Purification of Organic Compounds: from Crude Product to Purity

Raania Ajmal Rana

19700921

Bachelor of Science

in

Chemistry

Eastern Mediterranean University

July 2023

Gazimağusa, North Cyprus

Approval of the Department of Chemistry

I certify that this report satisfies all the requirements as a CHEM401 Graduation Project report for the degree of Bachelor of Science in Chemistry.

Digitally signed by Prof. Dr. İzzet Sakallı
DN: CN=Prof. Dr. İzzet Sakallı, C=CY, OU=Physics, O=Eastern Mediterranean University, E=izzet.sakalli@emu.edu.tr
Reason: I am the author of this document
Location: NORTHERN CYPRUS
Date: 2023-10-03 17:01:28

Prof. Dr. İzzet Sakallı

Chair, Department of Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a CHEM401 Graduation Project report for the degree of Bachelor of Science in Chemistry.

Digitally signed by Huriye ICIL
DN: cn=Huriye ICIL, o=Eastern Mediterranean University, ou=Chemistry, email=Huriye.icil@emu.edu.tr, c=CY
Date: 2023.10.03 17:53:01 +03'00'

Prof. Dr. Huriye İcil

Supervisor

Digitally signed by Huriye ICIL
DN: cn=Huriye ICIL, o=Eastern Mediterranean University, ou=Chemistry, email=Huriye.icil@emu.edu.tr, c=CY
Date: 2023.10.03 17:53:34 +03'00'

Prof. Dr. Huriye İcil

CHEM401 Graduation Project Coordinator

ABSTRACT

Purification of organic compounds is crucial to downstream application. Regardless of its source, an organic compound as rarely obtained pure. For example, a compound synthesized in lab contains impurities such as catalyst, unreacted reagents, solvents and by-products. For further use of the compound of interest in research or other purposes purity of the compound is essential. There are various techniques for purifying the compound of interest depending on the physical state of the compound as well the type of impurities present. Some of the most common techniques include crystallization, distillation, sublimation and chromatography. These will be described in detail below. In this experiment, N, N-bis(7H-purinyl)-1,4,5,8-naphthalene diimide, a naphthalene diimide derivative which had been previously synthesized was analyzed and purified. The compound was purified using recrystallization. The purity of the starting materials as well as the final product (after purification) was determined using IR spectroscopy and TLC.

Keywords: Naphthalene diimide derivative, recrystallization, IR Spectroscopy, TLC.

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LIST OF SYMBOLS AND ABBREVIATIONS

1	1,4,5,8-Naphthalenetetracarboxylic dianhydride
2	7H-purine-6-amine
3	N,N-bis(7H-purinyl)-1,4,5,8-naphthalene diimide
Ar	Aromatic
DNA	Deoxyribonucleic acid
HPLC	High Pressure Liquid Chromatography
IR	Infrared
mL	Milliliter
R_f	Retention factor
Str	Stretch
TLC	Thin Layer Chromatography
UV	Ultraviolent

INTRODUCTION

Importance of Purification in Organic Chemistry

As a general rule, any compound synthesized in the lab is initially impure. Purification of synthetic organic product is essential for downstream applications. Unreacted starting material, solvents and by-products all contribute to impurities. In order to make the desired product useful, removal of these impurities is imperative.

Purification of a compound increases accuracy of analytical techniques used to study the compound, so that valid data can be obtained for further research. Organic compounds to be used in organic syntheses require precise concentrations of the reactants. Presence of impurities can disrupt these levels and disturb the reaction system. Therefore, purification increases reliability and reproducibility of experiments by preventing inconsistencies in data resulting from such contaminants [1].

When it comes to biological applications of the compound, for instance in drug discovery the impurity may be involved in unexpected outcomes in the body or may cause toxicity. For example, certain residual impurities in synthesis of drugs are classified to be genotoxic, i.e. causing an insult to DNA. Such substances have the potential to induce carcinogenesis, along with other health hazards. Therefore, its removal from the final product is necessary, even if they are present in trace amounts [2].

Some common purification and separation techniques in organic chemistry include the following;

- Crystallization/recrystallization
- Chromatography
- Soxhlet Extraction
- Filtration
- Sublimation
- Distillation

Crystallization/recrystallization

Crystallization is a technique for purification of solid crude product. The selected solvent is heated close to its boiling point and the solid sample is completely dissolved in the solvent at this temperature. The solubility of solid sample must be considered in this process. The solvent must be chosen such that the sample has poor solubility at room temperature but is completely soluble in the solvent near its boiling point. Idea solvent selection may require multiple trials. Solubility data may be useful in solvent selection. Minimum amount of solvent should be used because the resulting solution must be saturated. Once the sample has been fully dissolved, it is filtered using vacuum filtration while still hot so that any insoluble impurities can be removed. The solution is then allowed to cool slowly to form pure crystals. Slow cooling is important as this will allow pure solid to crystallize, leaving the impurities behind. Cooling may be done at room temperature or a refrigerator. Crystal formation can take from a few hours up to days. Once crystals have deposited, the solution can be filtered using vacuum filtration or alternatively, by centrifugation. The process can be repeated again to get purer crystals (recrystallization).

Chromatography

Chromatography is a separation technique which can be used to monitor reaction progress and determine purity of a compound. It consists of a mobile phase (liquid or gas) and a stationary phase (solid or liquid) and separation of components occurs based on their affinity for mobile phase as well as their polarity. Some types of chromatography include TLC, column chromatography, paper chromatography and HPLC.

Thin Layer Chromatography

TLC consists of a solid stationary phase which is a thin layer of adsorbent, usually made of silica, alumina or cellulose. Mobile phase is made of a liquid eluent which allows for separation components as it travels up the TLC paper via capillary action. Components with high polarity have lower affinity for mobile phase and therefore travel shorter distances on the TLC plate. These spots are therefore found more towards the bottom of the plate. On the other hand, components with low polarity are found closer towards the top of the plate as they have a greater affinity towards mobile phase. Since all components of the sample may not be colorful, visualization of

components can be aided by certain chemicals e.g. vanillin bath or by the use of fluorescence, radiation and UV light etc.

Column Chromatography

Column chromatography can be used to separate both solid and liquid samples. It consists of a solid stationary phase which, like in TLC can be made of alumina, silica or cellulose etc. Adsorbent and the eluent are present in the column and sample is applied at the top of the column. Separation of components occurs according to their polarity. Components which have a greater ability to be adsorbed will be found at the upper portion of the column and compounds with lower adsorption ability will be found towards the bottom [4], [5].

Paper Chromatography

Paper chromatography is a form of liquid-liquid extraction where the stationary phase is made of an adsorbent paper and mobile phase is made of a selected solvent (or a mixture of solvents). The selected solvent may be polar or non-polar in nature. Components are separated based on their differential solubility in stationary and mobile phase. R_f value (distance covered by component/distance covered by solvent) may be used to identify the components [6].

HPLC

HPLC consists of solid stationary phase made of silica or polymer granules and the mobile phase is the solvent pumped at a pressure between 10 atm to 400 atm. The granular nature of stationary phase increases surface area of contact with sample components. The high pressure increases ability of components to separate. HPLC is much more efficient compared with the techniques describes above, however it is also more expensive as it consists of more components such as pressure pump [4].

Soxhlet Extraction

Soxhlet extraction is solid-liquid extraction technique. It was invented by a German chemist, Franz Ritter von Soxhlet in 1879. It works by continuous circulation of a solvent across the solid sample in the extractor. The extractor is set up by adjusting the sample in multiple layers of filter paper or a thimble, and placing it on a round bottom flask containing a solvent. Heating the solvent vaporizes it. The vapors travel up the extractor, condense and come into contact with the solid sample. The condensed solvent containing dissolved components continuously circulate through the extractor, collecting the soluble components in the round bottom flask over time. The cycle can be repeated multiple times. The longer the extractor is allowed to run, the more concentrated the solution in the round bottom flask becomes. Soxhlet extraction is a relatively inexpensive technique as it requires limited amount of solvent and does not require further addition of solvent. The extractor can also be left to run for hours or days and does not require continuous supervision [7].

Filtration

Filtration is a common purification/separation technique. It is often employed as part of other methods such as crystallization. It is used for solid-liquid extractions where the solid is separated from liquid by its passage through a porous region, such as a filter paper. The filter may also be a cloth, asbestos or sand particles. The pores are small in size in order to allow only the solvent to pass through, collecting the insoluble components in the filter medium. The solvent which is left behind is referred to as filtrate. The separation is based on the size difference in soluble and insoluble components in a solution. Some types of filtration include vacuum filtration/suction filtration, centrifugal filtration, hot filtration and gravity filtration [8].

Sublimation

Sublimation refers to the phenomenon where a solid substance directly transitions into the gaseous state without passing through the liquid state. This principle can be utilized for purification; sublimely volatile components in a mixture can be separated by deposition on a cold surface as solid form. This will leave the non-volatile components behind. Sublimation for a certain compound occurs at a specific range of temperature and pressures, below the triple point of the compound [9].

Distillation

Distillation is a purification technique based on the differences in boiling points of different compounds present in a sample. A component is allowed to boil and then condense, after which it can be collected leaving behind the remaining components. When the boiling points of the components to be separated lie far apart, simple distillation can be used. However, if the boiling points of the components are very close fractional distillation is used. Fractional distillation is more sophisticated and makes use of a fractionating column for separation. Other types of distillation include steam distillation, which can also be sued for solid samples and vacuum distillation [10], [11].

Spectroscopic Techniques

Spectroscopy involves identification of atoms and molecules present within a sample. It measures the interaction of compounds with electromagnetic radiation at the molecular level. The atoms/molecules in a sample absorbs or emits light of certain wavelength, which is recorded as a spectrum. Spectroscopy can be used to analyze both molecular and structural characteristics of the sample. Two types of spectroscopy used in this project IR and emission spectroscopy.

