

2013

Synthesis of 1-Pyrenyldiazomethane for Derivatization of Short-Chain Fatty Acids

Mary E. Kalvaitis

Follow this and additional works at: <http://commons.emich.edu/honors>

 Part of the [Chemistry Commons](#)

Recommended Citation

Kalvaitis, Mary E., "Synthesis of 1-Pyrenyldiazomethane for Derivatization of Short-Chain Fatty Acids" (2013). *Senior Honors Theses*. 341.
<http://commons.emich.edu/honors/341>

This Open Access Senior Honors Thesis is brought to you for free and open access by the Honors College at DigitalCommons@EMU. It has been accepted for inclusion in Senior Honors Theses by an authorized administrator of DigitalCommons@EMU. For more information, please contact lib-ir@emich.edu.

Synthesis of 1-Pyrenyldiazomethane for Derivatization of Short-Chain Fatty Acids

Abstract

Use of 1-pyrenyldiazomethane (PDAM) for derivatizing short-chain fatty acids (SCFA) is a successful way to analyze complex SCFA samples quickly and with minimal sample preparation. The costs associated with ordering PDAM from a commercial source can be high, typically more than \$300 not including shipping costs. This project developed a simple two-step synthesis procedure that can be used in undergraduate chemistry laboratories. We found that the PDAM produced by this procedure is pure enough to use for our derivatization needs without further purification. This synthesis can be used to further explore the use of PDAM for fast and simple analysis of SCFA without excessive material costs.

Degree Type

Open Access Senior Honors Thesis

Department

Chemistry

First Advisor

Heather Holmes

Second Advisor

Hedeel Evans

Keywords

fluorescence, gas chromatography, PDAM, Fatty Acids

Subject Categories

Chemistry

SYNTHESIS OF 1-PYRENYLDIAZOMETHANE FOR DERIVATIZATION OF
SHORT-CHAIN FATTY ACIDS

By

Mary E Kalvaitis

A Senior Thesis Submitted to the

Eastern Michigan University

Honors College

in Partial Fulfillment of the Requirements for Graduation

with Honors in Chemistry

Approved at Ypsilanti, Michigan, on this date April 25, 2013

Table of Contents

I. Introduction	5
Figure 1	7
II. Methods and Materials	7
1. Materials	7
Table 1: Reagents, Distributor, Particle Size (if applicable) and Purity	8
2. Instruments	8
3. Step 1: Formation of Pyrenecarboxaldehyde Hydrazone	8
3.1. Solvent Analysis	9
Table 2: Name and boiling point of solvents used in analysis	9
4. Step 2: Preparation of PDAM from PCH	9
III. Results	10
1. Step 1	10
1.1. Solvent Analysis	10
Table 3: Masses of reagents and products for Step 1 solvent analysis	10
1.2. Spectroscopic Analysis	11
Figure 2: H1 NMR spectrum of pyrenecarboxaldehyde	12
Figure 3: H1 NMR spectrum of hydrazine monohydrate	13
Figure 4: NMR Spectrum of crude PCH	14
Figure 5: H1 NMR spectrum of purified PCH	15
2. Step 2	15

2.1. Mass of PDAM produced and reaction yield	15
Table 4: Masses of reagents and PDAM produced from oxidation (step 2)	16
2.2. Spectroscopic Analysis	16
Figure 6: H1 NMR spectrum of synthesized PDAM	17
Figure 7: Fluorescence spectrum of pyrenecarboxaldehyde in ethyl acetate	19
Figure 8: Fluorescence spectrum of PDAM in ethyl acetate	19
Figure 9: Fluorescence spectra of pyrenyl esters of butyric acid in ethyl acetate .	20
Figure 10: Calibration curve for synthesized PDAM	21
IV. Conclusions	22
1. Step 1	22
2. Step 2	22
V. References	23
VI. Appendix: Practical Lab Procedure	29

Abstract

Use of 1-pyrenyldiazomethane (PDAM) for derivatizing short-chain fatty acids (SCFA) is a successful way to analyze complex SCFA samples quickly and with minimal sample preparation. The costs associated with ordering PDAM from a commercial source can be high, typically more than \$300 not including shipping costs. This project developed a simple two-step synthesis procedure that can be used in undergraduate chemistry laboratories. We found that the PDAM produced by this procedure is pure enough to use for our derivatization needs without further purification. This synthesis can be used to further explore the use of PDAM for fast and simple analysis of SCFA without excessive material costs.

I. Introduction

The human body is host to thousands of bacteria cells, mainly concentrated in the gastrointestinal system (GI). Some estimates indicate that 90% - 95% of the bacteria in the body are located in the colon. As the bacteria digest nutrients, they produce short-chain fatty acids (SCFAs). These organic acids are involved in regulatory processes of the GI tract such as water and electrolyte absorption (1). An individual with an imbalance or low concentration of intestinal bacteria may have an increased risk of inflammatory bowel disease, colon cancer, obesity and types 1 and 2 diabetes, mainly because of the changes in SCFA presence in the digestive system (2, 3). Monitoring SCFA in an individual exhibiting symptoms of any of the above conditions may ultimately help

diagnose a specific disease or evaluate the efficiency of a treatment (3).

Analysis of SCFAs in biological samples is complicated by extremely complex matrices. Accordingly, many methods of analysis have been developed. Direct sample injection without pretreatment for gas chromatographic analysis has been successful for some volatile fatty acids, though carryover in the injection port and peak tailing proved to be problematic. (4, 5, 6, 7, 8, 9) Capillary electrophoresis (CE) has been used for the determination of SCFAs in a variety of environmental and biological samples without sample pretreatment. (10, 11, 12, 13, 14, 15) More commonly, SCFAs are extracted prior to analysis using methods such as solid phase microextraction (16), single-drop microextraction (17), hollow fibre liquid-phase microextraction (18), high-throughput syringe solvent extraction (19) Rose-Gottlieb (20) and Folsch (21) extraction, and a number of other methods (22). Chemical derivatization of SCFAs is routine; the resulting analyses often yield greater sensitivity and lower limits of detection than for underivatized acids. Common strategies include alkylation, acylation, silylation, or esterification followed by gas or liquid chromatography, or electrophoresis (23, 24, 25, 26, 27, 28, 29). A large number of fluorescent reagents have been developed as well (30), including 1-pyrenyldiazomethane (PDAM), which has been used widely for analysis of biological samples. (31, 32, 33, 34, 35, 36, 37, 38).

