absorption of the drug in the gastrointestinal tract may be low or unpredictable. While a traditional pill or syrup might be preferred intake for a drug that is absorbed into the bloodstream from the small intestine, the drug may be susceptible to degradation in the stomach. A request for proposal of research from NineSigma reported data that ephedrine is one such drug.

1.2 Ephedrine: Medicinal Uses and Chemical Properties

Ephedrine is an alkaloid, originally extracted in China as far back as 5000 years ago from ma huang (*Ephedra sinica*), and from other various plants in the genus *Ephedra* (family Ephedraceae).[41] It is now mass produced synthetically because the extraction procedure is not cost effective and practical.[6] The chemical formula for ephedrine is $C_{10}H_{15}NO$, it has a formula weight (F.W.) of 165.19 g/mol, and the structure is illustrated in Figure 5.

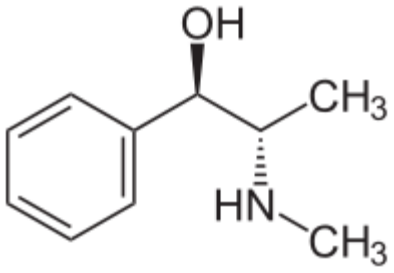

Figure 5. Chemical structure of ephedrine. It is often in hydrochloride or sulfate salt form, whereby the nitrogen has a positive charge due to another bonded hydrogen, and a counter anion.

There are two chiral centers on the molecule, allowing for four stereoisomers. The enantiomers with opposite stereochemistry around the chiral carbons, (1R,2S and 1S,2R) are designated ephedrine, and the enantiomers with stereochemically equal chiral centers, (1R,2R and 1S,2S), are designated pseudoephedrine. The structures of each are illustrated in Figure 6. Ephedrine is a good candidate for this research because it contains an amine group which will exchange with the clay. (1R,2S)-(-)-Ephedrine was the specific isomer selected as the analyte for this study because it is the most potent isomer,[21] and therefore the most marketed.

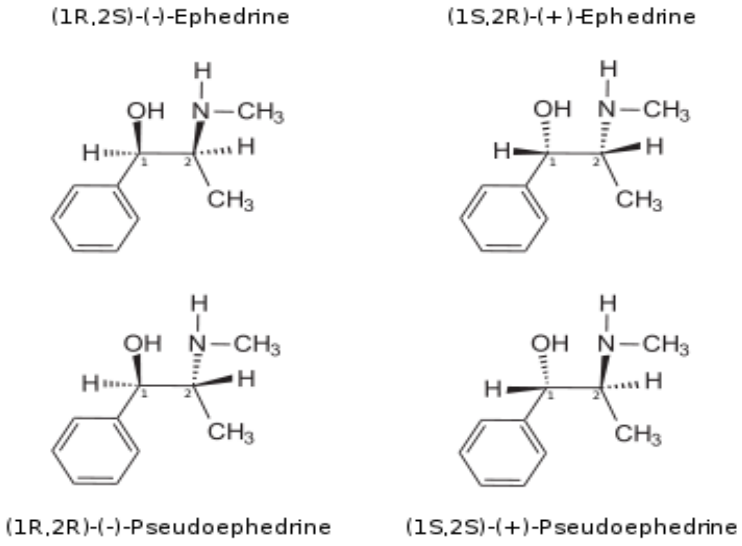

Figure 6. Four stereoisomers of ephedrine/pseudoephedrine.

Ephedrine functions as a sympathomimetic amine, meaning it mimics the effects of the sympathetic nervous system.[42] Physiologically it acts as an α- and β-adrenergic receptor agonist. Pharmacologically ephedrine is classified as a stimulant; exhibiting increased blood pressure, dilated coronary blood vessels and bronchi, reduced inflammation of mucosa, stimulated respiratory system and central nervous system[3], and suppressed appetite.[35] These physiological responses have made ephedrine a classically ascribed diaphoretic and antipyretic drug, circulatory stimulant, and bronchodilator, making it an effective agent for treatment of bronchial asthma, acute bronchospasm, idiopathic orthostatic hypotension, anesthesia-induced hypotension, urticaria,[3,4] and in modern times it is widely distributed mixed with caffeine as a diet supplement.[35]

1.3 N-nitrosoephedrine from Nitrosation of Ephedrine

In order for the effects of ephedrine to be achieved, the drug must pass through the stomach and into the small intestine where it is absorbed into the bloodstream.[3] However, the secondary amine group in ephedrine is susceptible to reaction by N-nitrosation in the stomach.[8-10] This reaction is instigated in humans by the everyday dietary consumption of nitrates and nitrites found in food, most especially meats since it is commonly used as a preservative, and about 5% of nitrates ingested are reduced to nitrite by salival bacteria.[12] In the presence of aqueous hydrochloric acid found in the stomach the nitrite ion is converted to nitrous acid and then is further reduced to the nitrosonium ion.[26] The reaction is illustrated in Figure 7.

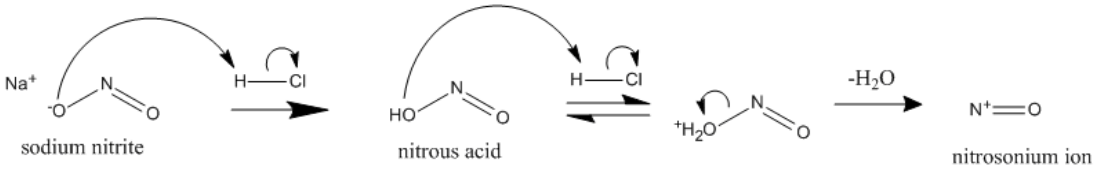

Figure 7. Conversion of nitrite ion to nitrosonium ion.

Nitrosonium has an affinity for primary, secondary and tertiary amines and it has been demonstrated both *in vitro* and *in vivo*.[8-10] The reaction of a nitrosonium ion with a secondary amine like ephedrine is illustrated in Figure 8. The expected product after reacting with the nitrosonium ion is a derivative of ephedrine, N-nitrosoephedrine, (NEP). NEP is classified as a β-hydroxy-N-nitrosamine and exists as (E)- and (Z)-rotamers, due to the N-N=O stereochemistry. Figure 9 illustrates both configurations as well as the resonant forms which hinder rotation.[28]

It has been reported that nitroso compounds cause carcinogenic activity in lab animals,[13-16] and NEP has carcinogenic tendencies also.[17,18] Additional research has been done to demonstrate NEP's mutagenic activity.[19]

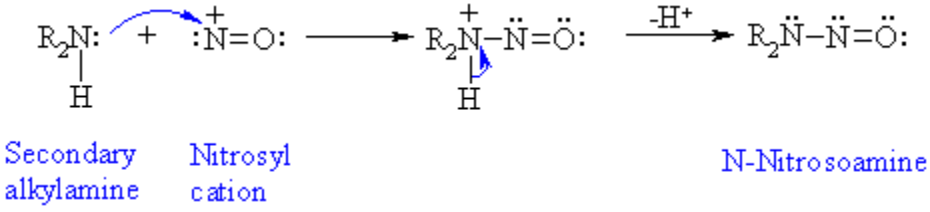

Figure 8. N-nitrosation reaction of a secondary amine.[26]

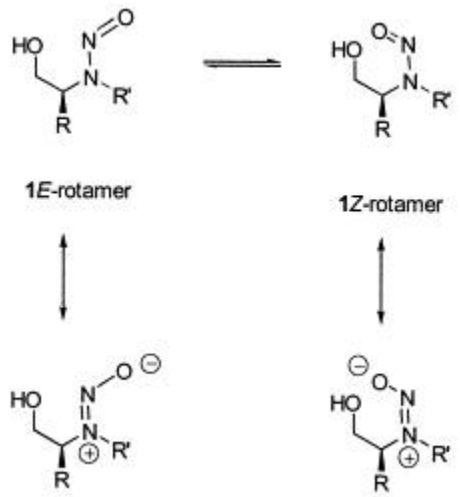

Figure 9. (E)- and (Z)-NEP. The two rotamers of NEP and resonant forms.[28] For this project, both R and R' are methyl groups. There is also a phenyl group bonded to the β-hydroxyl carbon.

1.4 Hypothesis

The hypothesis of this study is that ephedrine, when intercalated into Cloisite® Na^+, avoids N-nitrosation to a significant degree compared to unbound ephedrine when each are exposed to the same nitrosonium ion rich environment for thirty minutes.