IR Spectroscopy

IR spectroscopy helps determine and identify bonds and functional groups present in a sample. A particular bond absorbs a certain wavelength of IR waves, causing the covalently bonded atoms to vibrate. The bonds may stretch, bend or rotate as they are excited from ground state to vibrational energy state. The wavelength absorbed by a particular type of bond/functional group gives a distinct spectrum for a compound. Two compounds can not have the same IR spectrum. Therefore, IR spectroscopy is a valuable tool in distinguishing a compound from another. It may be used to monitor reaction progress or determine sample purity. IR spectrometer requires a radiation source, monochromator which disperses the radiation into a broad spectrum and a detector [12].

Emission Spectroscopy

Emission spectroscopy is based on excitation of atoms or molecules using electron impact, photon absorption or heat. The atoms/molecules then emit certain frequency of light waves upon returning

to their ground- state. This principle can be explained using the Jablonski diagram, as shown below.

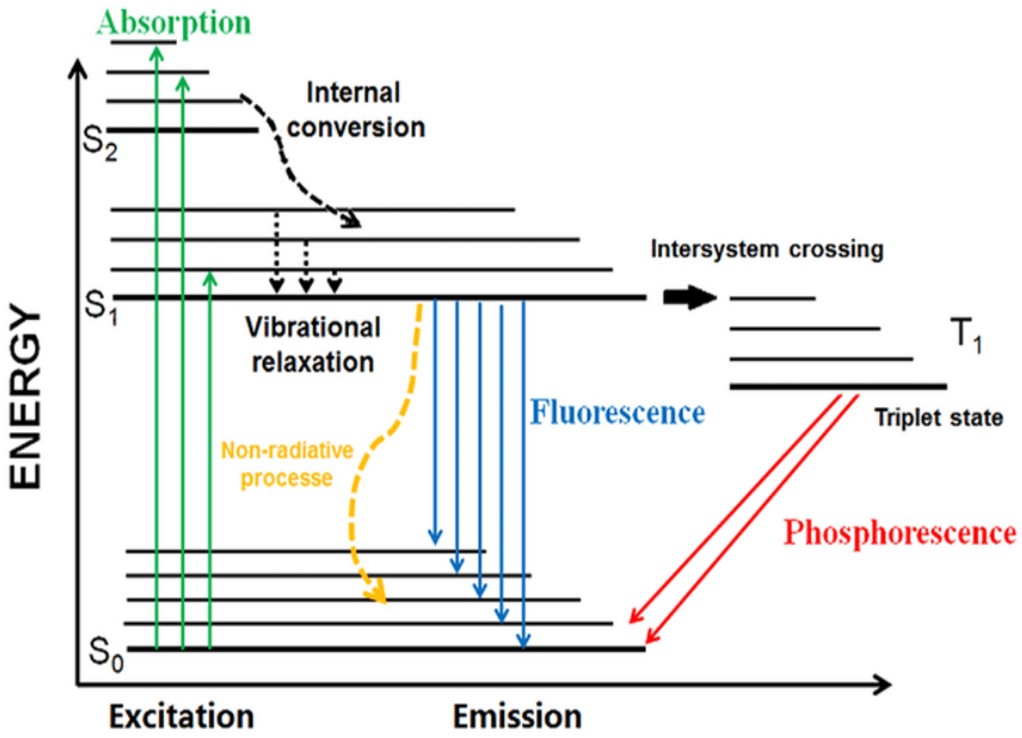

Fig 1: Energy level diagram for electron transitions during emission spectroscopy.

The diagram shows different types of electronic transitions which can occur upon excitation of molecules. Electron transition from ground state to higher energy state occurs when a molecule absorbs sufficient energy for the transition. The molecule may undergo internal conversion, where energy transfer occurs within the same energy level so no energy is emitted during this process. However, if the molecule returns to a lower energy level, it emits fluorescence with a wavelength longer than the absorbed wavelength. Intersystem crossing may occur in which the electron transitions from higher energy level to lower energy level with a different spin state. This gives access to the triplet state, where the promoted (excited) electron has a spin orientation parallel to the electron at the lower energy level. When electron transitions back to the low energy state from triplet state, it releases energy in the form of phosphorescence [13].

Synthesis Scheme for N, N-bis(7H-purinyl)-1,4,5,8-naphthalene diimide (3)

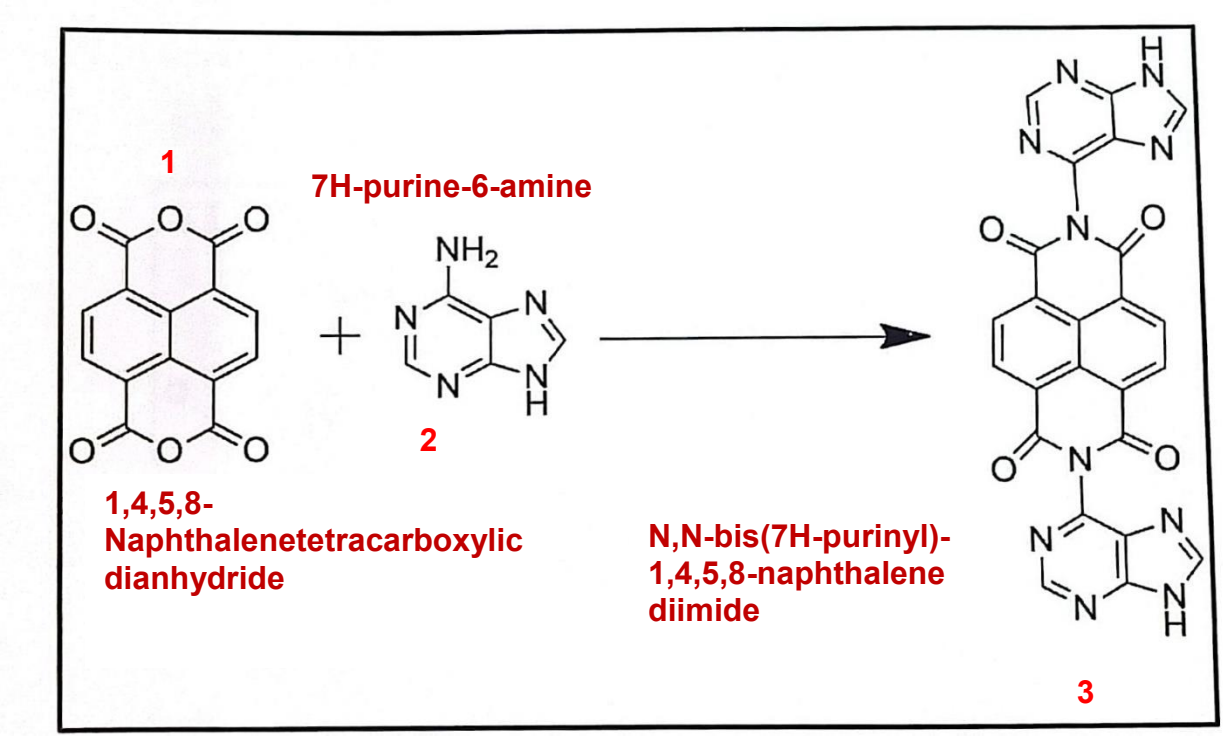

Fig 2: Synthesis scheme for the naphthalene diimide derivative (3).

The compound that was purified in this project had been already synthesized. The synthesis requires 1,4,5,8-Naphthalenetetracarboxylic dianhydride (NTCDA) (1) and 7H-purine-6-amine (adenine) (2) as starting materials. NTCDA has the molecular formula $C_{14}H_4O_6$. It is an aromatic compound. Two carboxylic anhydride groups are attached with the naphthalene core. The electron deficient carbon (C=O) gives the molecule electron accepting ability. The compound appears light brown and melts at temperatures above 300 degrees Celsius.

Adenine has the molecular formula $C_5H_5N_5$. It is a small molecule consisting of two aromatic rings and primary and secondary amine functional groups. It contributes to the two purinyl groups present in the final product (3). It appears yellowish white and has a melting point of 360 degree Celsius. Adenine is one of the nitrogenous bases present in DNA and RNA and also serves as part of the adenosine triphosphate (ATP) molecule, the energy carrying molecule in living things [15].

The final product, N, N-bis(7H-purinyl)-1,4,5,8-naphthalene diimide has the molecular formula

$C_{14}H_{10}N_2O_6$. The naphthalene core is attached with the purinyl groups via iimide groups. The compound appears greenish black [16].

Mechanism of the Reaction

The reaction occurs in four steps. Adenine acts as a nucleophile and the solvent, isoquinoline catalyzes the reaction.

Step 1: Adenine acts as a nucleophile in the reaction. The nitrogen atom of the primary amine, having a lone pair of electrons makes a nucleophilic attack on the electropositive carbonyl carbon (C=O) of the NTCDA. This breaks the C-O bond of the carboxylic anhydride, forming positively charged amide groups and negatively charged carboxylate groups.