Derivatization of SCFA samples by attaching PDAM can aid in recovering the SCFA's from the matrix and give better sensitivity and limits of detection. This reaction is simple, rapid, and easily performed in a small vial at room temperature (31, 32, 33, 34,

35, 36, 37, 38). Unfortunately, PDAM itself can be costly to obtain, with some distributors charging more than \$300 for less than 1 g of material. Additionally, the molecule can degrade from exposure to light and heat (39). Any unused PDAM from one derivatization could go to waste if improperly stored.

The synthesis method proposed here can be used to make PDAM on demand in practically any organic chemistry laboratory. All of the techniques used can be mastered quickly by an undergraduate chemistry student, allowing students to get involved in SCFA projects and gain experience with multi-step synthesis methods and saving the program money by reducing the need to order and ship pre-made PDAM from an outside source.

The general form of the synthesis is based on information found in (31) and is shown in Figure 1. The first step utilizes the aldehyde group of pyrenecarboxaldehyde to create a hydrazone from hydrazine monohydrate. The second step oxidizes the pyrenecarboxaldehyde hydrazone (PCH) with manganese dioxide to form the final product, 1-pyrenyldiazomethane (PDAM).

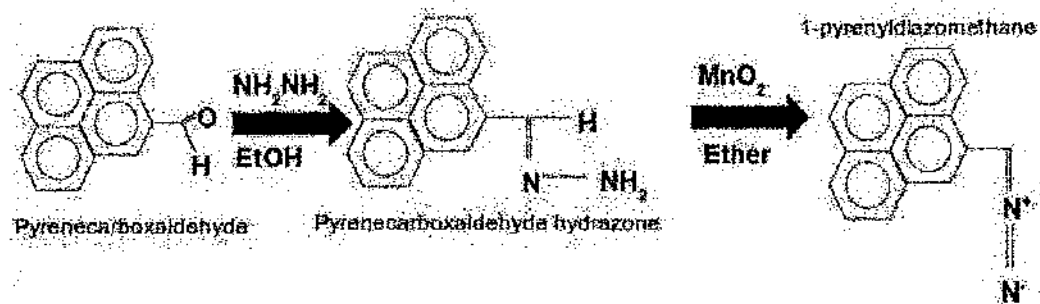


Figure 1: The synthesis begins with pyrenecarboxaldehyde. Using hydrazine monohydrate, the aldehyde group is converted to a hydrazone. The pyrenecarboxaldehyde hydrazone is oxidized to give 1-pyrenyldiazomethane.

II. Methods and Materials

1. Materials

Reagents used were obtained from Sigma-Aldrich and Fisher distributors at the highest purities. Details about each reagent are listed in Table 1.

Table 1: Reagents, Distributor, Particle Size (if applicable) and Purity.

Compound	Distributor	Particle Size	Purity
Hydrazine Monohydrate	Sigma-Aldrich	N/A	Reagent Grade
Pyrenecarboxaldehyde	Sigma-Aldrich	Not Specified	99%
Manganese Dioxide	Sigma-Aldrich	< 5 μm activated	85%
Ethanol	Fisher	N/A	Absolute
Diethyl Ether	Fisher	N/A	Reagent Grade

Note that activated manganese dioxide was used.

2. Instruments

All nuclear magnetic resonance (NMR) spectra were obtained from an Oxford NMR AS400 with a field strength of approximately 9.3897 T operating at a frequency of 400 MHz.

Fluorescence spectra were obtained using a Jasco FP-6300 Spectrofluorometer with an excitation wavelength of 340 nm, excitation and emission bandwidths of 2.5 nm, a data pitch of 0.5 nm, and a scanning speed of 200 nm/min.

3. Step 1: Formation of Pyrenecarboxaldehyde Hydrazone

A 10% scale of the procedure used by Nimura et al. was used for these experiments (31). In a small vial, approximately 0.5 g pyrenecarboxaldehyde and 0.34 g hydrazine monohydrate were added to 8-10 mL of pure ethanol. The mixture was stirred by magnetic stir plate for three hours. The solution was filtered by vacuum and collected for recrystallization. This crude pyrenecarboxaldehyde hydrazone was recrystallized using various solvents and collected again by vacuum recrystallization. The recrystallized pyrenecarboxaldehyde hydrazone was left to dry on a watch-glass overnight. The yield was approximately 0.4 g pyrenecarboxaldehyde hydrazone.

3.1 Solvent Analysis

Several solvents were examined for use in the formation of pyrenecarboxaldehyde hydrazone (PCII) from pyrenecarboxaldehyde and hydrazine monohydrate (Figure 1, Step 1). The particular solvents and their boiling points are shown in Table 2 were used as a solvent for Step 1 and for recrystallization of PCH.

Table 2: Name and boiling point of solvents used in analysis

Solvent	Boiling Point (°C)
Diethyl Ether	34.60
Ethanol	78.37
Ethyl acetate	77.10
Methanol	65.00
Toluene	110.6
Water	100.00

4. Step 2: Preparation of PDAM from PCH

Approximately 0.2 g of the pyrenecarboxaldehyde hydrazone and 0.65 g activated manganese dioxide were added to 30-50 mL of dry ether. The solution was sonicated for two hours and the manganese dioxide removed via gravity filtration. The resulting solution was a suspension of 1-pyrenyldiazomethane in ether. Allowing the solution to evaporate completely gave about 0.1 g 1-pyrenyldiazomethane as reddish-brown crystals.

III. Results

1. Step 1

Results from the solvent analysis and obtained spectra were used to adjust the reaction conditions of the first step as well as determine the success of the reaction.

1.1 Solvent Analysis

Table 3 shows the exact masses of reagents used for the solvent analysis study along with the mass of PCH obtained before and after recrystallization.

Table 3: Masses of reagents and products for Step 1 solvent analysis

Solvent	Pyrenecarbox-aldehyde	Hydrazine Monohydrate	Crude PCH	Recrystallized PCH
Diethyl Ether	0.507 g	0.349 g	0.708 g	---
Ethanol	0.494 g	0.336 g	1.448 g	0.925 g
Ethyl acetate	0.498 g	0.346 g	0.691 g	0.122 g
Methanol	0.500 g	0.347 g	1.063 g	0.942 g
Toluene	0.499 g	0.340 g	1.227 g	0.488 g
Water	0.498 g	0.343 g	0.864 g	0.674 g

It appears that all reactions gave PCH. However, it was determined that the

reagents did not fully react in water most likely due to low solubility of the pyrenecarboxaldehyde. The reason for the low yield from the ethyl acetate is undetermined, but may also be due to solubility issues. Diethyl ether evaporated too quickly to be used as a solvent for the first step.

Methanol, ethanol, and toluene all gave over 1 g of PCH, likely because they are polar organic solvents so the pyrenecarboxaldehyde would easily dissolve. Success with ethanol was expected based on previous publications (31) and gave the most crude product.