2.0 EXPERIMENTAL MATERIALS AND METHODS

2.1 Raw Materials and Equipment

Southern Clay Products, Inc. supplied the montmorillonite clay, refined to Cloisite® Na^+. No additional modifications to the clay were performed prior to experimentation. The (1R,2S)-(-)-Ephedrine hydrochloride (99%) was purchased from Sigma-Aldrich. All other chemicals used were purchased from various sources before this project was created.

All pH readings were done with a Corning pH Meter 120. Wide angle powder X-ray diffraction was performed on a Bruker AXS D8 Focus diffractometer using Cu Kα radiation to measure the d-spacing distance of the clay to confirm ephedrine intercalation. Results are discussed in Section 3.1. To calculate the weight percentage of ephedrine to clay, thermal gravimetric analysis (TGA) was performed on a Thermal Advantage (TA) TGA Q50 scanned at a rate of 10 degrees per minute from room temperature up to 800°C with argon as a purge gas and results are illustrated in Section 3.2.

Gas chromatography (GC) was performed on an Agilent Technologies 6890N Network GC System. The system is comprised of two separate columns, one of which fed into a standard flame ionization detector (FID), and one into a mass spectrometer (MS), which was an Agilent Technologies 5793 Network Mass Selective Detector. The column for FID was Agilent 19091Z-433 HP-1, 100% dimethylpolysiloxane, non-polar,

general purpose capillary of dimensions 30 m (length) x 250 μm (diameter) x 0.25 μm (thickness). The column for MS was Agilent 19091J-433 HP-5MS, with 5% phenyl – methylpolysiloxane of the same dimensions. Chromatogram peak areas were integrated with the GC's accompanying software, Agilent Technologies Enhanced MSD ChemStation program, software edition D.01.02.16.

Proton Nuclear Magnetic Resonance (NMR) was performed on a Bruker Avance III 400MHz NMR Spectrometer.

2.2 Preliminary Preparations and Methods

2.2.1 Intercalation of Ephedrine HCl into Cloisite® Na^+

Cloisite® Na^+ has a 95 meq/100 g exchange capacity. Three separate batches of the composite were prepared at 75%, 100%, and 110% of the total exchange capacity of Cloisite® Na^+. Ephedrine HCl has a valence of 1 and formula weight of 201.69 g/mol. Therefore the milliequivalent weight of ephedrine HCl is 0.20169 g. At 100% exchange capacity 19.16 g ephedrine HCl is required if 100 g of Cloisite® Na^+ is used. Since only 5 g of Cloisite® Na^+ was used for each batch, the results were divided by 20 so that 0.958 g of Ephedrine was added for 100% exchange capacity, 0.719 g was added for 75%, and 1.05 g for 110%.

Five grams of Cloisite® Na^+ was gradually dispersed in three separate beakers containing 250 mL of deionized water (diH_2O) and heated to 75°C and stirred with a stir bar until each solution was a homogeneous slurry free of clumps. The premeasured ephedrine HCl was first dissolved in a small amount (~20 mL) of diH_2O and slowly added to each respective clay solution with continued stirring. By dissolving the

ephedrine HCl in diH_2O, all chloride ions dissociated from the positively charged amine sites, and since the pKa of ephedrine is 9.6,[5] the amine will remain positively charged in the aqueous solution and exchange on the clay surface. This allows for maximum interaction potential.

Each light yellow solution of clay slowly thickened and turned slightly lighter in color as the ephedrine was gradually added. The dispersions were then placed in 250 mL centrifuge containers and spun down at approximately 3500 rpm for 5 minutes. The supernatant was discarded and the compacted clays were spread out and allowed to air dry on watch glasses. Upon 2-3 days of drying, the clay composites were individually ground into finer particles with a mortar and pestle, and stored in labeled vials.

2.2.2 Simulated Gastric Fluid

Simulated gastric fluid (SGF) without pepsin was prepared according to the industry standard, which is 0.2% (w/v) NaCl and 0.7% (v/v) concentrated HCl in diH_2O. 40 mM potassium nitrite was also added to the SGF. This is 1600 times what is considered the upper limit of normal nitrite concentration in the stomach, which is 25 μM.[29] This was in congruence with the World Health Organization Nitrosation Assay Procedure. The pH of the final solution was approximately 1.5. After mixing, the solution was stored in a labeled one liter glass storage container. The nitrite concentration according to this procedure is obviously not intended to be representative of normal gastric nitrite levels. Thus, the nitrite concentration was later increased to approximately 120 mM by the addition of more potassium nitrite, which raised the pH to 2.3, close to the optimum pH (2.0) for N-nitrosation of secondary amines to occur.[11] This

was done to force the reaction in short periods of time in the SGF. In preliminary tests, ephedrine was not degraded to any quantifiable degree, even after 5 hours soaking in the original 40 mM nitrite/SGF solution based upon GC testing. The concentration was increased to force the reaction to occur which ultimately created quantifiable and worthwhile results. Further reference to use of SGF containing 120 mM nitrite will be abbreviated MSGF, for "modified SGF".

2.2.3 Extraction Solvent and Incorporation of Internal Standard

The original extraction solvent chosen was diethyl ether. Though ephedrine is readily soluble, it proved to be impractical because of such high vapor pressure and it was difficult to maintain accurate volumes. Some of the storage vials were not air tight, which allowed for rapid evaporation and the concentration of analyte was no longer accurate. It was later discovered that common impurities in diethyl ether react with and degrade ephedrine.[7] Toluene was eventually chosen as the most practical extraction solvent. Toluene has a lower vapor pressure, is insoluble in the aqueous chemicals used in the reacting stage of the experiment, will solvate neutral compounds, especially aromatic functional group containing compounds, and does not nitrosate under any conditions.[23] An internal standard (IS) was required for GC analysis. The reason for this is explained in Section 2.2.4. The best available compound was methyl benzoate because its retention time was baseline resolved and did not overlay with the analyte or by-product peaks.

The extraction solvent/IS solution (ES/IS) was prepared by placing 250 μL of methyl benzoate (1.084 g/mL, 136.15 g/mol) into a 250 mL volumetric flask via an

Eppendorf 200-1000 μL pipet and filling the flask to volume with anhydrous toluene. The solution was mixed thoroughly and stored in a labeled storage flask. The concentration of IS in solvent was 8.0 mM.

2.2.4 Gas Chromatography Parameter Optimization

GCMS was performed on ancillary test samples to determine the retention order and identifying each peak in the chromatogram by comparing the spectrum to the National Institute of Standards and Technology (NIST) spectrum reference library.[24, 25] Once the peaks were identified by MS, the FID was used for extraction method testing and analysis of peak areas. Because both columns were reverse phase (non-polar stationary phase) the order of retention did not change, however retention times did. All integration was done on smooth, baseline resolved peaks, and for any data series requiring comparative results, the start and end retention time points were uniform.

Injection volume for MS was approximately 0.1 μL. The run method for MS was "MS Default 1", with an initial temperature of 50°C held for 3 minutes and ramped 15°C per minute to 200°C and held for 30 minutes. This method never changed throughout the research.

Each FID injection was approximately 1μL. Once the optimum FID run parameters were determined, the method was saved as "MJR" and ran with initial oven temperature of 80°C held for 1 minute then ramped 15°C per minute to 200°C and held for 15 minutes.

Since the GC inlet ports require manual injections by means of syringe, and because of such minute injection volumes, the potential for error due to variance in true

volume injected was corrected by the IS. By ensuring that the concentration (mol per unit volume) of IS is the same for every injection, the variance in actual injection volume can be normalized based upon the IS peak response for every injection.

2.3 Experimental Procedure

Some preliminary methods were determined to be fallible. For instance, there were previously mentioned problems choosing the extraction solvent. Another error was mixing the clay and ephedrine HCl without first dissolving the ephedrine HCl in diH_2O. Adding dry drug resulted in large clumps forming in the clay solution and precipitating out quickly. It was evident that the intercalation did not occur uniformly.