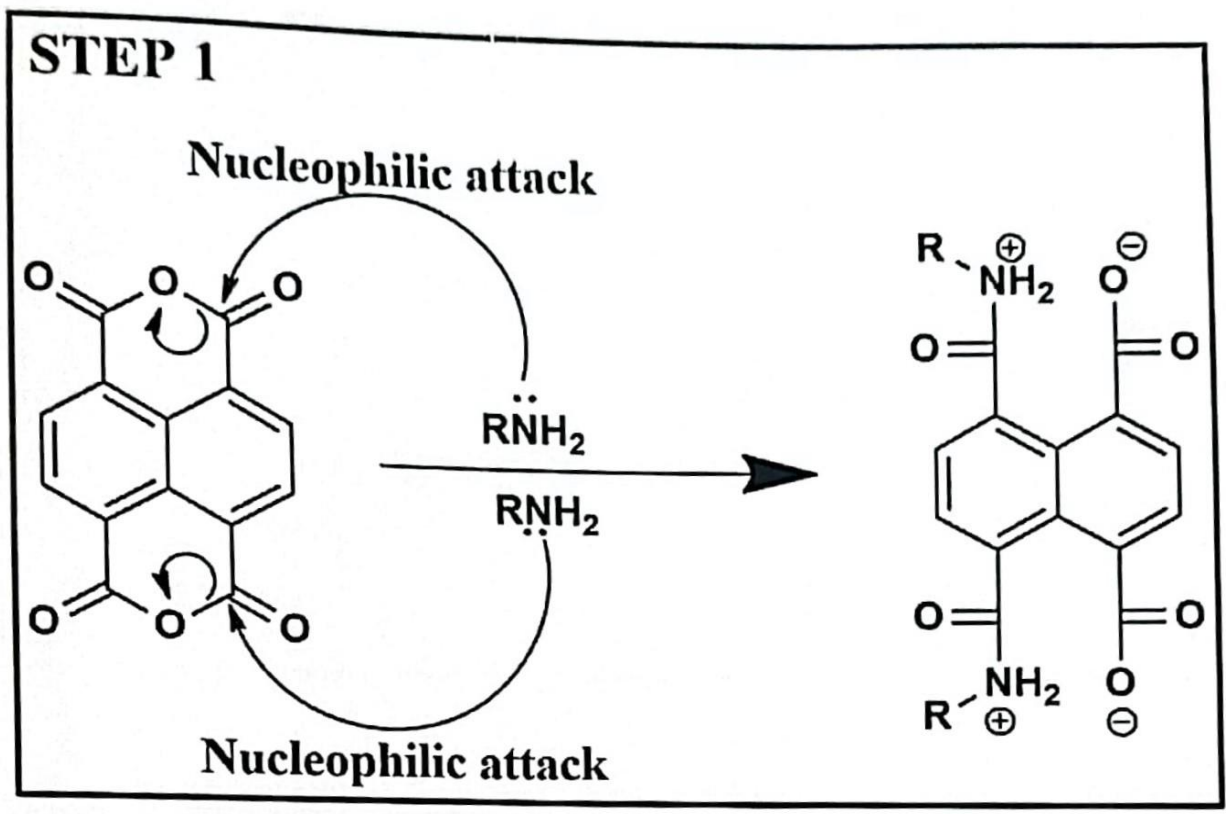

Fig 3: Step 1 for the synthesis of compound 3.

Step 2: In this step the charge is neutralized at the negatively charged oxygen of carboxylate and positively charged nitrogen at the primary amine. The oxygen makes a proton abstract at a hydrogen at the primary amine. By accepting a proton, carboxylic acid (-COOH) is obtained.

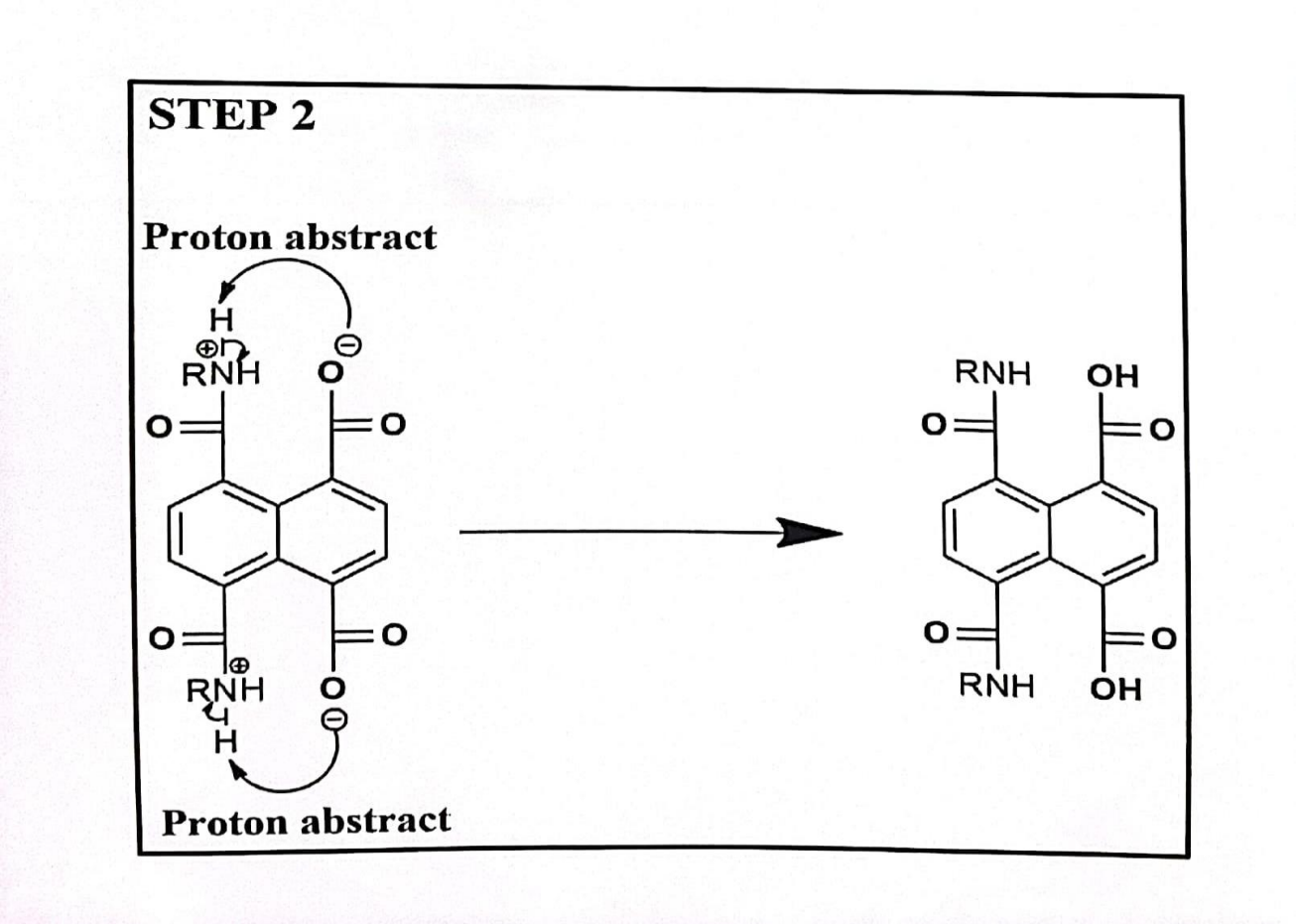

Fig 4: Step 2 for the synthesis of compound 3.

Step 3: The product (3) is formed in this step. Isoquinoline, having a nitrogen atom with a lone pair of electrons makes a proton abstract at the hydrogen of the amide. The catalyst gets a temporary positive charge as nitrogen forms a bond with hydrogen. This allows the nitrogen of the amide to attack the carbonyl carbon of the carboxylic acid group. As a result, bond formation occurs between the nitrogen and carbon. 2 hydroxide molecules are given out.

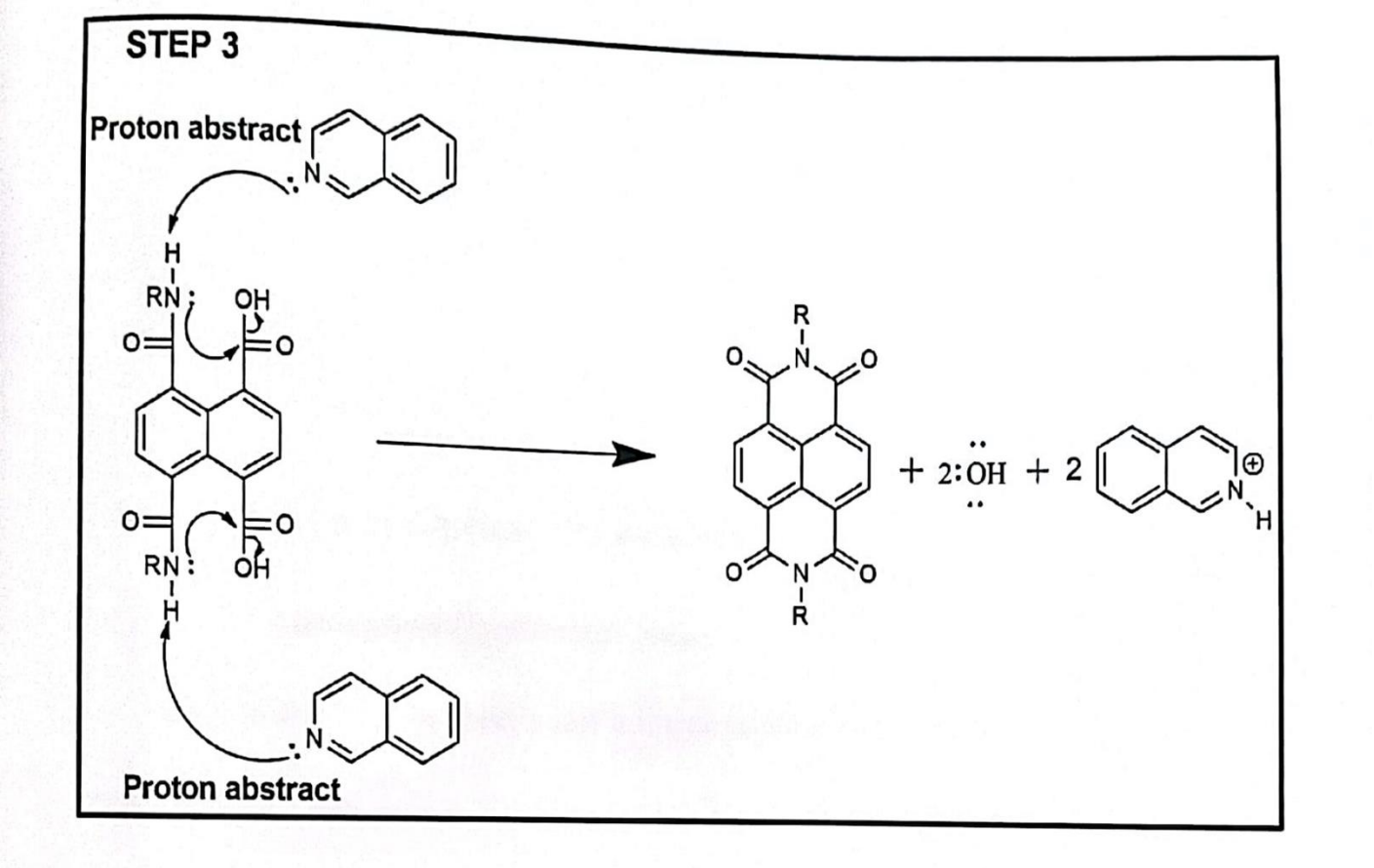

Fig 5: Step 3 for the synthesis of compound 3.