Toluene, ethyl acetate, and water had significant sample loss during recrystallization. Diethyl ether evaporated too quickly to complete any recrystallization. Ethanol and methanol had the least sample loss and were easiest to work with for recrystallization.

Ethanol was used as the solvent for all subsequent reactions because it gave the highest yield of crude PCH, was easy to work with during recrystallization, and was less expensive and easier to dispose of than toluene.

1.2 Spectroscopic Analysis

Success of step 1 of the synthesis reaction was determined by nuclear magnetic resonance (NMR) image comparison of the reactants and products.

Figure 2 shows the ¹H NMR of pyrenecarboxaldehyde. Note the aldehyde proton at 10.75 ppm and the peaks of the conjugated pyrene group from 8 to 9 ppm. During step

1 of the synthesis, the aldehyde on the pyrenecarboxaldehyde should be converted to a hydrazone. This would correspond to disappearance of the aldehyde peak on the NMR. The pyrene group should remain unchanged.

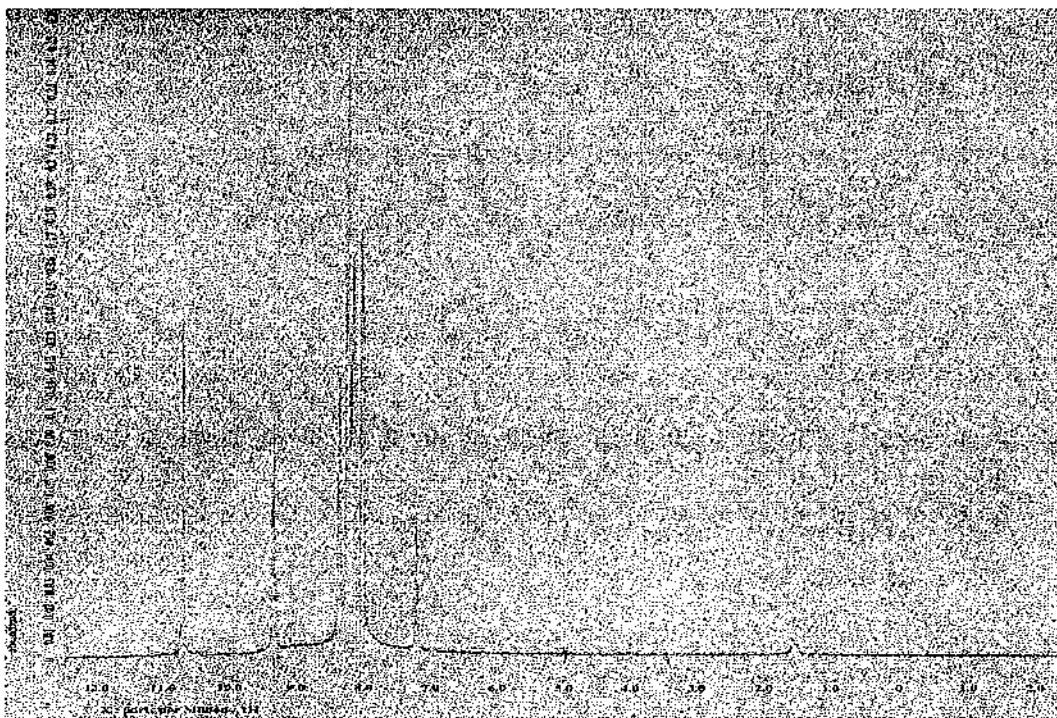


Figure 2: ^1H NMR spectrum of pyrenecarboxaldehyde. Important peaks are the aldehyde peak at 10.75 ppm and the pyrene peaks from 8-9 ppm. Success of the first step of the synthesis would correspond to the aldehyde peak disappearing and the pyrene peaks remaining unchanged.

The ^1H NMR image of hydrazine monohydrate (Figure 3) shows several water and solvent peaks, and also the N-H peak at 4.6 ppm. After the aldehyde on the pyrenecarboxaldehyde is converted to a hydrazone, the N-II peak should disappear.

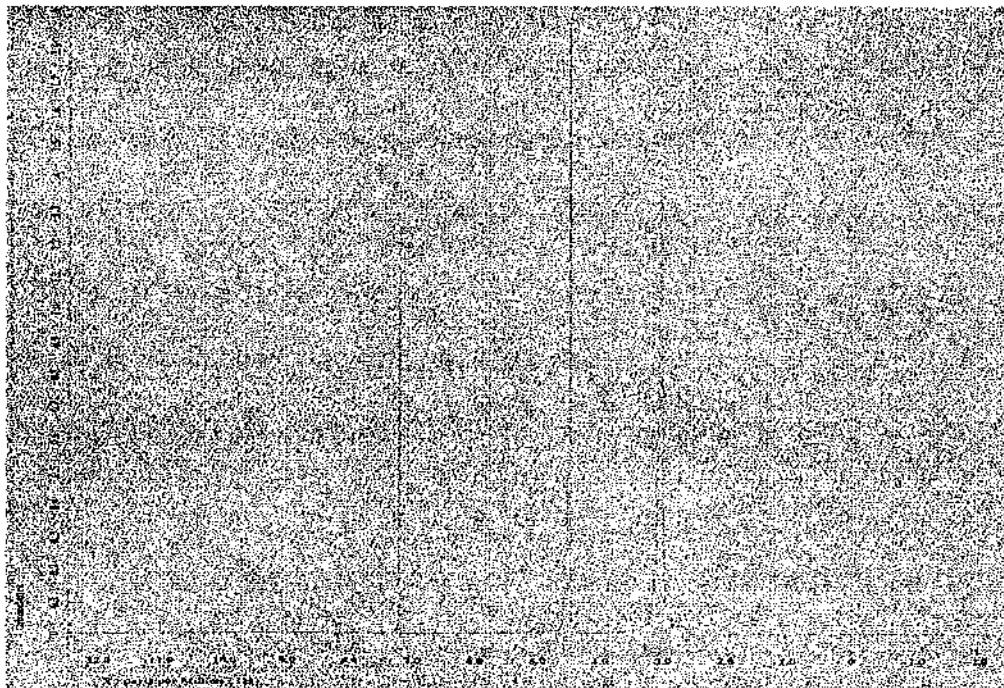


Figure 3: ^1H NMR spectrum of hydrazine monohydrate. Note the N-H peak at 4.6 ppm. All other peaks are attributed to solvents or water in the sample tube. For the first step of the synthesis to be considered successful, the N-H peak should disappear.