The final experiment was weighing two equal 90 mg measurements of pure ephedrine HCl, and one 900 mg measurement of 100% composite batch. Two vials were filled with 15 mL MSGF, and one vial with 15 mL diH_2O. The first 90 mg pure measurement was placed in one of the MSGF vials (Sample 1), the 900 mg of 100% composite was placed in the other MSGF vial (Sample 2), and the second 90 mg pure measurement was placed in the vial with only diH_2O (Control). These were sealed and shaken vigorously for exactly 30 minutes each.

At time, 12 M NaOH was added dropwise to each until the pH was raised to above 9.6, thereby neutralizing any acid content, nitrosonium ion content, and all remaining ephedrine, and terminating any reactions. The organic material; ephedrine and any degradation products were solvated in the aqueous supernatant.

Using a volumetric pipet, exactly 5 mL of ES/IS was placed in each vial, sealed, and shaken vigorously for a few minutes. Using a Pasteur pipet, some of the ES/IS

supernatant from each sample was removed and placed in separate storage vials for GC analysis, the results of which are discussed in Section 3.3.

2.3.1 NMR

An NMR was needed to assist the mass spectrum data in identifying the structure of the unknown by-product (discussed in Section 3.3). The procedure for prepping the sample was a modified procedure to that described in Section 2.3. This was done by soaking pure ephedrine HCl in MSGF for several days in order to maximize by-product concentration. Toluene was used to extract the organic material and then evaporated. The dried residue was then reconstituted in an amount of $CDCl_3$ suitable for the NMR test vial (~1.5 mL). All run parameters are included on the NMR spectra found in Section 3.4.

3.0 RESULTS AND DISCUSSION

3.1 XRD Results

All three composite batches were run on the XRD at 2-Theta scale to confirm intercalation, and the d-spacing was determined to be approximately 15.2 Å for 75% exchange capacity batch, 15.0 Å for 100%, and 14.9 Å for 110%, expanded from the standard approximate 11.9 Å of raw Cloisite® Na^+. All of the scans are compiled onto one graph which is illustrated in Figure 10. The peaks in the intercalated system are sharper and more intense and this implies that the intercalated system is more ordered than the hydrated clay. The absolute increase of 0.3 nm in the gallery spacing can only occur if the benzene ring in the ephedrine is lying flat between the plates. In order for the benzene to be standing upright to the surface, a minimum of a 0.7 nm increase in the d-spacing would be required. This orientation of the ephedrine in the gallery could very well present a very protective environment.

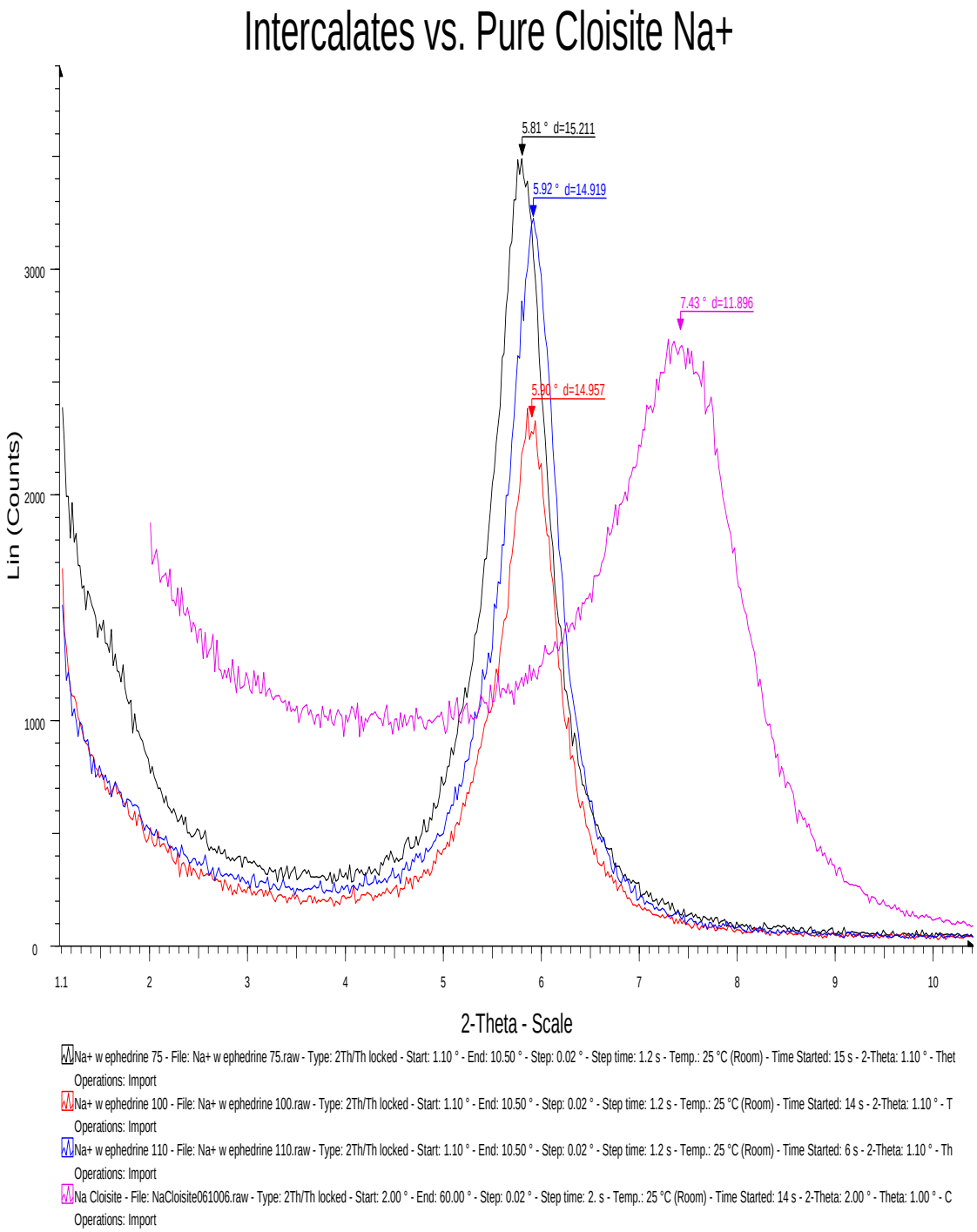

Figure 10. XRD Results. X-ray diffraction results for all three batches of ephedrine/Cloisite® composites compared to pure Cloisite® Na^+. 75% exchange capactity is the peak with d=15.211, 100% exchange capacity is the peak with d=14.957, and 110% exchange capacity with d=14.919. Pure Cloisite® Na^+.is the peak with d=11.896. The shift in peak position demonstrates an increase in the spacing between platelets.

3.2 TGA Results

TGA was performed to determine the weight percent of all three composite batches and to compare and contrast versus each pure component. Each run was performed under argon gas, started at room temperature and ramped 10°C per minute to 800°C. The graphs display two trend lines; the first (green), corresponds with Y-1 (left) axis, showing the percent of the original mass remaining as the temperature increases (x-axis). The other (blue) corresponds with the Y-2 (right) axis shows percent mass loss per degree Celsius.

Figure 11 shows that pure ephedrine HCl begins to melt and/or degrade at 187-188°C, which is consistent with literature[20], and it is completely destroyed and evaporated at approximately 290°C. Figure 12 demonstrates that the raw Cloisite® Na^+ is about 6.7% water due to the first significant loss of mass up to 100°C, and at approximately 550°C the dehydroxylation of the octahedral layer occurs with a 5.66% weight loss on a dry weight basis, leaving behind a residue, comprised of the silicon oxide coated remnants of the octahedral layer.[22]

In Figures 13-15, it can be seen in every case that significant water of hydration are present in the intercalates as evidenced by the weight loss below 150^{o}C. The weight loss between 150^{o}C and 800^{o}C can be attributed to two sources. The first is from the degradation of the ephedrine and the second from the dehydroxylation of the clay. In order to calculate the amount of contribution from the two sources in this region requires the measurement of the percent contribution of dehydroxylation in pure Cloisite® Na^+.

To determine the loading of ephedrine in each case requires first to put the weight loss on a dry weight basis. This is easily done since the water peak is completely

resolved. The next step is to determine the residual amount of dehydroxylated clay. This is done by subtracting the total dry weight adjusted percent weight loss from 100%. This is the percent of dehydroxylated clay residue. This is divided by the percent residue from the pure Cloisite® Na^+ TGA run to yield the theoretical mass of hydroxylated clay in the original sample. Subtracting this theoretical number from the clay residual yields the dehydroxylation contribution to the weight loss between 150°C and 800°C. This number is the percent mass of ephedrine on the various intercalated composites. Table 1 is a compilation of these results. It can be seen that the actual percent loadings of ephedrine are 75%, 81 % and 86% for 75%, 100% and 110% respectively. The lack of full exchange could be a combination of the high water solubility of the ephedrine hydrochloride and the lack of full exfoliation of the clay platelets.