Step 4: Negatively charged hydroxide abstracts proton from the catalyst. This returns the catalyst back to its original form and water is given off as by product.

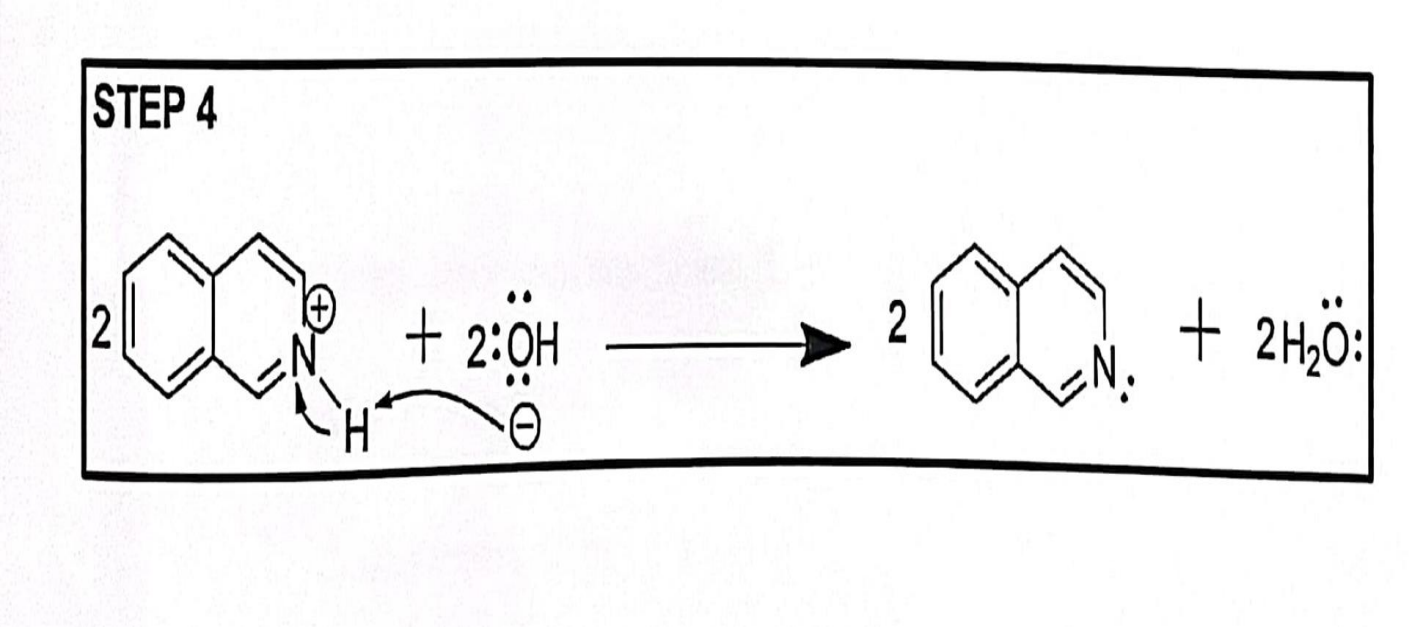

Fig 6: Step 4 for the synthesis of compound 3.

EXPERIMENTAL

Procedure

The experimental procedure involved analysis of the starting materials (compound 1 and 2) as well as the crude (impure) product, 3. This was followed by purification of the crude product by recrystallization, and its analysis and measurement so that purity could be determined. This was done by comparing the results with the starting material. Firstly, IR spectroscopy of the reactants and the crude product was done (separately). This was followed by TLC of the crude product. The crude product was then purified by recrystallization, and Soxhlet extraction (H_2O) was done to further enhance purity. Once the product (3) was purified, its IR spectroscopy and TLC were done. These results (IR spectroscopy and TLC) were then compared with the starting materials. Finally, the purified product (3) was then further analyzed by emission spectroscopy.

IR Spectroscopy

Potassium bromide (KBr) was used as the carrier in sample preparation as it is optically transparent (does not show peaks in IR spectrum). Mortar and pestle were cleaned with chloroform. A small amount of sample and KBr (about 1:100 sample:KBr ratio) were added using a spatula and crushed to fine powder. A dye was not used in this experiment and all samples had a color distinguishable from KBr (1-light brown, 2-pale yellow and 3-greenish black). Once a homogenous mixture was obtained, it was transferred to a sample bottle and dried using vacuum oven for 30 minutes to dehydrate the sample. The sample powder was used to create a pellet with a pellet machine. The IR spectrum was then recorded.

Recrystallization

A suitable solvent for recrystallization was chosen; solubility among four different solvents, N-Methylpyrrolidone (NMP), dimethylacetamide (DMAc), dimethylformamide (DMF) and dimethylsulfoxide (DMSO) was tested. These solvents were chosen considering the polarity of the compound (3), which is expected to be soluble in the above-mentioned polar solvents. DMSO was chosen as the appropriate solvent as it was observed that compound 3 was poorly soluble in DMSO

at room temperature but showed complete solubility around the boiling point of DMSO (about 189 degree Celsius). DMSO was heated up to its boiling point and compound 3 was slowly added. Once all the compound had dissolved, the solution was filtered using vacuum filtration to remove soluble impurities. Following this, the filtrate was cooled to room temperature and then refrigerated to allow crystal formation. On the second day after refrigeration, crystal formation was not observed therefore the inside of the Erlenmeyer flask was scratched with a glass rod. This successfully induced crystal formation, the solution was kept back into the refrigerator for one more day. On the third day, the crystals had deposited and the solution was filtered using vacuum filtration. Once filtered, the crystals were dried in order to remove any traces of DMSO.

Vacuum filtration was used instead of gravity filtration as it is more efficient for recrystallization. Unlike gravity filtration, a pressure gradient drives the liquid through the filter paper which is much faster. Recrystallized solution requires slow filtration as it allows crystals to be collected at the center of the filter paper rather than being dispersed throughout the filter paper. This makes collecting the crystals from the filter paper easier. Moreover, vacuum filtration gives a purer filtrate and prevents clogging of filter paper which slows down filtration.

Soxhlet Extraction

The round bottom flask was filled with distilled water and the Soxhlet extractor was assembled. After compound 3 was purified and dried, it was covered in 3 layers of filter papers folded upon each other. The sample was then positioned in the main chamber of the apparatus. The round bottom flask was placed on a heating mantle and the extractor was adjusted onto the round bottom flask. The reflux condenser was attached and water supply was turned on so cold water could enter from the bottom and exit at the top of the extractor. The extractor was allowed to run overnight for removal of water-soluble impurities, e.g. DMSO traces, unreacted adenine etc.

TLC

Compounds 1,2, impure 3 and purified 3 were added to 4 separate vials and mixed with acetone. The vials were labeled with the respective samples. Trials were conducted to choose the best eluent. A 10:1 chloroform: methanol mixture was first tested. This eluent showed little separation of spots. A second trial was conducted using 1:1 chloroform:methanol mixture. This mixture also gave. little resolution of spots. Other trials were conducted using only methanol and only

chloroform as eluents. Methanol gave the best separation of spots; therefore, it was chosen as the eluent for TLC. 10mL methanol was added to the TLC chamber. Silica Gel 60 F_{254} TLC paper was used. Lines were drawn gently with a pencil and ruler, 1cm from the edge at the top and bottom of the paper. Four different spots were marked and labeled (NTCDA, adenine, impure compound 3 and purified compound 3). Capillary spotters were used to spot the samples onto the TLC paper. The TLC paper was then placed in the chamber using forceps and allowed to develop. Once the plate was developed, it was removed using forceps and allowed to dry. The plate was visualized using UV lamp and vanillin bath.

Emission Spectroscopy

360nm excitation wavelength was chosen and samples were prepared using NMP, DMAc, DMF and DMSO with a small amount in purified compound 3 in separate vials. Cuvettes were left in hydrochloric acid solution overnight for cleaning. The next day they were washed with acetone, filled with samples and emission spectrum for each sample was recorded.

RESULTS AND DISCUSSION

IR Spectroscopy

The IR spectrum of NTCDA (1) is shown below.