The NMR spectrum of the crude PCII from recrystallization in ethanol is shown in Figure 4. Note the peak at 5.8 ppm, descriptive of a hydrazone. Also, the pyrene peaks appear from 8-9 ppm. These were identified in the pyrenecarboxaldehyde NMR spectrum in Figure 2, and are unchanged as expected. The reduced aldehyde peak at 10.75 ppm seems to indicate that some reagent remains in the product.

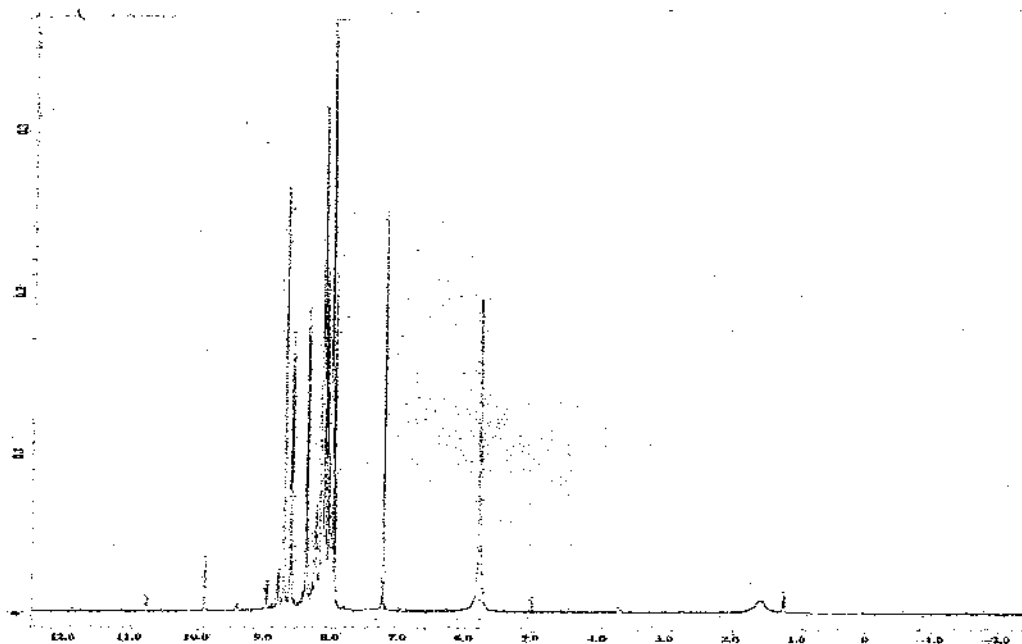


Figure 4: NMR Spectrum of crude PCH. Note the aldehyde peak at 10.75 ppm, similar to Figure 3.

Crude PCH was synthesized and recrystallized with ethanol several times with similar results. The proton NMR spectrum of the purified product after recrystallization in ethanol is shown in Figure 5. There was little to no change in the NMR spectra, indicating no change in sample purity between the crude and recrystallized PCH (Figures 4 and 5). Recrystallization resulted in large sample loss without improving purity.

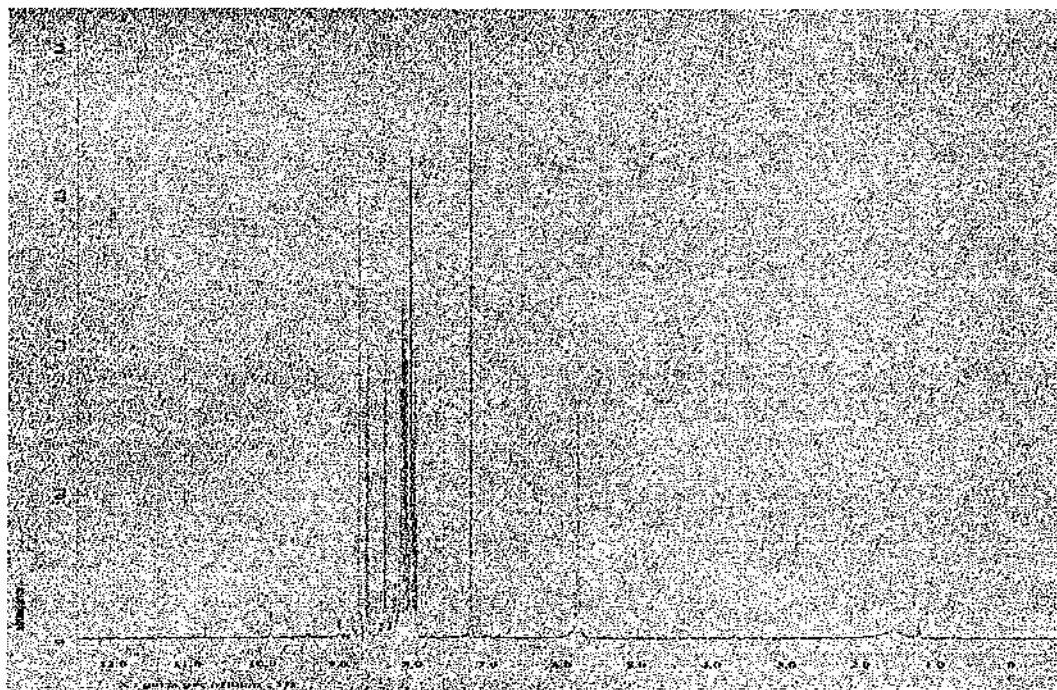


Figure 5: ^1H NMR spectrum of purified PCH. Note the reduction of the N-H peak from the hydrazine (see Figure 3) and of the aldehyde peak (see Figure 2). These imply the first step of the reaction was successful but imply some residual starting materials. As expected the pyrene group (see Figure 3) was unaltered.

2. Step 2

The oxidation of the pyrenecarboxaldehyde hydrazone (PCII) (see step 2 in Figure 1) produced PDAM as determined by NMR spectroscopic analysis and the yield of step 2 was determined.

2.1 Mass of PDAM produced and reaction yield

Two batches of PDAM were successfully produced via the synthesis shown in

Figure 1. Table 4 shows the masses of (PCH) and manganese dioxide used to complete step 2 of the synthesis and the mass of PDAM produced.

Table 4: Masses of reagents and PDAM produced from oxidation (step 2)

PCH	Manganese Dioxide	PDAM produced
0.2701 g	0.6615 g	0.07588 g
0.1968 g	0.6739 g	0.0988 g

The synthesized PDAM was obtained as rust-red crystals after the diethyl ether solvent evaporated completely. The mass of PDAM obtained from Trial 1 is artificially low because some of the crystals were removed for NMR analysis before weighing the sample. Based on results from Trial 2, the yield of the reaction is approximately 19%.