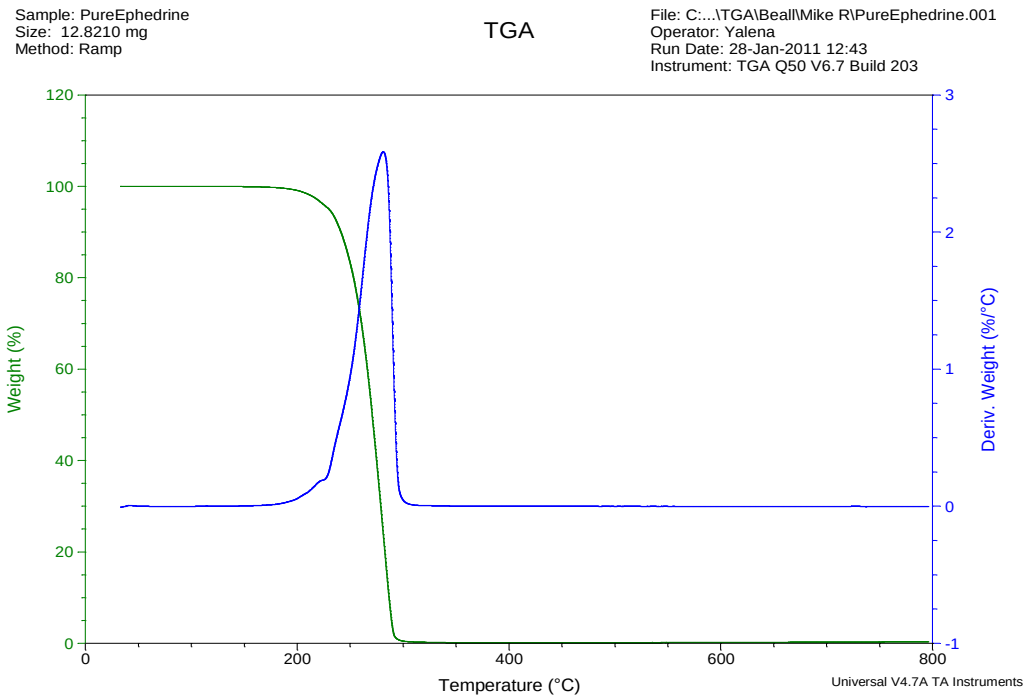

Figure 11. TGA of pure ephedrine HCl.

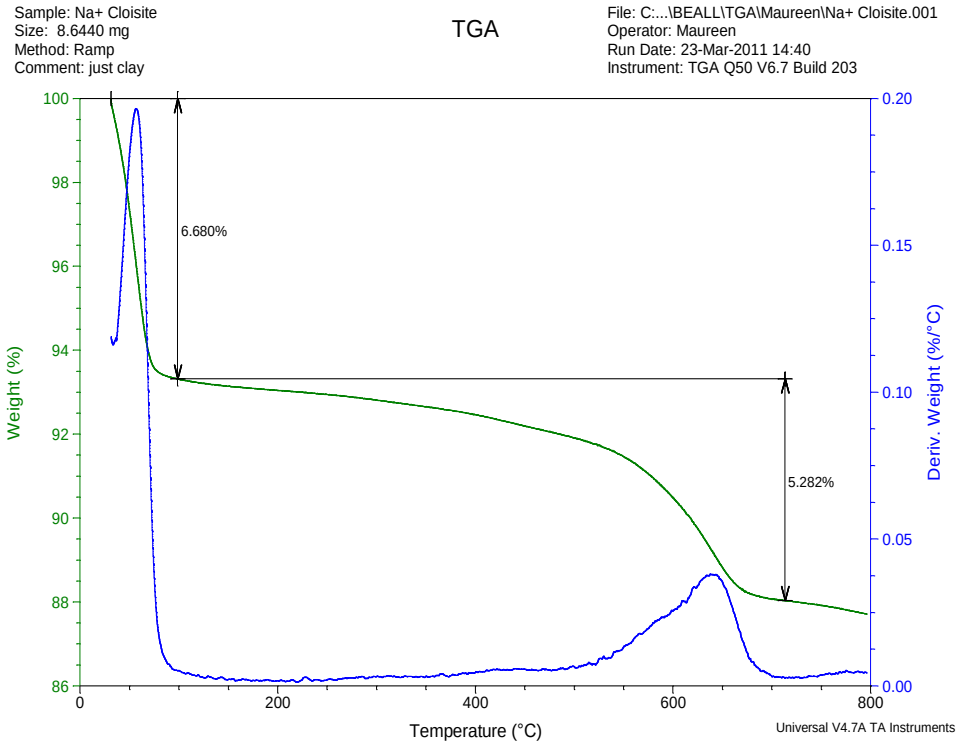

Figure 12. TGA of pure Cloisite® Na^+.

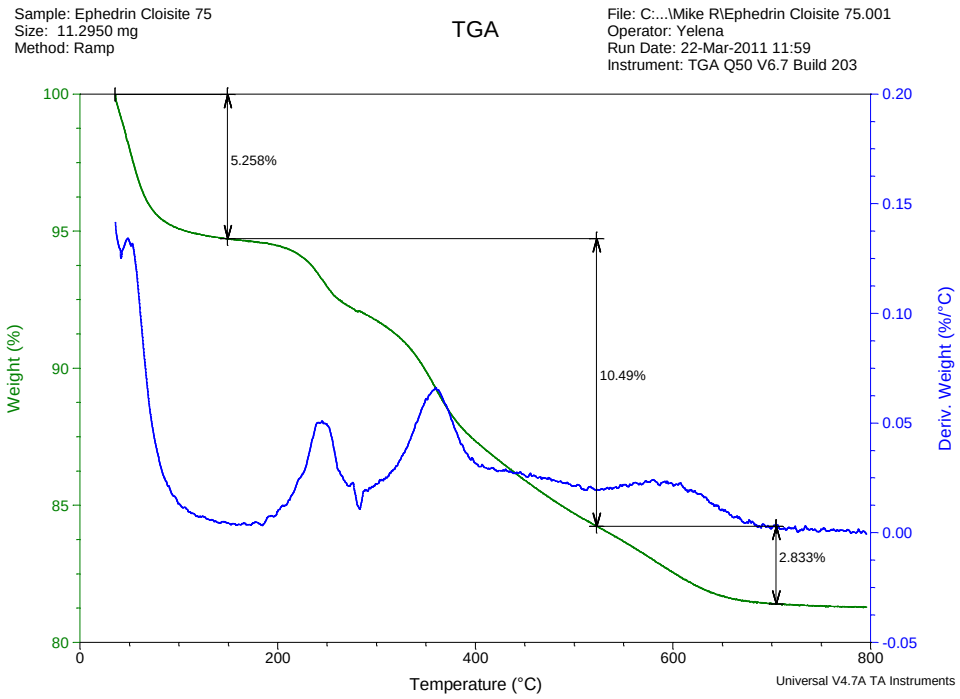

Figure 13. TGA of 75%. TGA of Cloisite® Na^+ intercalated with ephedrine HCl at 75% exchange capacity.