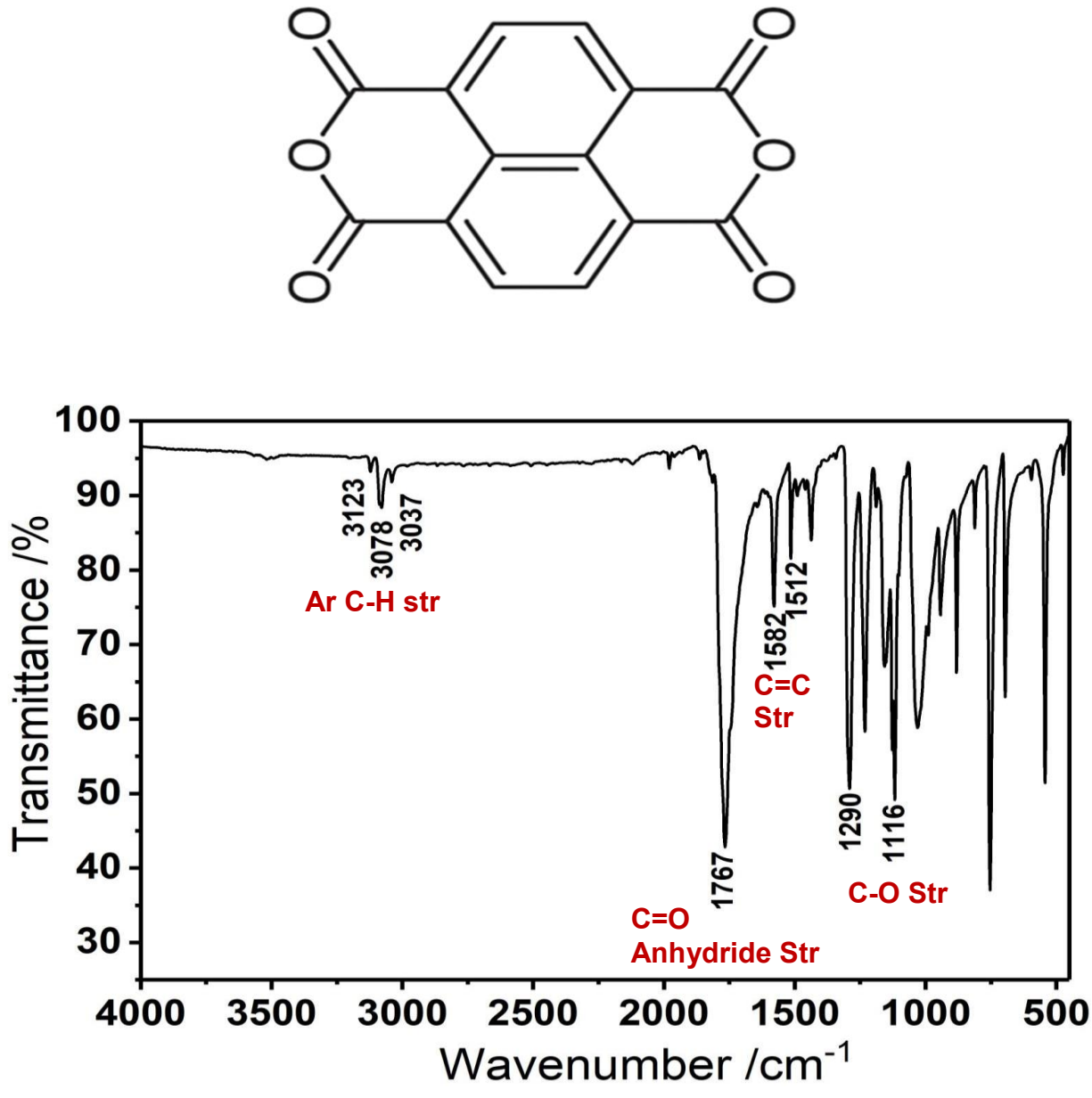

Fig 7: IR spectrum of compound 1.

The IR spectrum of adenine (2) is shown below.

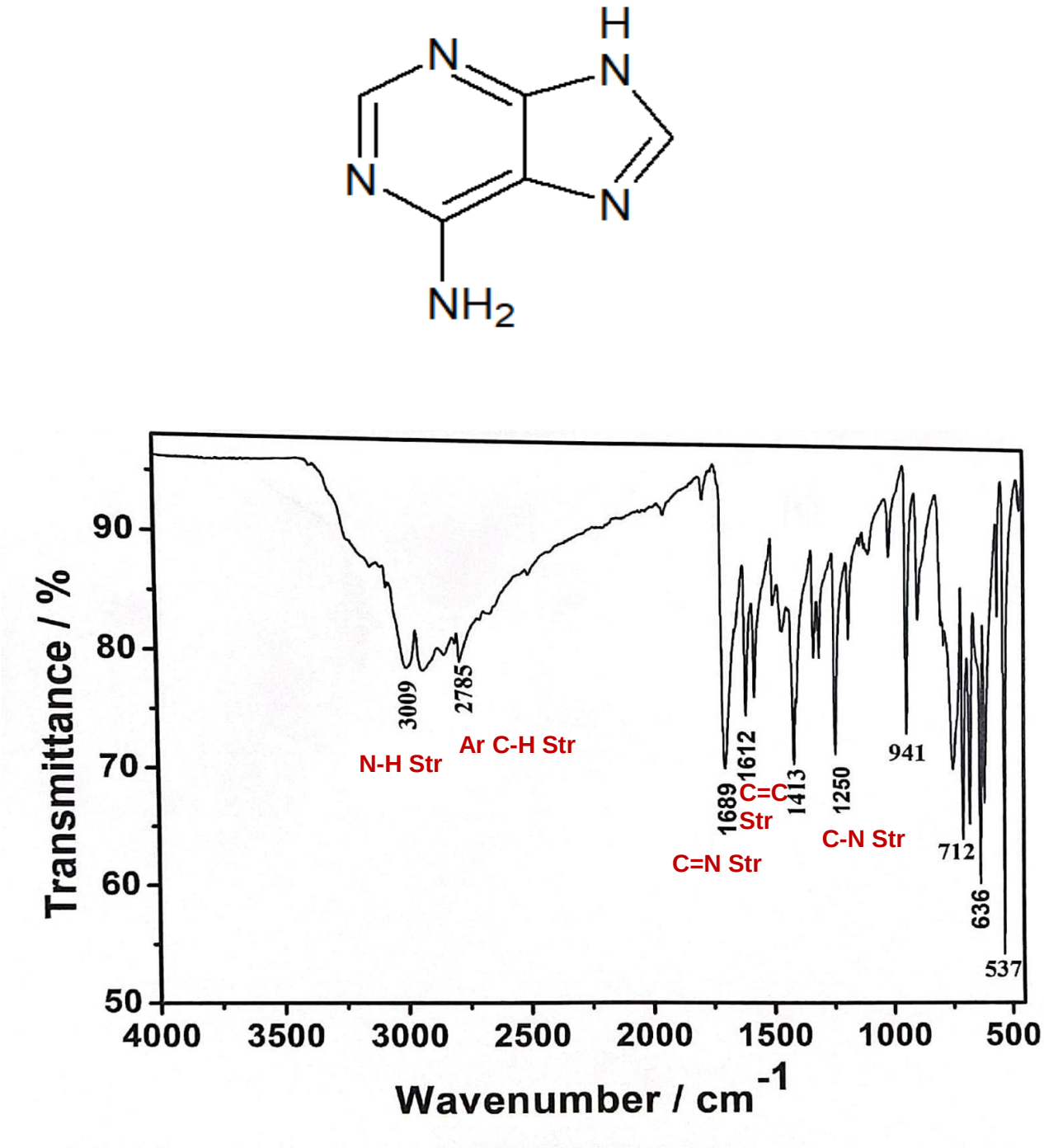

Fig 8: IR spectrum of compound 2.

The IR spectrum of the impure compound (3) (i.e before recrystallization) is shown below.

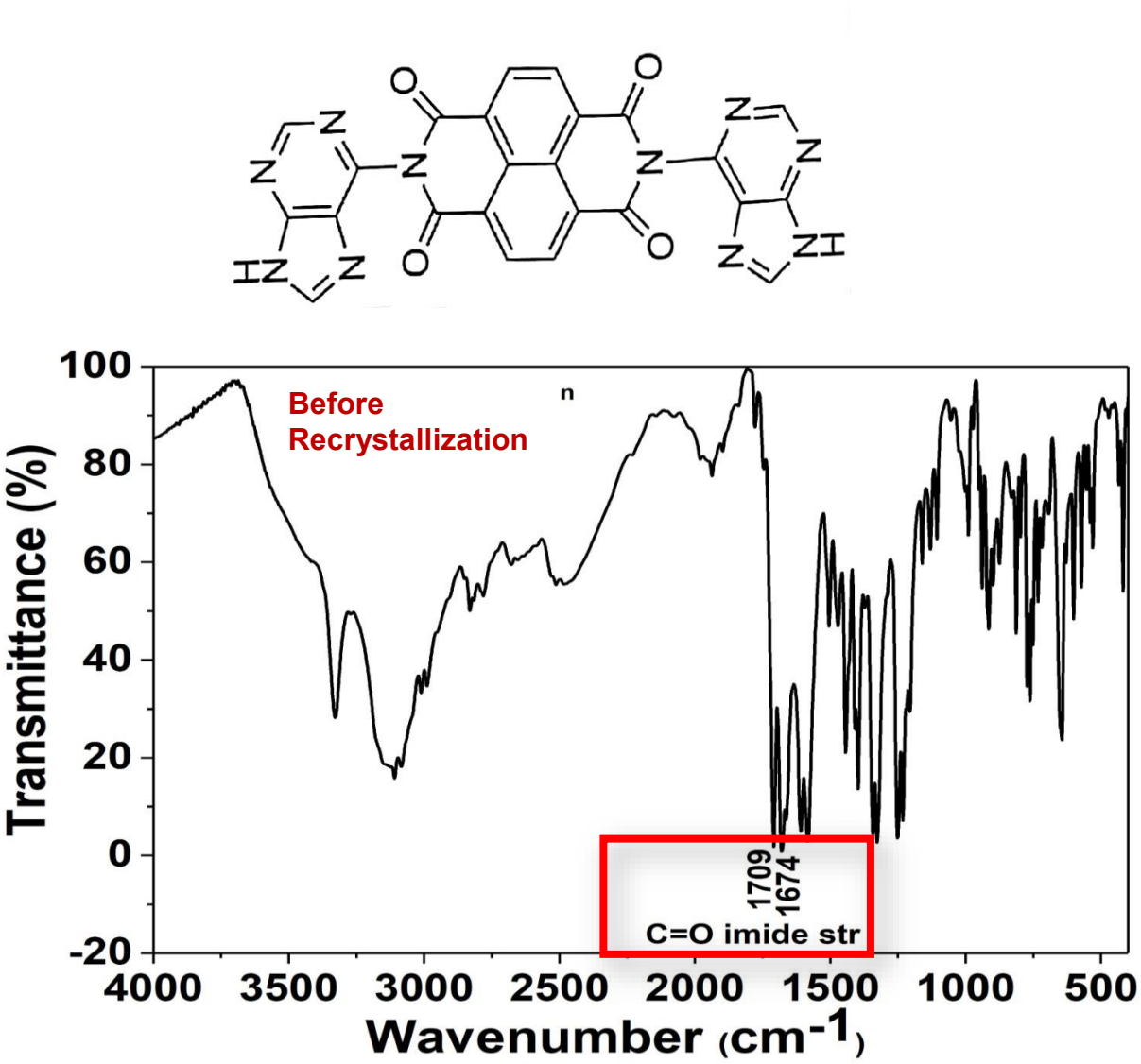

Fig 9: IR Spectrum of compound 3 before purification.