2.2 Spectroscopic Analysis

The ¹H NMR spectrum of the synthesized PDAM is shown in Figure 6. Note the reduction in the N-H peak from the PCH (see Figure 5) which implies additional conjugation and formation of the diazo group. There remain some peaks from the aldehyde at 10.75 ppm and also diethyl ether at approximately 1.1 ppm and 3.5 ppm which would imply residual starting materials and solvent in the PDAM crystals. The pyrene group at 8-9 ppm remained unchanged from previous spectra, Figures 2 and 4, as anticipated.

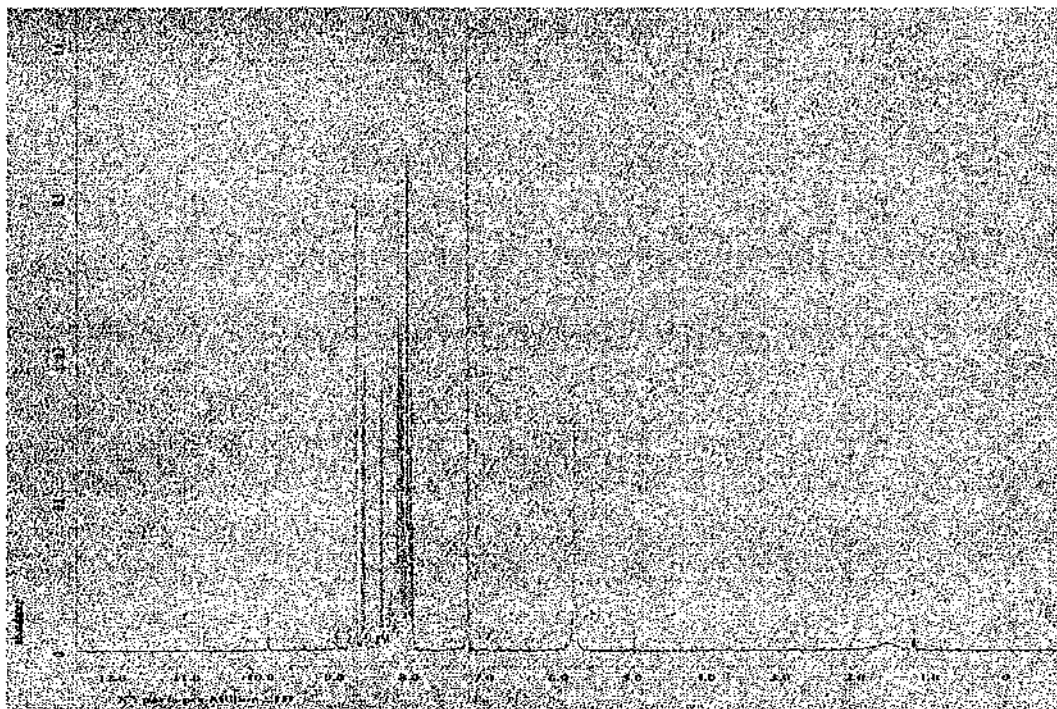


Figure 6: ^1H NMR spectrum of synthesized PDAM. Note the reduction of the N-H peak (from Figure 5) which shows the increased conjugation and formation of the diazo group. Some peaks from the aldehyde at 10.75 and diethyl ether at approximately 1.1 ppm and 3.5 ppm show some starting materials and solvent may be trapped in the PDAM crystals. However, the pyrene group remained unchanged (see Figures 2 and 4) as

Pyrenecarboxaldehyde and synthesized PDAM were analyzed using fluorescence spectroscopy to confirm the success of the reaction. The fluorescence spectrum obtained from pyrenecarboxaldehyde (PCA) is shown in Figure 7. The fluorescence intensity steadily increases over a wavelength range of approximately 380 nm through 405 nm, where a maximum is observed. This matches fluorescence spectra previously observed for PCA (42), and is significantly different than the fluorescence spectrum collected under the same conditions for PDAM (Figure 8). Using an excitation wavelength of 340

nm, PDAM has a fluorescence maximum at approximately 395 nm (35), which is clearly evident on the spectrum of PDAM in Figure 8.

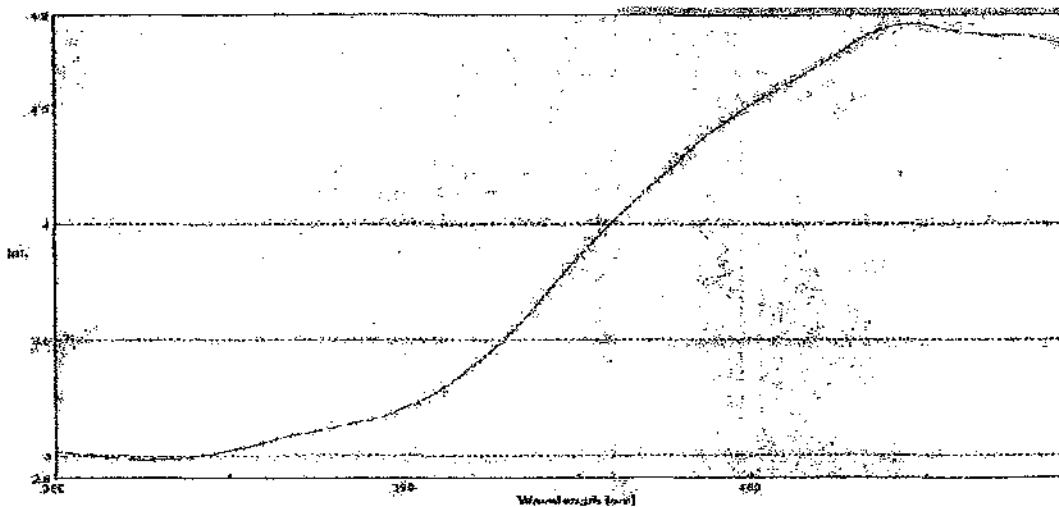


Figure 7: The fluorescence spectrum of pyrenecarboxaldehyde in ethyl acetate. Note there is a maximum at a wavelength of approximately 405 nm, from an excitation wavelength of 340 nm.