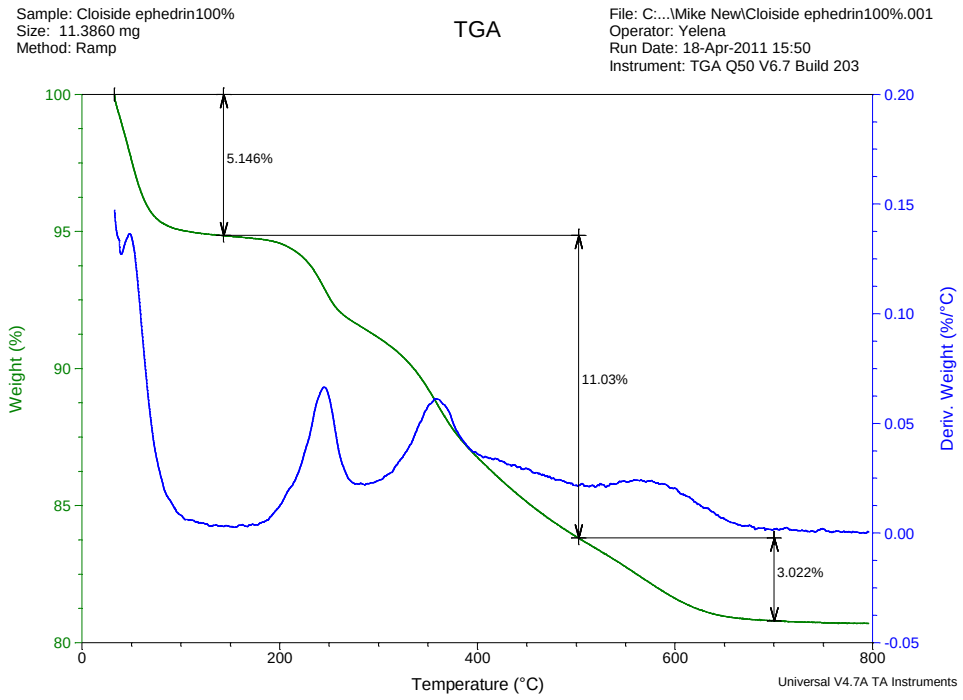

Figure 14. TGA of 100%. TGA of Cloisite® Na^+ intercalated with ephedrine HCl at 100% exchange capacity.

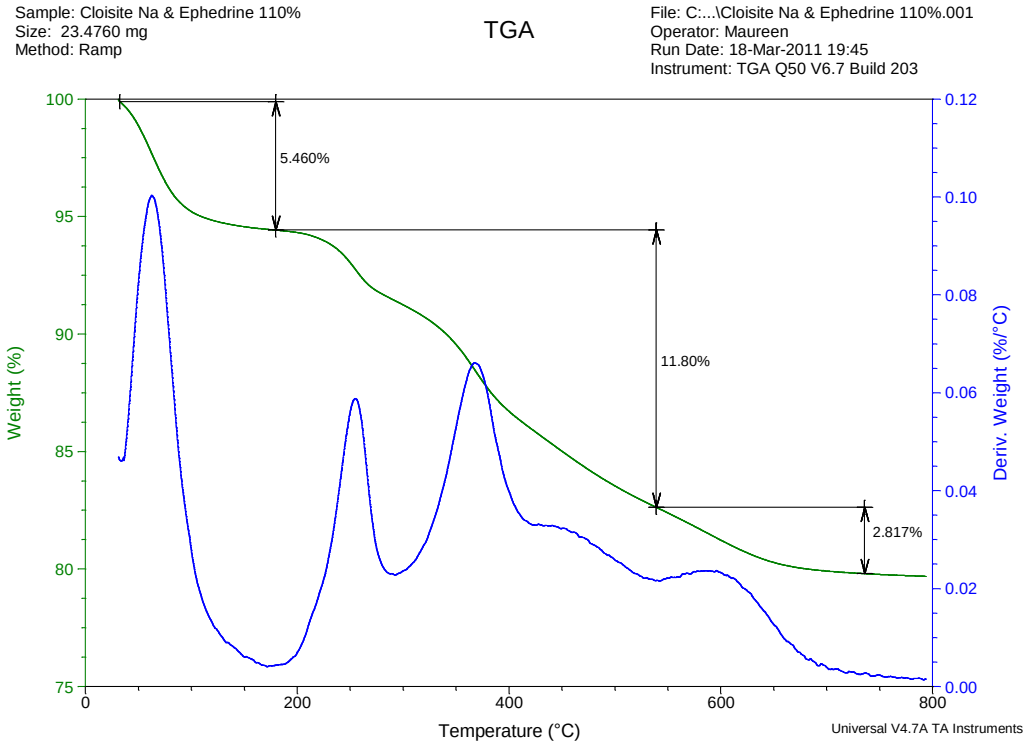

Figure 15. TGA of 110%. TGA of Cloisite® Na^+ intercalated with ephedrine HCl at 110% exchange capacity.

Table 1. TGA calculations.

Composite Batch	Water(%)	Ephedrine + OH (%)	Residual (%)	Ephedrine (%)
75% Raw	5.528	13.323	81.419	0
75% Dry Weight	0	14.062	85.958	10.27
100% Raw	5.146	14.052	80.802	0
100% Dry Weight	0	14.814	85.185	11.06
110% Raw	5.46	14.617	79.923	0
110% Dry Weight	0	15.461	84.539	11.74

These results in addition to the results from XRD (Section 3.1), it can be inferred that the d-spacing for all loadings are statistically the same and this implies that the cation exchange capacity has not been fulfilled in any case. However, it is quite clear that the interaction of the ephedrine with the clay has altered the decomposition of the ephedrine. The decomposition initiates at about the same temperature but yields three different decomposition steps that extend all the way above 500^{o}C.

3.3 Post-MSGF Results with GC

Upon analysis of the three samples prepared in Section 2.3, the FID chromatogram of Sample 1 clearly contains three distinct peaks (Figure 16). The first peak at 2.6 minutes is methyl benzoate, the IS. Peak two at 5.2 minutes is ephedrine. Peak 3 at 7.7 minutes is some unknown by product, predicted to be NEP, but that must be confirmed by the MS.

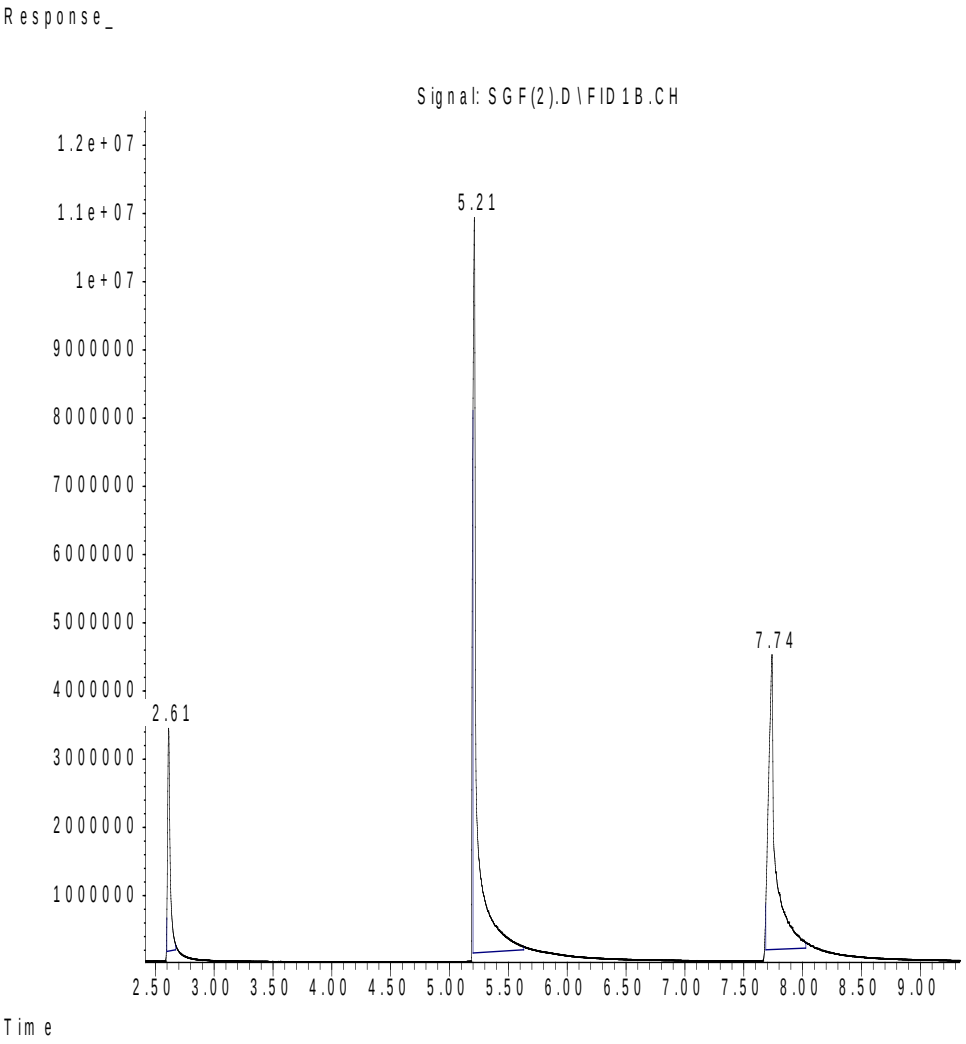

Figure 16. FID chromatogram of Sample 1. FID of pure ephedrine soaked in MSGF for 30 minutes and extracted.

