ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

GRADUATE PROGRAM

Dynamic variable temperature NMR investigation of tautomerism in nicotinamide

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August 2024.
Addis Ababa, Ethiopia

Dynamic variable temperature NMR investigation of tautomerism in nicotinamide

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A Thesis Submitted to

The Department of Chemistry

Presented in Partial Fulfillment of the Requirement for the Degree of Master of Science in Chemistry (Physical Chemistry)

Aug, 2024.

Addis Ababa University

Ethiopia

ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

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This is to certify that the thesis prepared by Dejene Girma Dadi entitled "Dynamic Variable temperature NMR investigation of tautomerism in nicotinamide "submitted in partial fulfillment of the requirements of the degree of Master of Science in chemistry (physical chemistry) complies with the regulation of the university and meets the accepted standards concerning originality and quality.

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Abstract

Dynamic Variable temperature proton nuclear magnetic resonance (1H NMR) spectrometry has been used to study variable temperature nicotinamides. A functional group with limited rotation around the C-N bond, the dynamic nicotinamide bond is studied using variable temperature (VT) 1H NMR spectroscopy.

The majority of the structural and stereochemical characteristics of an organic compound can be revealed by 1H NMR spectroscopy, which is categorized under the category of 1H NMR spectroscopy or proton magnetic resonance (PMR) spectroscopy in conjunction with 13C NMR. A compound's potential carbon atom composition and number of non-equivalent carbon atoms can both be ascertained from its 13C-NMR spectra. As a result, 13C-NMR offers precise information about a molecule's carbon skeleton.

The amide molecule in the studies exhibited two separate peaks, each of which corresponded to the amine group. The peak separation decreased with increasing temperature, suggesting the presence of a rotating barrier around the amide bond.

At 328 K, the two peaks were seen to combine. Acetonitrile and chloroform, two generally nonpolar solvents, cause amide and related compounds to undergo substantial temperature-dependent structural changes, according to variable-temperature 1H NMR investigations.

The behavior that has been seen indicates that the rotational barrier about the C=N bond cannot be the cause of the peak splitting. Instead, it implies that a tautomeric equilibrium is the cause of the two peaks, which come from two distinct chromophores (O-H and =N-H chromophores).

Keywords: ^{1}H NMR, amide, tautomeric equilibrium, Variable temperature, rate 13C NMR,

Acknowledgment

I take this opportunity to express my deepest appreciation to Dr. Mesfin Redi, who not only served as my advisor, providing valuable and countless resources, and insight, but also constantly gave me support, encouragement, and reassurance. I am grateful to my co-advisor Hagos (Msc.) for his inspirational and timely support, technical expertise, and understanding throughout the project. I am grateful to Dr. Mesfin for his genuine encouragement, and support in the spectra of different samples, during the entire work.

Last but not least, I would like to express profound gratitude to my family.

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List of Abbreviations

^{1}H NMR	Proton nuclear magnetic resonance
C_6D_6	Deuterated benzene
C-N	Carbon Nitrogen bond
CO	Carbonyl bond
MHz	Megahertz
TMS	Tetramethylsilane
NLO	Nonlinear optics
Eq	Equation
K_{rate}	Exchange rate constant
E_a	Activation Energy
ΔH	Enthalpy Change
ΔG	Free Energy Change
ΔS	Entropy Change
K	Kelvin

1 Introduction

Amides are organic compounds with a functional group made up of a nitrogen atom and a C=O (carbonyl) link.

To model and anticipate the behavior of amides in larger molecules, such as proteins, which are crucial to biological processes because they serve as structural supports, catalysts, signal transmitters, and other roles, it is vital to understand the structure of amides.

Their three-dimensional architectures, which move constantly across a broad variety of time and length scales, dictate their biological roles [1]. Aside from that, amides are fascinating functional substances that are very important to the pharmaceutical and organic synthesis industries.

The amide molecule planarizes around the -N-CO moiety as a result [2]. There will be a rotating barrier surrounding the amide bond due to the amide's partial double bond feature. Due to the previously noted impeded rotation about the C-N bond, Ramiro Quintanilla-Licea et al. [3] propose that the two protons on the nitrogen atom are non-equivalent at the rotational barrier of the C-N partial double bond of form amide. Two protons on the nitrogen atom signals are detected at $\delta = 7.21$ and 7.48 ppm in the matching 1H NMR spectra, along with a singlet for the formyl proton at $\delta = 8.21$. As a result, this causes the two protons on the amide's nitrogen to absorb chemical shifts differently [3].

Using NMR spectroscopy methods, amides' structure and bonding form can be examined experimentally. To find out the environment in which a particular atom resides, NMR is often employed.

Therefore, in this experiment, dynamic proton NMR spectroscopy was used to explore the rotational barrier of nicotinamide. Because of the characteristic period of the NMR measurements, which makes a range of reaction rates commonly found in laboratories easily accessible, this method is aesthetically pleasing (10^{-1}-10^{-5} s^{-1}). This method can also be used to study the exchange rate in rotational barriers.