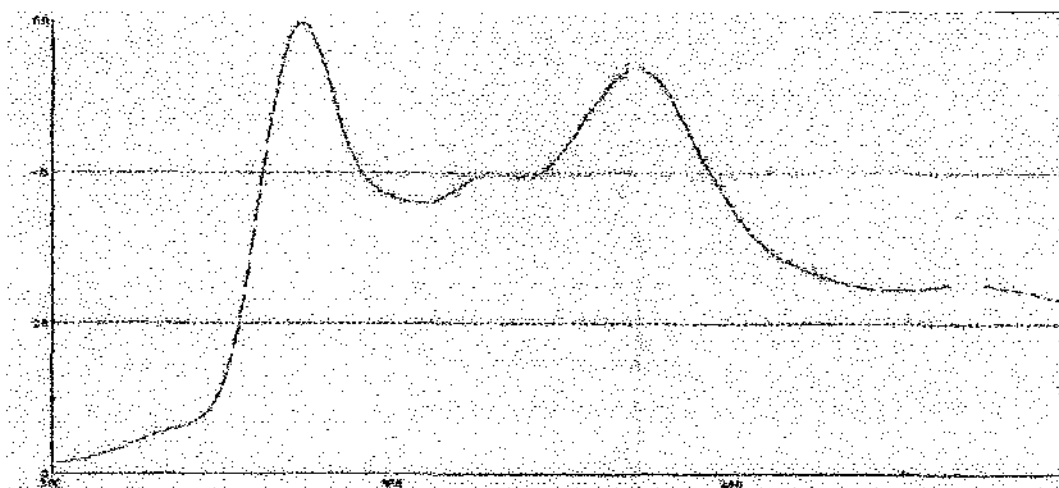


Figure 8: The fluorescence spectrum of PDAM in ethyl acetate. There is an emission maximum at 395 nm, from an excitation wavelength of 340 nm.

The synthesized PDAM was used to derivatize standards of butyric acid, and fluorescence spectra were obtained for the solutions of the pyrenyl esters (Figure 9).

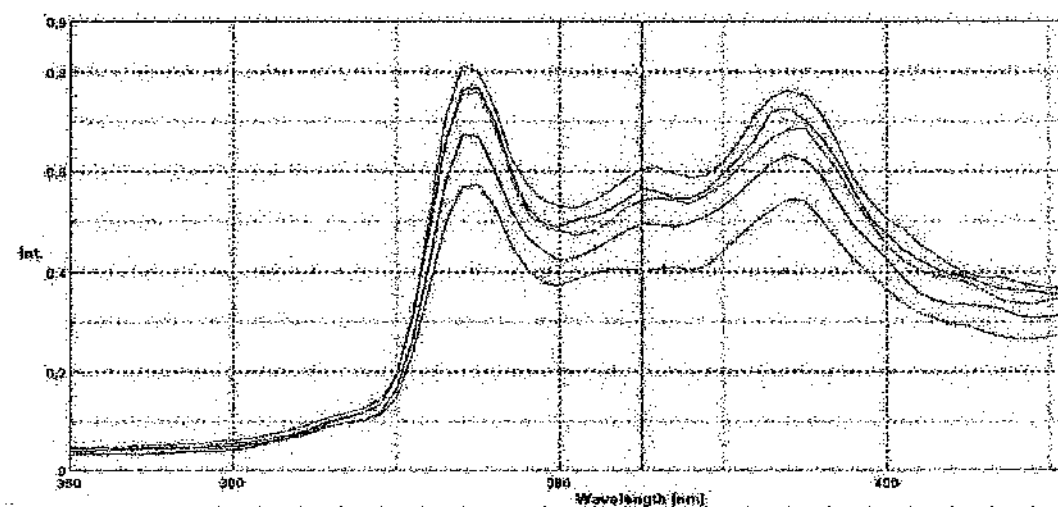


Figure 9: The fluorescence spectra of pyrenyl esters of butyric acid in ethyl acetate. The concentrations of butyric acid prior to derivatization were from 1 to 10 ppm. The emission maxima appear at 395 nm from an excitation wavelength of 340 nm.

A calibration curve, shown in Figure 10, was created from the fluorescence intensities at 395 nm. The fluorescence intensity over the range 1 to 10 ppm of the pyrenyl derivative was approximately linear, indicating that the purity of the synthesized PDAM was sufficient for derivatization of butyric acid.

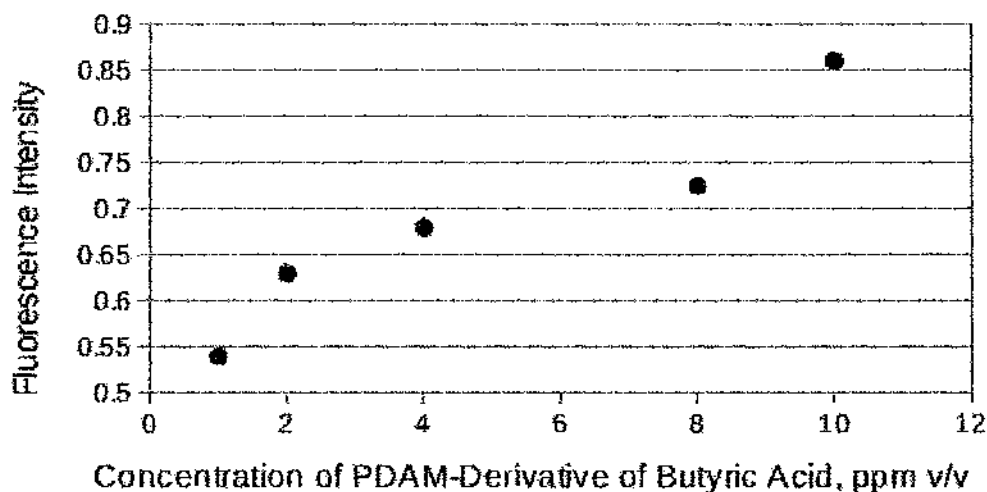


Figure 10: Calibration curve for synthesized PDAM. Results were approximately linear, indicating effective PDAM purity as a derivatizing agent for butyric acid.

PDAM from recrystallized PCH was pure enough to be used in derivatization reactions without further purification. Because the recrystallization of PCH generated little to no improvements to PCH purity, it seems reasonable that the recrystallization of PCH is not necessary and wouldn't affect the purity of PDAM. However, purity of PDAM synthesized from crude PCH was not examined but is likely not to affect the yield or purity of the PDAM created. Recrystallization of PDAM was deemed unnecessary because it had already been successfully used in derivatization reactions. It also seemed likely that based on the results of PCH recrystallization, recrystallizing PDAM would result in large sample loss and minimal or no improvement to purity.

IV. Conclusions

1. Step 1

From the solvent analysis it was determined that ethanol was the optimal solvent for step 1 of the synthesis in which PCA and hydrazine monohydrate are used to create PCII. Using NMR spectroscopy, it was determined that step 1 successfully produced PCII based on changes in key peaks on the H1 NMR spectra. It was also determined via NMR spectroscopy that recrystallization did not improve PCH purity and so was unnecessary after step 1. However, the affect of using crude product on the mass and purity of synthesized PDAM was not examined.