Sample 2 also shows 3 distinct peaks (Figure 17), the IS, a large ephedrine peak, and a small peak for the unknown compound.

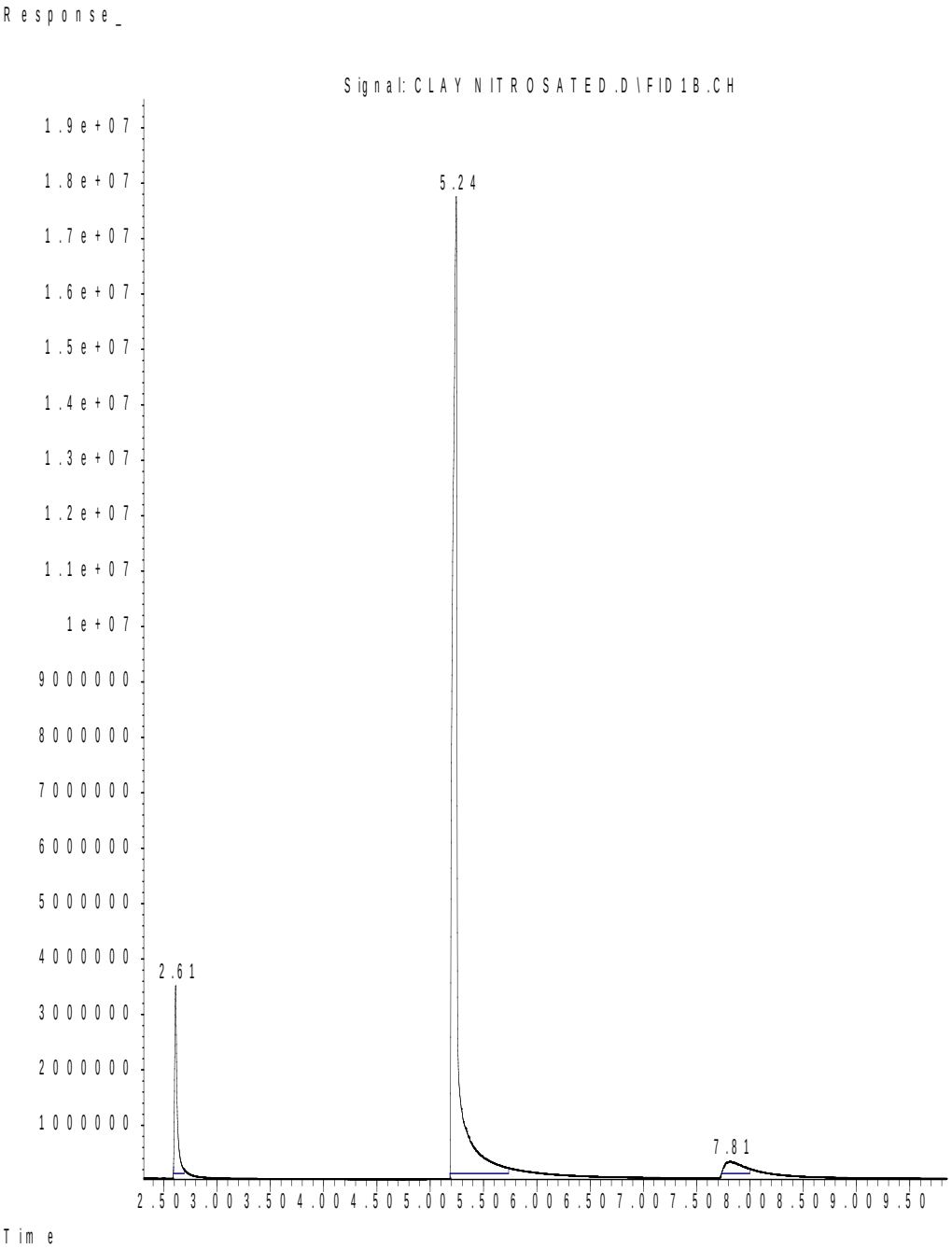

Figure 17. FID chromatogram of Sample 2. FID of 100% exchange capacity composite batch soaked in MSGF for 30 minutes and extracted.

The control (Figure 18) shows only two peaks, IS, ephedrine, and no third peak of unknown compound.

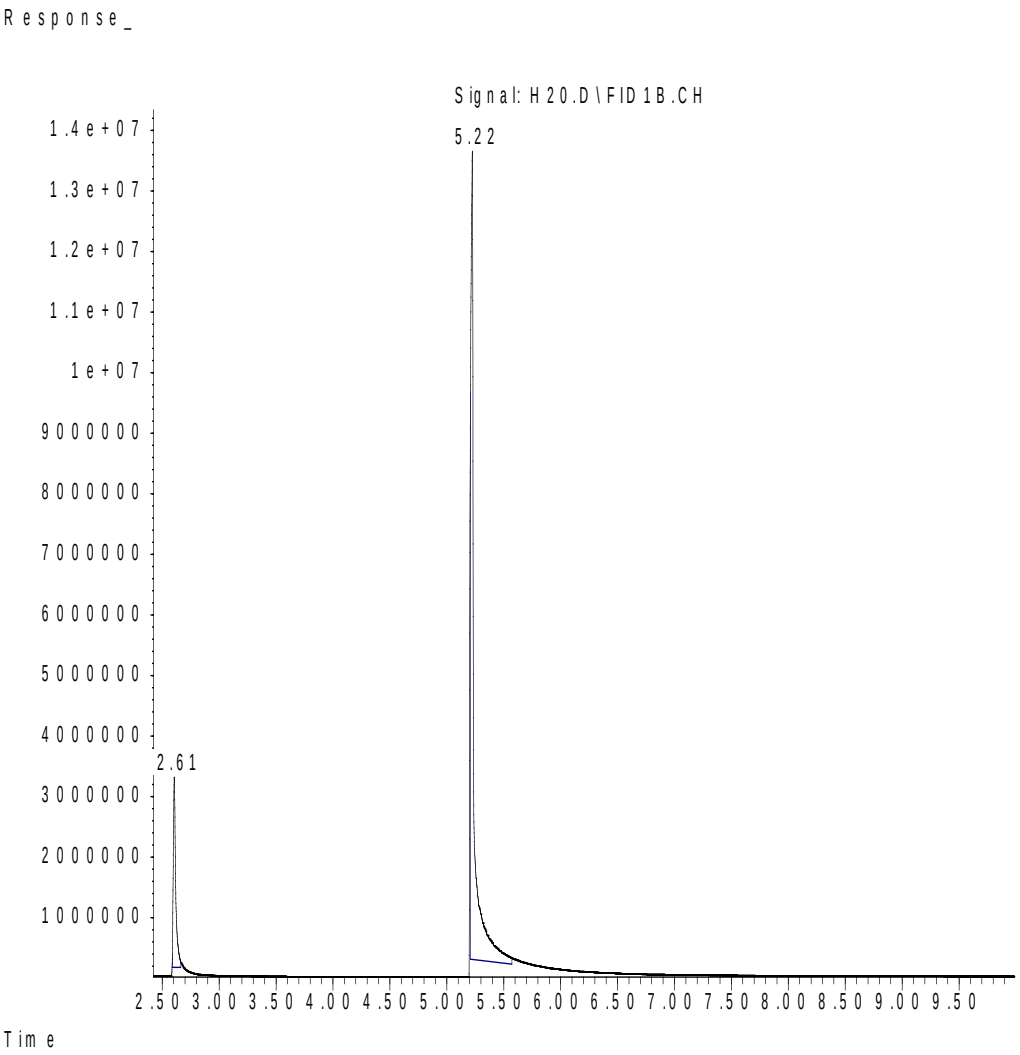

Figure 18. FID chromatogram of Control. FID of pure ephedrine soaked in diH_20 for 30 minutes and extracted.