The IR spectrum of purified compound 3 (i.e. recrystallized) is shown below.

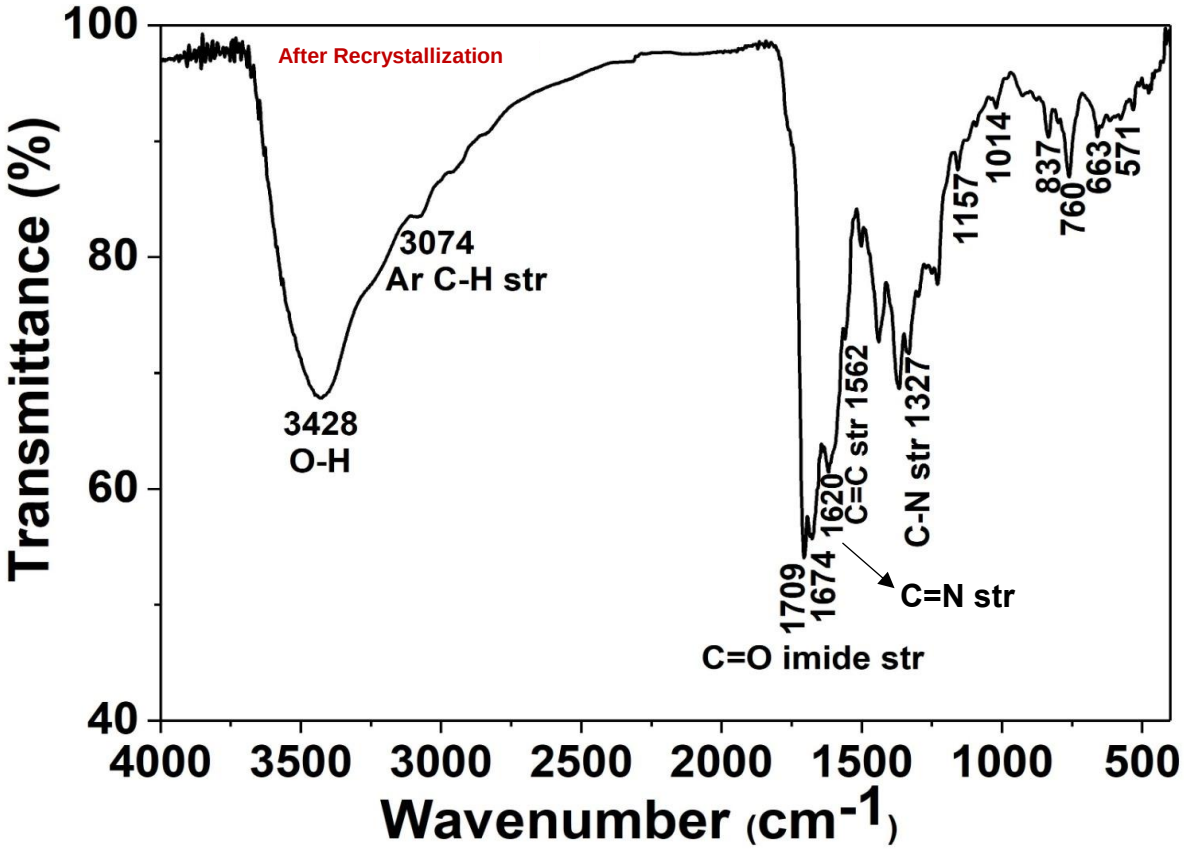

Fig 10: IR Spectrum of compound 3 after purification.

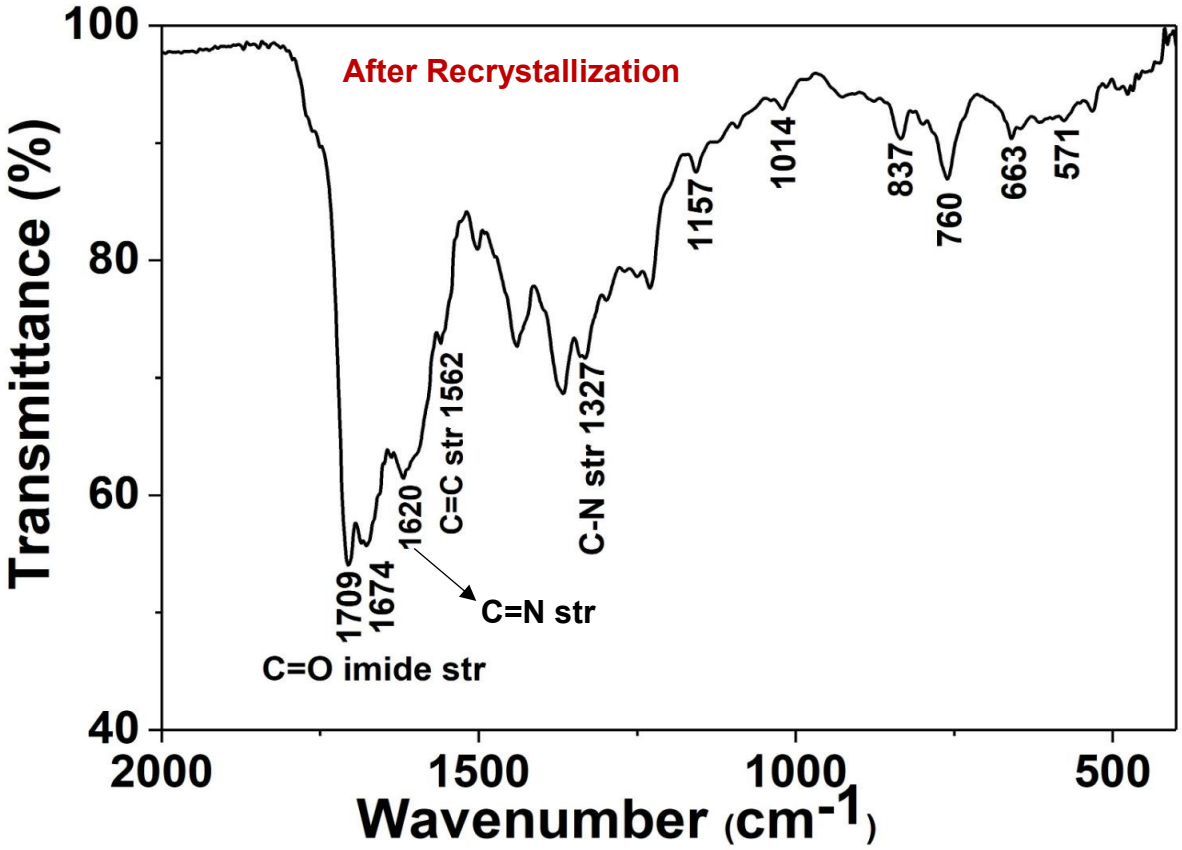

Fig 11: IR spectrum of compound 3 after purification before 2000/cm.

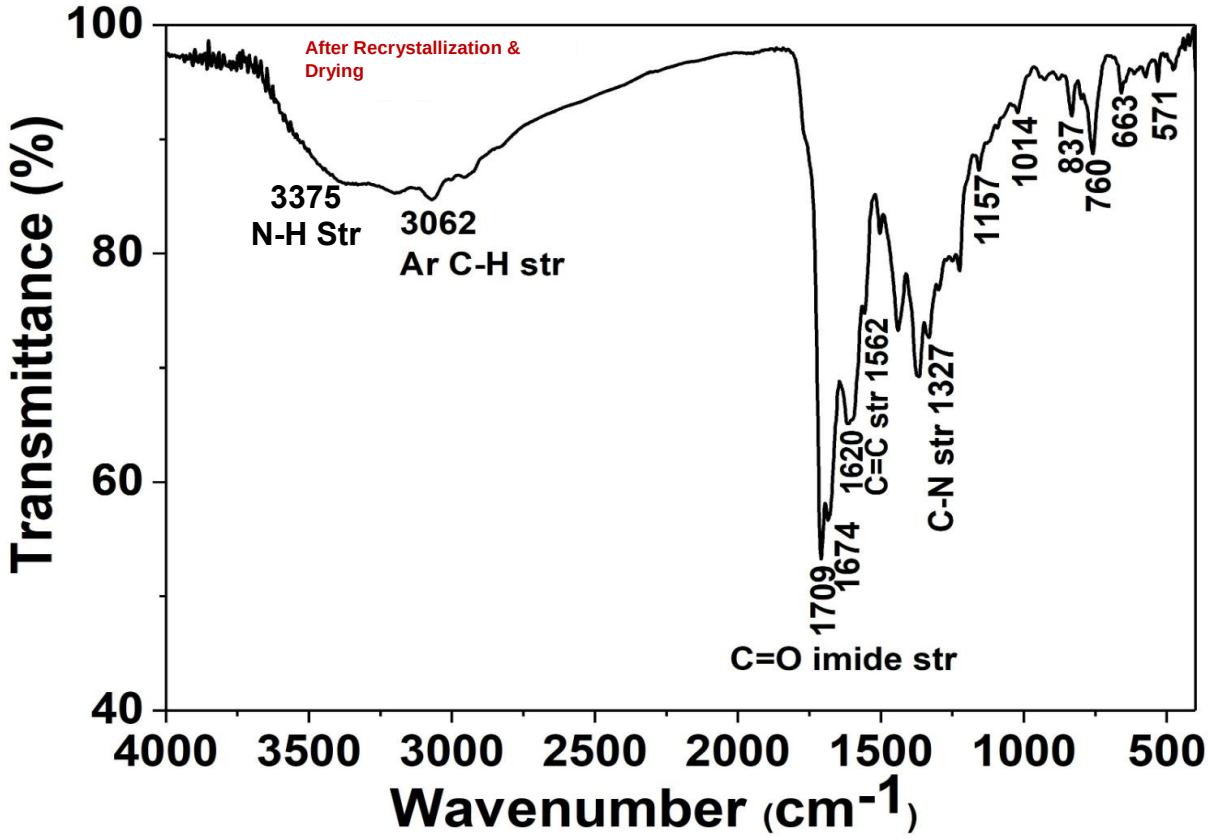

Fig 12: IR spectrum of compound 3 after purification and dehydration.