2. Step 2

PDAM was successfully produced from the intermediate product using the synthesis outlined in Figure 1. The yield of the reaction was low, 19%, but PDAM was produced in sufficient quantities to use for derivatization of short-chain fatty acid samples in ethyl acetate. The purity of the product was high enough to be used successfully as a derivatizing agent for these short-chain fatty acid samples.

Nimura et al. (31) claim an 85% yield of PDAM from this procedure. The yield in our procedure may be low from the smaller scale or purity and particle size of the manganese dioxide. The article doesn't specify the particle size of manganese dioxide used to complete the reaction.

V. References

- (1) Vinolo, M., Rodrigues H., Nachbar R., Curi R. (2011) Regulation of Inflammation by Short Chain Fatty Acids. *Nutrients*: 3 858-876
- (2) Kim, Y. (1998) Topics in Clinical Nutrition: Short-Chain Fatty Acids in Ulcerative Colitis. *Nutrition Reviews*: 56 (1) 17-24
- (3) Augcnlicht, I., Mariadason J., Wilson A., Arango D., Yang, W., Hccrdt B., Velcich, A. (2002) Short Chain Fatty Acids and Colon Cancer. *The Journal of Nutrition*: 132 (12) 3804-3808
- (4) Pompei, A., Cordisco, L., Raimondi, S., Amaretti, A., Pagnoni, U. M., Matteuzzi, D., et al. (2008). In vitro comparison of the prebiotic effects of two inulin-type fructans. *Anaerobe*: 14 80-286.
- (5) Steward, M. L., Timm, D. A., & Slavin, J. L. (2008). Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system. *Nutrition Research*: 28 329-334.
- (6) Summanen, P., Baron, E.J., Citron, D.M., Strong, H.M., Wexler H.M., Finegold, S.M. (1993) *Wadsworth Anaerobic Bacteriology Manual*, 5th Ed.; Star Publishing Co: Belmont, CA
- (7) Socolowsky, S., Hohne, C., Sandow D.Z., (1990) The direct detection of volatile fatty acids by gas chromatography in microbiological diagnosis. *Medizin. Labor. Diagn.*: 31 445-452
- (8) Gas chromatographic analysis of short-chain fatty acids and lactic acids in microbiological diagnostics. (1989) *Zeitschrift fur die Gesamte Hygiene und Ihre*

Grenzgebiete: 35 (10), 596-598

- (9) Wiggins, R.J., Wilks, M., Tabaqchali S. (1985) Analysis by gas liquid chromatography of production of volatile fatty acids by anaerobic bacteria grown on solid medium. *Journal of Clinical Pathology: 38* 933
- (10) Arellano, M., Jomard, P., El Kaddouri, S., Roques, C., Nepveu, F., Couderc, F. (2000) Routine analysis of short-chain fatty acids for anaerobic bacteria identification using capillary electrophoresis and indirect ultraviolet detection, *Journal of Chromatography B: Biomedical Sciences and Applications: 741* (1) 89-100
- (11) Arellano, M., Andrianary, J., Dedieu, F., Couderc, F., Puig, P. (1997) Method development and validation for the simultaneous determination of organic and inorganic acids by capillary zone electrophoresis. *Journal of Chromatography: 765* 321-328
- (12) Arellano, M., El Kaddouri, S., Roques C., Couderc F., Puig, Ph. (1997) Capillary electrophoresis and indirect UV detection as a fast and simple analytical tool for bacterial taxonomy. *Journal of Chromatography: 781* 497-501
- (13) Li, K., Li S.F.Y., (1994) Determination of Anions in Water Samples by Capillary Zone Electrophoresis with Indirect UV Dctection. *Journal of Liquid Chromatography: 17* 3889
- (14) Romano, J.P. and Krol, J. (1993), Capillary ion electrophoresis, an environmental method for the determination of anions in water. *Journal of Chromatography A: 640* (1-2) 403-412

- (15) Zuriguel, V., Causse, E., Bounery, J.D., Nouadje, G., Simeon, N., Nertz, M., Salvayre, R., Couderc, F. (1997) Short chain fatty acids analysis by capillary electrophoresis and indirect UV detection or laser-induced fluorescence. *Journal of Chromatography A*: 781 (1-2) 233-238.
- (16) Vitali, B., Ndagijimana, M., Cruciani, F., Carnevali, P., Candela, M., Guerzoni, M. E. (2010). Impact of a symbiotic food on the gut microbial ecology and metabolic profiles. *BMC Microbiology*: 10 4–17.
- (17) Li, Y., Xiong, Y., Liang, Q., Fang, C., Wang, C. (2010) Application of headspace single-drop microextraction coupled with gas chromatography for the determination of short-chain fatty acids in RuO₄ oxidation products of asphaltenes. *Journal of Chromatography A*: 1217 3561-3566
- (18) van Pinxteren, D., Teich, M., Herrmann, H. (2012) Hollow fibre liquid-phase microextraction of functionalised carboxylic acids from atmospheric particles combined with capillary electrophoresis/mass spectrometric analysis. *Journal of Chromatography A*: 1267 178–188
- (19) Stromberg, N., Sahlin, E. (2012) Determination of the short-chain fatty acid pattern in biodiesel using high throughput syringe solvent extraction and ion exclusion chromatography. *Fuel*: 97 531–535
- (20) AOAC. (1995). *In Official methods of analysis Association of Official Agricultural Chemists*. Washington D.C: AOAC.
- (21) Folch, J., Lees, M., Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*:

226 497-509.

- (22) Martini, M., Altomonte, I., Salari, F., Ichihara, K., Fukubayashi, Y. (2010) Evaluation of the fatty acid profile from the core and membrane of fat globules in ewe's milk during lactation. *LWT - Food Science and Technology*: 50 (2013) 253-258
- (23) Chen, S., Chuang, Y. (2002) Analysis of fatty acids by column liquid chromatography, *Analytica Chimica Acta*: 465 145-155
- (24) Lima, E.S., Abdalla, D.S.P. (2002) High-performance liquid chromatography of fatty acids in biological samples. *Analytica Chimica Acta*: 465 81-91
- (25) Toyo'oka, T. (1995) Use of derivatization to improve the chromatographic properties and detection selectivity of physiologically important carboxylic acids. *Journal of Chromatography B*: 671 91-112
- (26) Galli, V., Garcia, A., Saavedra, L., Barbas, C. (2003) Capillary electrophoresis for short-chain organic acids and inorganic anions in different samples. *Electrophoresis*: 24 1951-1981
- (27) Christie W. (1993) *Advances in Lipid Methodology – Two*, Oily Press, Dundee 69-111
- (28) Atapattu, S., Rosenfeld, J (2013) Solid phase analytical derivatization as a sample preparation method. *Journal of Chromatography A*: 1218 (51) 9135-9141
- (29) Ken'ichi, I., Yumento, F. (2010) Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research*: 51 (3) 635-640
- (30) Toyo'oka, T. Fluorescent tagging of physiologically important carboxylic acids,