The first peak to reach the detector is the IS, methyl benzoate confirmed by mass spectrum (Figure 19), and verified by the online NIST MS reference library.[24] The 136 m/z peak is the F.W. of methyl benzoate; $C_8H_8O_2$. Peaks 77, 91/92, and 105 m/z are benzene; $C_6H_5^+$, tropylium; $C_7H_7^+$ /$C_7H_8^+$, and methyl tropylium; $C_8H_9^+$, respectively.

The second peak is confirmed ephedrine[25] (Figure 20). 147 m/z as the molecular ion peak accounts for the ephedrine; $C_{10}H_{12}N^{+}$; F.W. 165 m/z less 18 m/z for the hydroxyl leaving as H_2O. The parent ion peak at 58 m/z is $C_3H_8N^{+}$, fragmented from the most favorable cleavage bond between the two chiral carbons. It also contains the same series 77, 91, 105 m/z as previously described in methyl benzoate. This easily characterizes the structure of ephedrine. It is possible that the third peak with a molecular ion peak of 159 m/z is NEP^{+} (F.W. of 195 g/mol), having lost 36 m/z as two water molecules due to the hydroxyl group, and also the oxygen on the N-nitroso group (Figure 21), but it is unverifiable in the NIST mass spectrum library. The fragments suggest that it is a derivative of ephedrine, with the same fragment peaks described in Figure 20. To confirm what the by-product is, proton NMR (^{1}H-NMR) was done, and the results are described in Section 3.4.

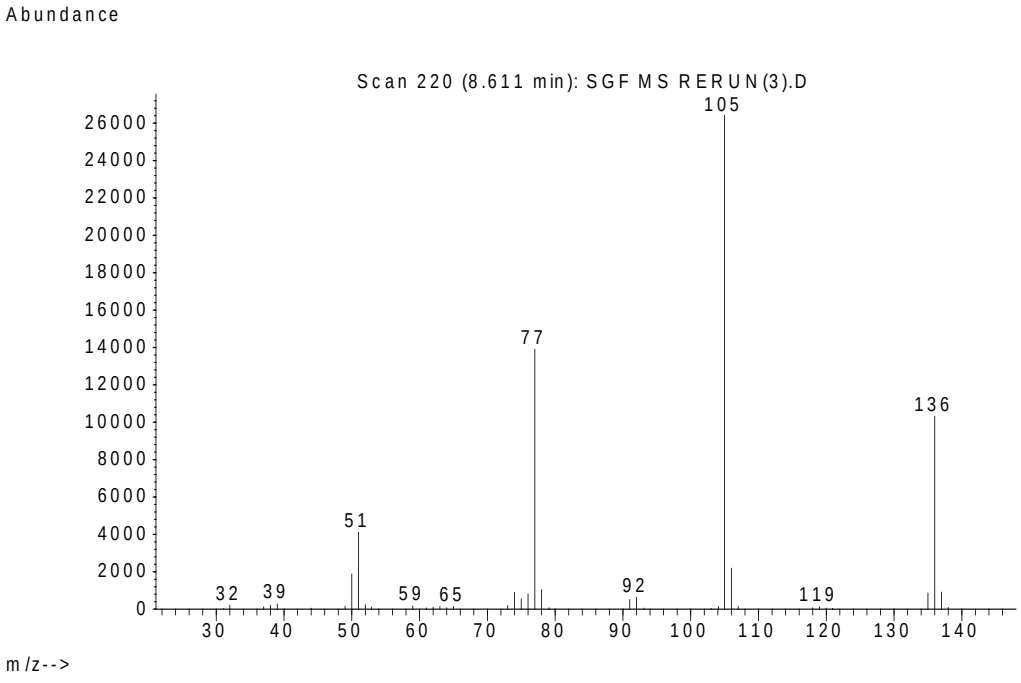

Figure 19. Mass spectrum of IS. Methyl benzoate.[24]

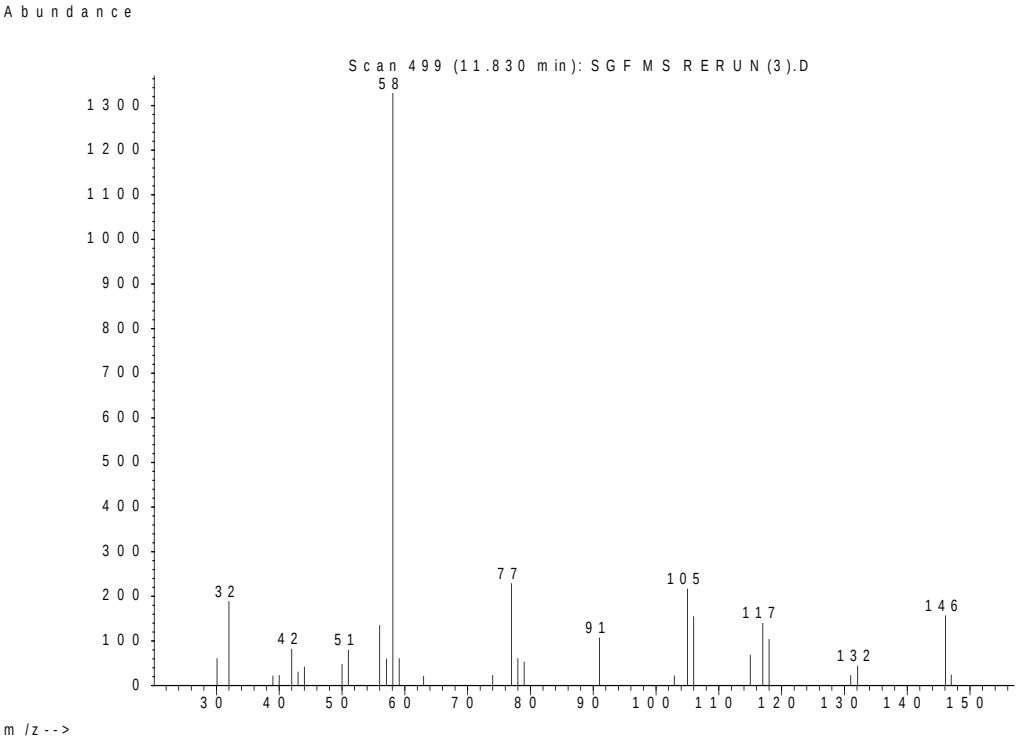

Figure 20. Mass spectrum of ephedrine.[25]

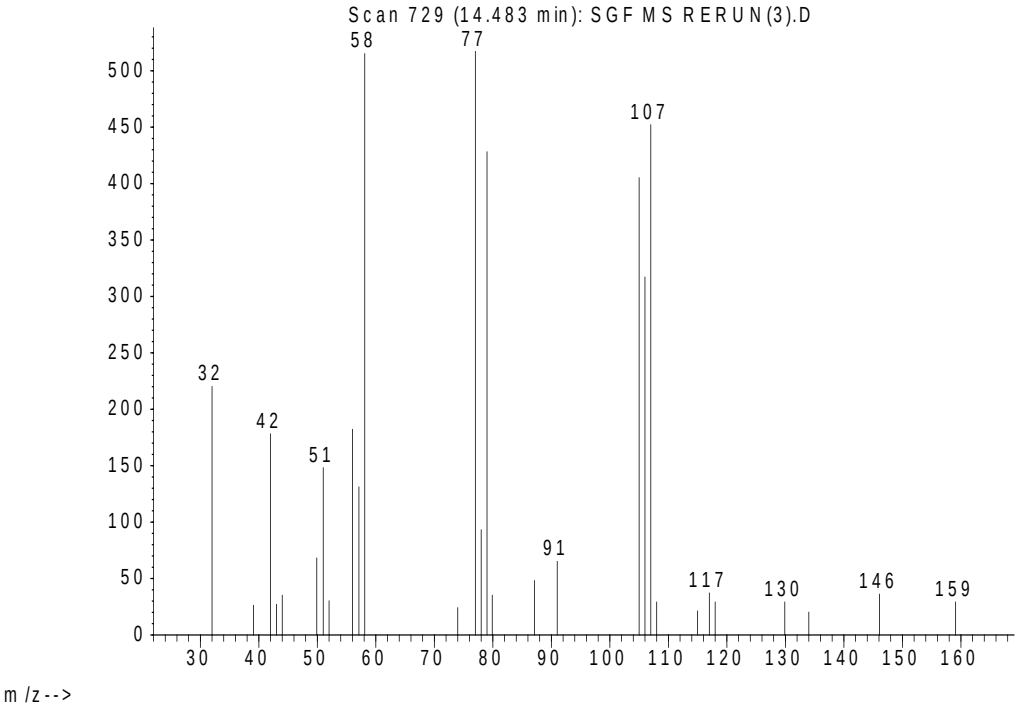

Figure 21. Mass spectrum of unknown by-product.