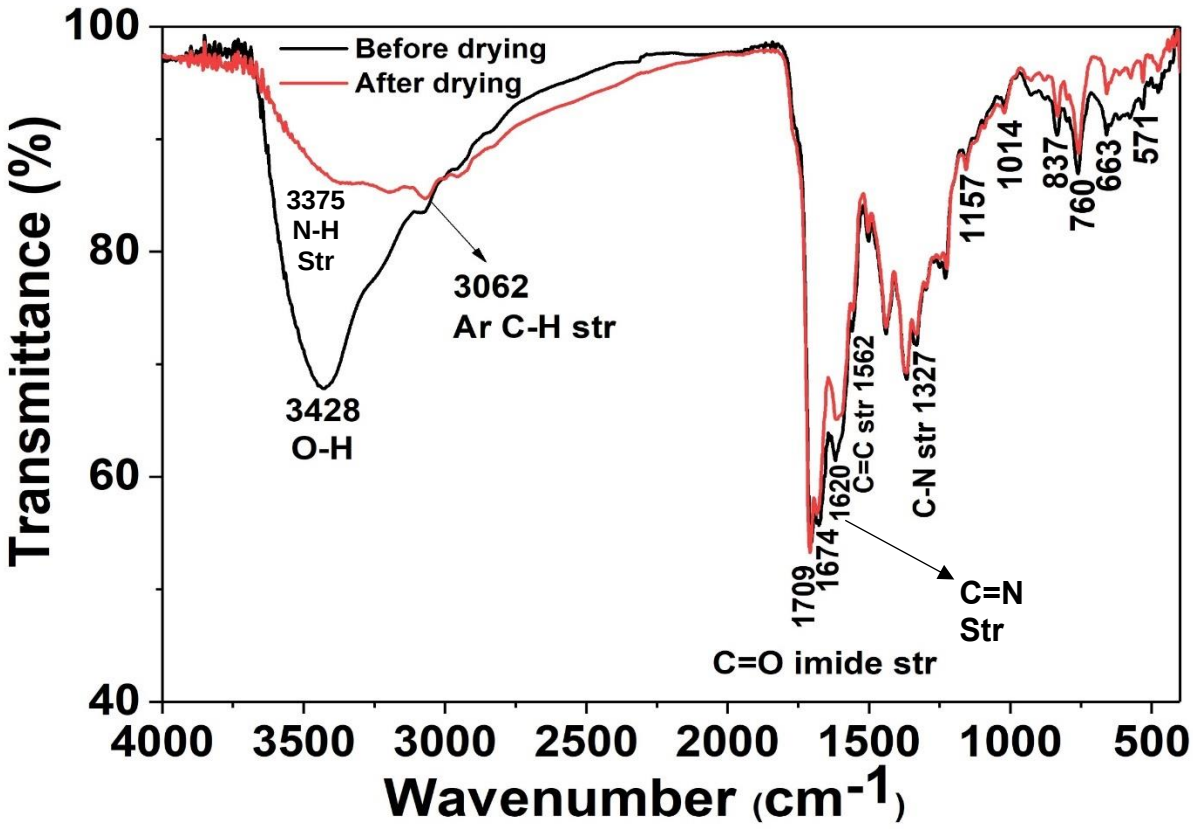

Fig 13: Comparison of IR spectrum of purified compound 3 before and after dehydration.

The IR spectrum of NTCDA (1) shows stretches of C-H belonging to the aromatic region (naphthalene core) in 3100-3000/cm region having weak peaks. Aromatic C=C stretches are observed at 1582/cm and 1512/cm giving weak peaks. Anhydride stretch of C=O gives a sharp peak at 1767/cm while C-O present in the anhydride gives a peak at 1160/cm.

The IR spectrum of adenine (2) shows a broad peak in 3500-2000/cm region. Weak peak at 3009/cm belongs to the N-H stretch while the peak at 2785/cm belongs to aromatic C-H bonds. The peak is broader, resulting in aromatic C-H stretch appearing at smaller wavenumber than normally expected (in 3100-3000/cm region) due to hydrogen bonding between nitrogen and hydrogen present in adenine. C=N shows peak at 1689/cm while C-N shows peak at 1250/cm. C=C bonds present in the aromatic region shows its peak at 1612/cm.

IR spectrum of the impure compound (3) appears ambiguous, especially before 2000/cm which are difficult to identify. Therefore, these peaks were left unlabeled. This is because the spectrum also shows peaks corresponding to the impurities. Impurities may be coming from the catalyst,

unreacted reagents and by-product (water). It is important to note here that the spectrum does distinguish the product from the reactants due to the presence of the carbonyl peaks at 1709/cm and 1674/cm instead of 1796/cm as it was observed in the spectrum of compound 1. It shows that these peaks belong the amide functional group rather than anhydride functional group. This shows that IR spectroscopy can be used both to determine purity as well as monitor reaction progress and ensure product formation by comparing starting material with end product.

IR spectrum of compound 3 after recrystallization appears much clearer, and the peaks are identifiable. Aromatic C-H peak is seen at its usual region. A broad peak at 3428/cm appears due to O-H from the presence of moisture in the purified product, and not because of any other impurity. This is proven in the spectrum that will be discussed below.

The rest of the peaks before 2000/cm correspond to the functional groups present in the final product. 1620/cm shows C=N stretch and 1562/cm shows C=C stretch in the aromatic regions. Weak peak at 1327/cm shows C-N stretch.

The IR spectrum obtained after further drying the purified compound 3 in vacuum oven proves that moisture from the compound had successfully been removed. The large O-H peak which was observed earlier at 3428/cm had disappeared, revealing the N-H peak at 3375/cm.

TLC

The result of TLC under UV light is shown below.

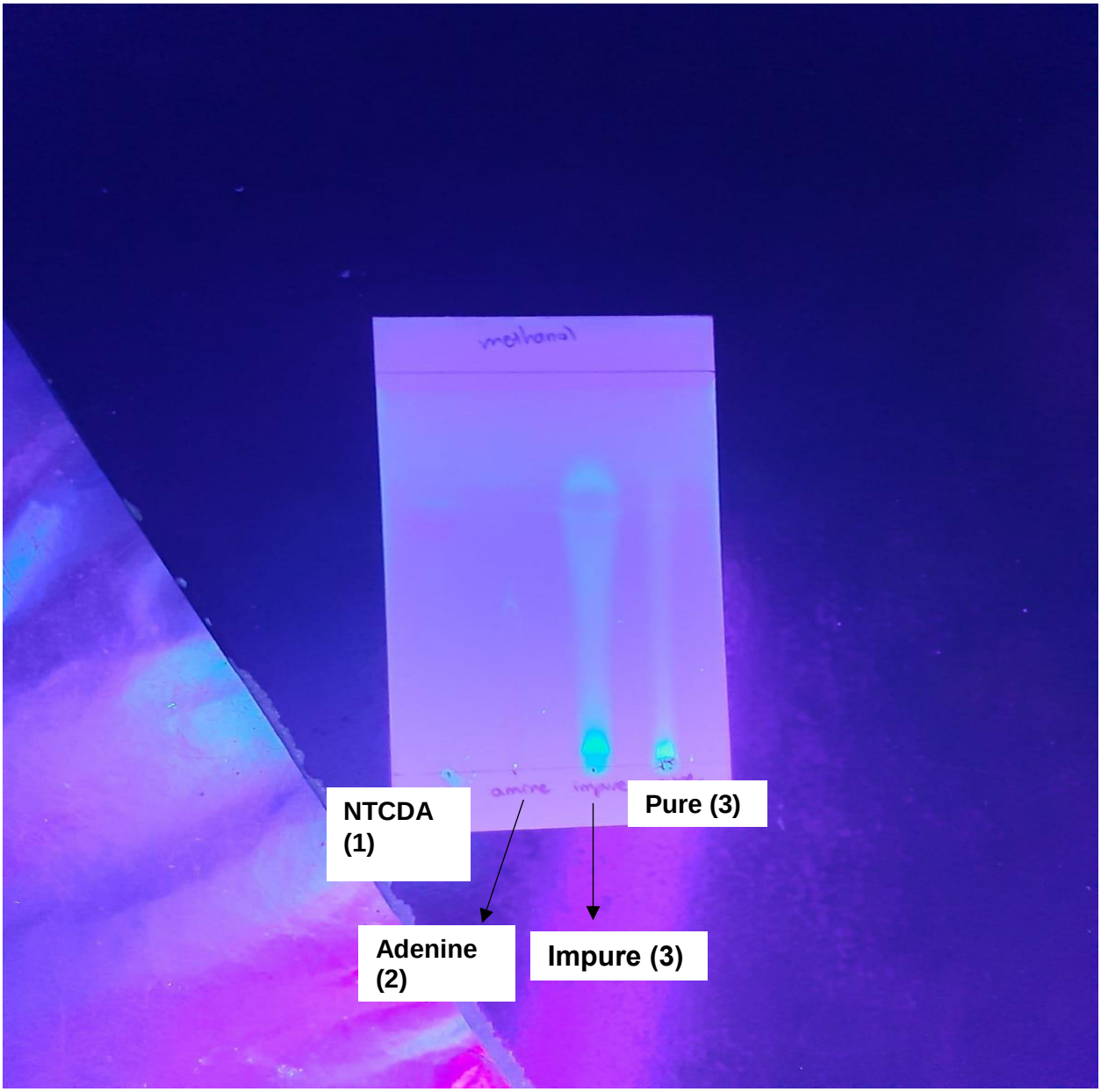

Fig 14: TLC results under UV lamp.