- including fatty acids, for their detection in liquid chromatography. *Analytica Chimica Acta*: 465 111–130
- (31) Nimura, N., Kinoshita T. (1998) 1-Pyrenyldiazomethane as a Fluorescent Labeling Reagent for Liquid Chromatographic Determination of Carboxylic Acids *Analytical Chemistry*: 60 2067-2070.
- (32) Schneede, J., Ueland, P.M. (1993) Automated Assay of Methylmalonic Acid in Serum and Urine by Derivatization with 1-Pyrenyldiazomethane, Liquid Chromatography, and Fluorescence Detection. *Clinical Chemistry*: 39 (3) 392-399
- (33) Pan, L., Adams, M., Pawliszyn, J. (1995). Determination of fatty acids using solid phase microextraction. *Analytical Chemistry*: 67 4396–4403.
- (34) Mills, G. A., Walker, V., Mughal, II. (1999) Headspace solid-phase microextraction with 1-pyrenyldiazomethane on-fibre derivatisation for analysis of faecal short-chain fatty acids. *Journal of Chromatography B*: 730 113–122.
- (35) Vladimir, R., David, K., Valerian, K. (2002) Derivatization of F2-isoprostanes with 1-pyrenyldiazomethane and their subsequent determination by fluorescence high-performance liquid chromatography. *Analytical Biochemistry*: 311 10-18
- (36) Sporkert, F., Pragst, F., Hübner, S., Mills, G. (2002) Headspace solid-phase microextraction with 1-pyrenyldiazomethane on-fibre derivatisation for analysis of fluoroacetic acid in biological samples. *Journal of Chromatography B*: 772 45-51
- (37) Li, Y., Lander, R., Manger, W., Lee, A. Determination of lipid profile in

meningococcal polysaccharide using reversed-phase liquid chromatography.

Journal of Chromatography B: 804 353-358

- (38) Kotera, M., Dhcu, M., Millet, A., Lhomme, J., Laayoun, A. (2005)

Pyrenyldiazomethane: a versatile reagent for nucleotide phosphate alkylation.

Bioorganic and Medicinal Chemistry Letters: 15 705-708

- (39) PDAM for HPLC derivatization (2013) *Sigma-Aldrich* Retrieved From:

<http://www.sigmaaldrich.com/catalog/product/fluka/p6863?lang=en®ion=US>

- (40) Bianchi, F., Dall'Asta, M., Dcl Rio, D., Mangia, A., Musci, M., Scazzino, F. (2011)

Development of a headspace solid-phase microextraction gas chromatography

mass spectrometric method for the determination of short-chain fatty acids from

intestinal fermentation. *Food Chemistry: 129* 200–205

- (41) Pan, L., Pawliszyn, J. (1997) Derivatization/Solid-Phase Microextraction: New

Approach to Polar Analytes, *Analytical Chemistry: 69* 196-205

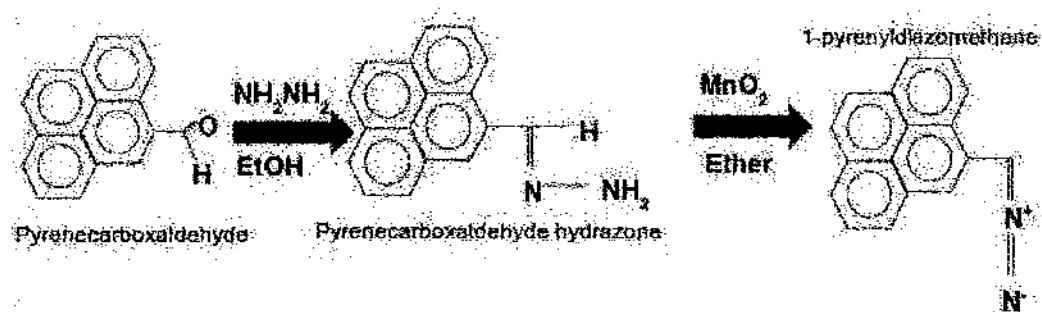
- (42) Fletcher, K., Hendricks, E., Pandey, S. (2002) Preferential Solvation within Binary

and Ternary Solutions Containing Room-Temperature Ionic Liquids. *AGI 13th*

International Symposium on Molten Salts: May 14 Abstract 1404.

V. Appendix: Practical Lab Procedure

Overview of synthesis reaction:



The diazo group reacts with carboxylic acids on the short-chain fatty acids in samples. Removal of this polar group from the PDAM makes the compound insoluble, moving it into the headspace where it can be readily collected by gas-tight syringe.

Supplies

Reagents	Glassware	Other Equipment
0.5 g Pyrenecarboxaldehyde	Small vial (~10 mL capacity)	Stir/Heat Plate
0.34 g Hydrazine Monohydrate	200 mL beaker	Small Stir Bar
0.65 g Activated Manganese Dioxide	Vacuum Filtration Apparatus	Glass Filter
About 15 mL Absolute Ethanol	Buchner Funnel	Paper Filter
5. Anhydrous Ether	Watch Glass Funnel 200 mL flask	Sonicator

Detailed Procedure:

- To small vial add:
 - 0.5 g pyrenecarboxaldehyde

- 0.34 g hydrazine monohydrate
 - 8 mL absolute ethanol
 - small stir bar
2. Stir at room temperature for ~3 hours
 3. Separate via suction filtration, wash with ethanol
 4. Allow intermediate compound (pyrenecarboxaldehyde hydrazone) to dry on watch glass
 5. To beaker add:
 - 0.2 g pyrenecarboxaldehyde hydrazone
 - 0.65 g activated manganese dioxide
 - 50 mL anhydrous diethyl ether
 6. In sonicator, mix ice and water into slurry-like mixture
 7. Place beaker into sonicator, cover top with a watch glass or other vented cover
 8. Sonicate solution for ~90 minutes, checking frequently that the ether has not evaporated completely. Add more ether if necessary
 9. Separate manganese dioxide from product by gravity filtration. PDAM will be suspended in the ether solution
 10. Put the beaker in a dark place and allow the ether to dry
 11. Collect PDAM crystals from the beaker. Place in a small vial, wrap in foil and freeze until use