Integration of the IS peaks (Table 2), for each sample are close enough in response that the injection volumes were all very close to 1 μL. No normalization is necessary because the adjustment would be too small to make a significant difference in the concentration ratio of ephedrine and the by-product. Also, the complete lack of a third peak in the control confirms that it is a by-product of a reaction of ephedrine with something in the MSGF. The experiment was repeated, with the results being quite similar and is presented in Table 3.

Table 2. Integrated peak responses and retention time intervals (Experiment 1).

Experiment 1	**Sample 1**	**Sample 2**	**Control**
IS Response	62,152,095	64,471,683	64,936,276
IS Time Interval (min)	(2.585 - 3.0)	(2.583 - 3.0)	(2.589 - 3.0)
Ephedrine Response	213,971,564	684,009,194	553,646,070
Ephedrine Time Interval (min)	(5.186 - 7.0)	(5.184 - 7.0)	(5.191 - 7.0)
Unknown Response	279,816,916	85,570,613	0
Unknown Time Interval (min)	(7.659 - 9.0)	(7.690 - 9.0)	-
PERCENT EPHEDRINE UNDEGRADED @ 30min	**43.33**	**88.88**	**100**

Table 3. Integrated peak responses and retention time intervals (Experiment 2).

Experiment 2	**Sample 1**	**Sample 2**	**Control**
IS Response	62,121,038	67,935,452	63,618,978
IS Time Interval (min)	(2.585 - 3.0)	(2.582 - 3.0)	(2.577 - 3.0)
Ephedrine Response	213,407,669	684,098,506	448,648,757
Ephedrine Time Interval (min)	(5.185 - 7.0)	(5.181 - 7.0)	(5.195 - 7.0)
Unknown Response	280,281,757	61,536,551	0
Unknown Time Interval (min)	(7.655 - 9.0)	(7.706 - 9.0)	-
PERCENT UNDEGRADED	**43.23**	**91.75**	**100**

3.4 ^{1}H-NMR On Unknown Compound

A ^{1}H-NMR was performed so as to assist the mass spectrum data with elucidating the unknown by-product. The full spectrum is illustrated in Figure 21. The unknown is confirmed to be a mixture of (E)- and (Z)-NEP. The most distinguishing factor of the rotamers on the spectrum is the N-methyl group. The (E)-rotamer, represented by the intense peak at 2.95 ppm is the major component and the less intense (Z)-rotamer peak at 3.72 ppm mimics the results by Hitchcock et al.[28] The signal is shifted upfield in (E) due to electron field shielding caused by the proximity to the nitroso moiety. The (Z) form is not shielded.[28] Integration reveals that (E) to (Z) ratio is approximately 6:1.

The benzene ring of each rotamer is represented in the 7.3 ppm region. A quartet of doublets represents the α-N-nitroso-CH at 4.67 ppm. At 1.45 ppm is a doublet for the α-N-nitroso carbon-CH_3 group. The β-hydroxy-N-nitroso-CH is the 5.03 ppm multiplet. A doublet 2.50 ppm is attributed to the hydroxyl hydrogen. The 1.6 ppm peak is simply water.

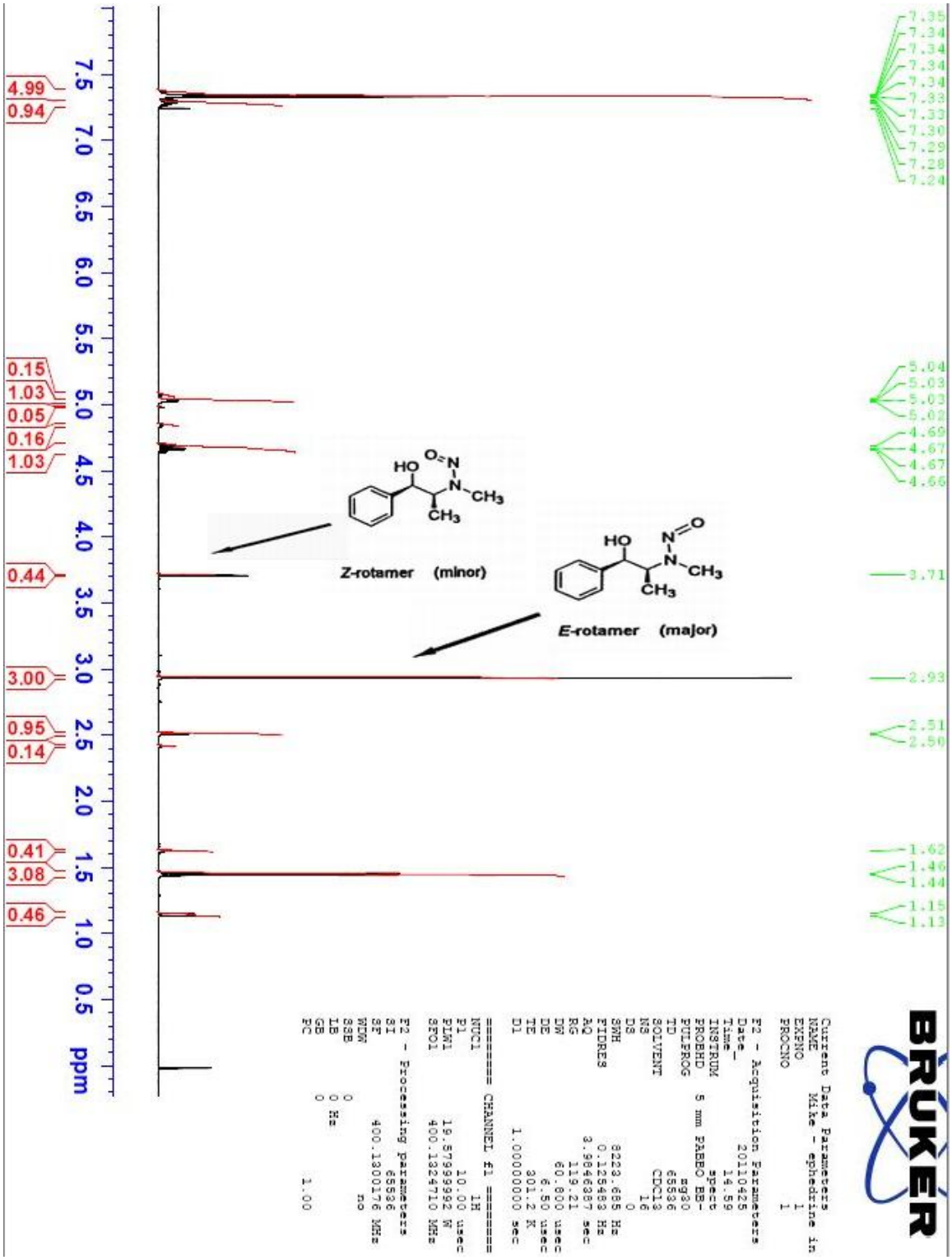

Figure 22. Full ^{1}H-NMR of (E)- and (Z)-NEP.

4.0 CONCLUSIONS AND FURTHER RESEARCH

4.1 Conclusions

These experiments have demonstrated that ephedrine hydrochloride intercalates readily into the montmorillonite gallery and that the intercalated ephedrine is much more thermally stable within the gallery. Also, it is evident ephedrine molecules orient with the benzene ring parallel to the surface of the clay. The hypothesis that ephedrine intercalated in the layers of Cloisite® Na^+ is protected from N-nitrosation was supported by the results described herein.

4.2 Future Study

Due to the success of this research, the type of molecules intercalated into analytical clays must be expanded and tested experimentally. Studies of particular interest would be determining how well clay protects amino acids, peptides, or entire proteins, most especially insulin. If insulin could be administered orally and survive the stomach, rather than intravenously injected, it could revolutionize treatment of the diabetes epidemic.

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VITA

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