The first two columns shown belong to the reactants. The third column shows compound 3 before purification and the last column shows compound 3 after it had been purified by recrystallization. The results show presence of unreacted adenine in the impure product. This spot is absent in the purified product, showing that this had successfully been removed.

The result of TLC after treatment with vanillin bath is shown below.

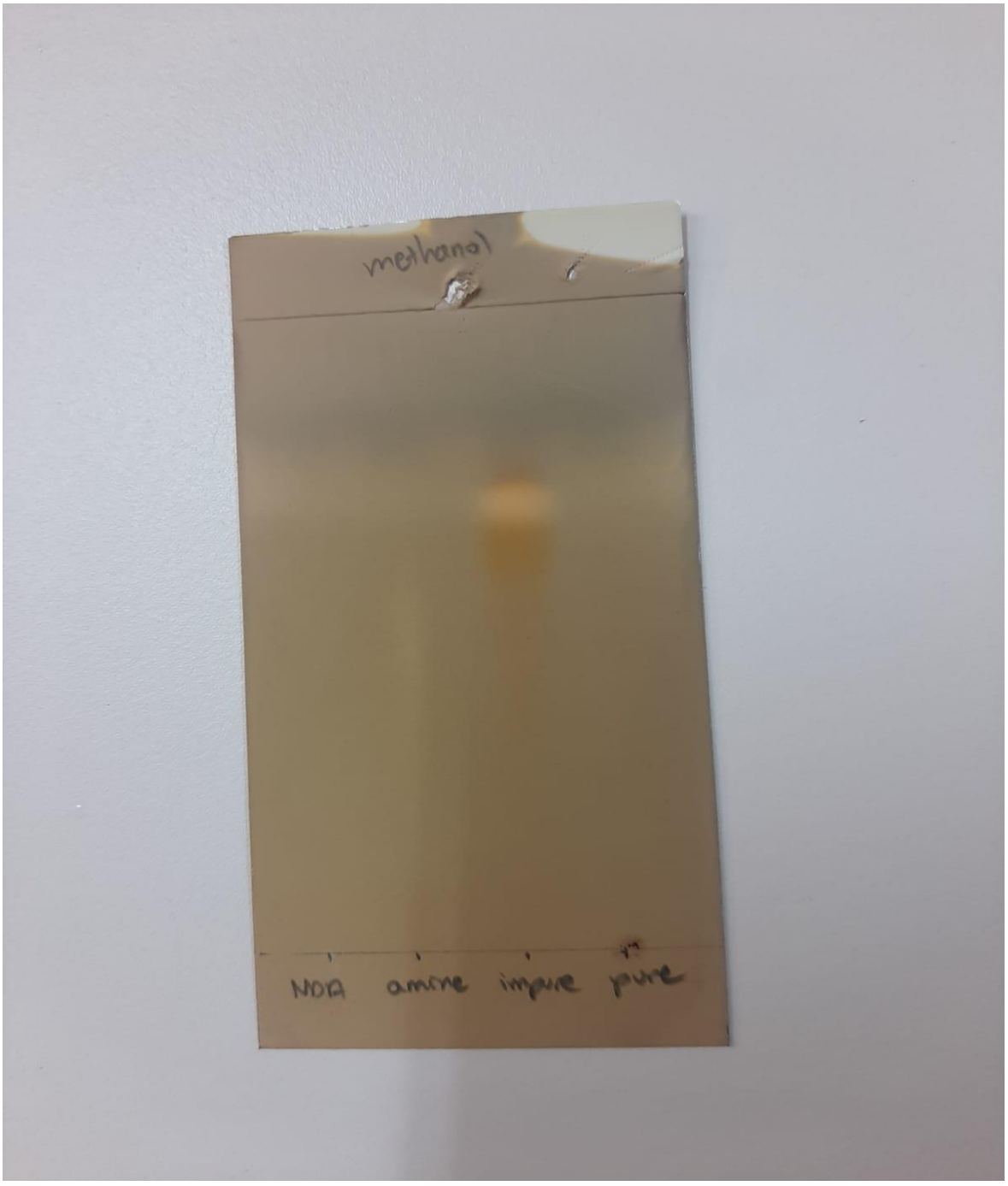

Fig 15: TLC results after vanillin bath and drying.

TLC result obtained after vanillin treatment makes the unreacted adenine spot more visible in the impure product, and its absence in the purified product is also observable.

Emission Spectroscopy

The result of emission spectroscopy is shown below.

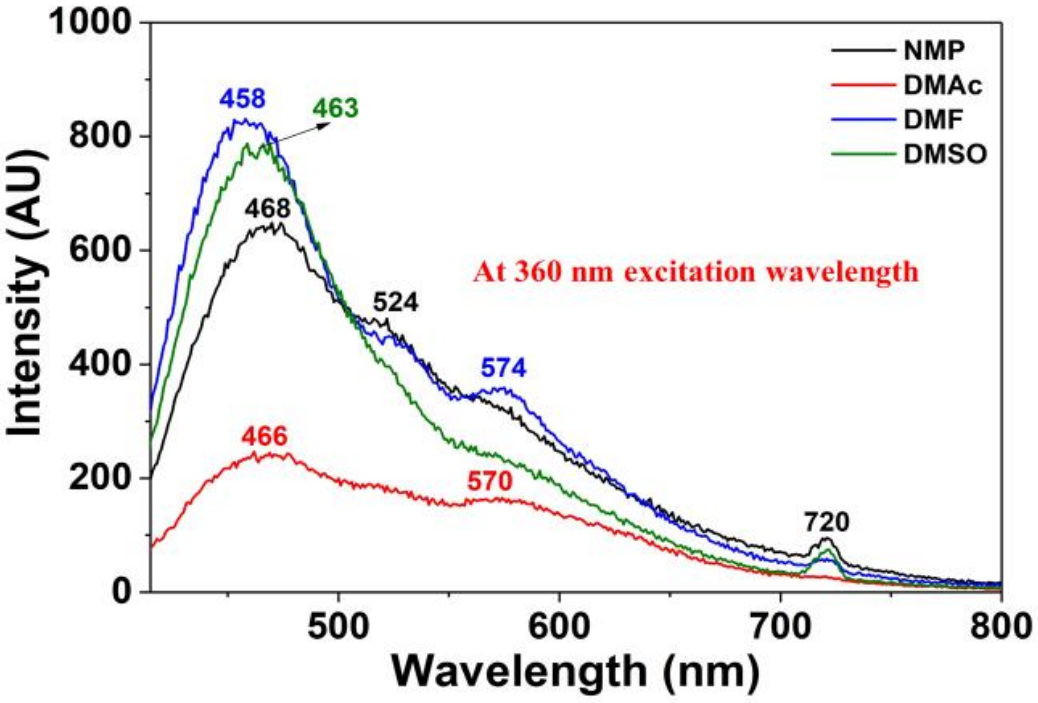

Fig 16: Emission spectrum of compound 3 in four different solvents.

As normally expected, the emission spectrum did not show three distinct peaks however a broad large peak from 0-520nm corresponds with excimer emission. A small peak at 720nm corresponds with charge transfer. An excimer is formed by dimerization of 2 atoms/molecules of the same species. Excimer is short-lived and forms only when one of the two atoms/molecules is excited. When the excimer returns back to its ground state, it releases energy which gives a peak in the emission spectrum such as the one shown above. Charge transfer refers to transfer of electrons from one atom/molecule to another, resulting in an excited state. The small peak at 720nm corresponds to the energy released when this excited state returns back to ground state.

These results show the electron accepting ability of the compound (3). The compound can be further used in photonic technology, e.g. organic photovoltaics and photonic sensors [17].

Naphthalene diimide derivatives have significant biological applications. They can be used for further syntheses of different derivatives such as this compound (3). Due to its ability to bind with DNA, they can be used to bind to specific sequences making them potential biomarkers for DNA and for studying its interactions with other molecules, e.g. DNA-protein interactions. Currently NDI derivatives are being used in cancer research as telomerase inhibitors and as anticancer agents [18].

CONCLUSION

Recrystallization is one of the most common purification techniques in organic chemistry. Briefly, a derivative of naphthalene diimide which had already been synthesized was purified by recrystallization. The data obtained showed that the compound was successfully purified. There are various purification techniques, the technique may be chosen according to the requirements. Recrystallization is appropriate for non-volatile solids. Furthermore, the process can be repeated multiple times to enhance purification. Recrystallization may be done multiple times, Soxhlet extraction can also be left to run for hours or days.

Once the compound has been purified, measurements can be done using IR spectroscopy along with both absorption and emission spectroscopy. Other types of chromatography such as column chromatography can also be used. This will increase the reliability of measurements. Other measurements such as cyclic voltammetry, differential pulse voltammetry and square wave voltammetry are more sensitive techniques which could be utilized.

A combination of different purification, separation and analytical techniques is ideal for purifying a compound and determining its purity. Use of multiple techniques will enhance purification and increase reliability of data obtained.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Prof. Dr. HURİYE İCİL, for giving me this opportunity and for her constant support and guidance. I would also like to mention ARWA ABOURAJAB, who helped me throughout and taught me valuable skills I will never forget. I would not have succeeded without their support. They are an important part of my academic journey, and I am truly fortunate to have worked with them.

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