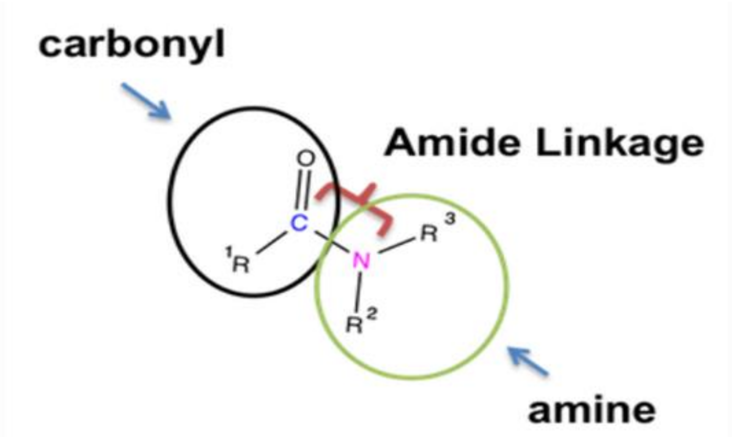
NMR spectra of protons are used to examine the impact of exchange at various temperatures. Because of the slow exchange at low temperatures, the rate constant is substantially smaller than the change in chemical shift, suggesting that the spectrum is made up of two distinct singlets.

The proton NMR spectra show only one peak because, at high temperatures, the exchange is quick, meaning the rate exchange constant is greater than the chemical shift change. In other words, there is a rapid rotation about the amide bond followed by the disappearance of magnetic non-equivalence. Additionally, there exists a middle-temperature range, referred to as coalescent temperatures, when the spectrum is comprised of two substantially broader overlapping lines. Using temperature-dependent proton NMR spectroscopy, the kinetic and thermodynamic parameters for the rotation of amide bonds have been assessed.

2. Literatures Review

2.1. Amides

Amides are organic molecules, and the synthesis of amide bonds is a crucial step in the organic synthesis process. In the pharmaceutical industry, amide bonds are crucial to the structure of biological systems like proteins and nucleic acids, which are large molecules made up of repeating amide units. They arise from the condensation of one amino acids carboxylic acid group with another amino acid group to create an amide linkage, commonly referred to as a peptide bond [4]. As a result, the nitrogen (N) and carbonyl (CO) atoms in an amide comprise its functional group.



Where R^1, R^2, and R^3 are hydrogen, aryl, or alkyl

Figure 1. The general structure of amides.

2.1.1. Structure and Classification of Amide

Figure 2 indicates how amides can be categorized as first order, second order, or third order based on the substituent on nitrogen.

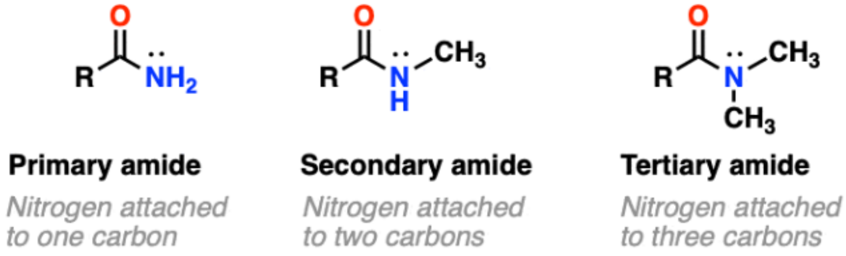


Figure2. General structure and classification of amides

Amides include three different types of hydrogen: hydrogen connected to the nitrogen atom of the amide, hydrogen on the carbonyl side, and hydrogen attached to the carbon atom on the alkyl (or aryl) group on the carbonyl side, which is known as α-hydrogen.

The backbone structure of proteins and similar molecules is largely determined by the conformation of the amide bond. The majority of amide qualities can be easily explained by assuming amide resonance structures [5]. Here, nicotinamide and nicotinamide, two first-order amides, are of importance (figure 3).

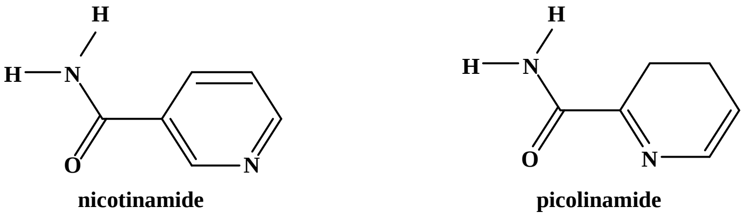


Figure 3. The structure of nicotinamide and picolinamide.

Amides are stable molecules. The resonance interaction between the carbonyl (CO) group's δ-bond and the nitrogen's nonbonding electron accounts for the stability of amides. The O-C-N bond in amides (Figure 4) has a double bond character due to this electron delocalization. As a result, the energy needed to rotate around the C-N partial double bond of amides (figure 5) is fixed and much larger than the energy needed to rotate about C-N single bonds [6].

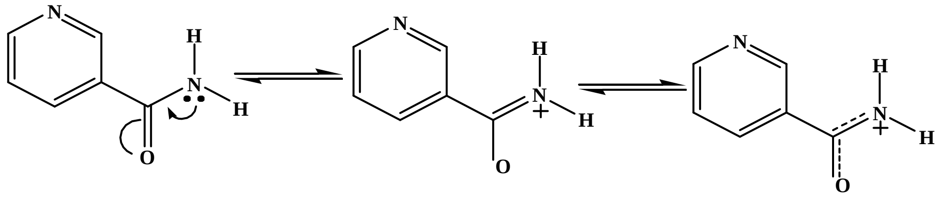


Figure 4. Resonance stabilization of nicotinamide.

Protons (Ha and Hb) on the amide's nitrogen (figure 5) have separate 1H NMR signals because they are in different chemical environments—that is, they are magnetically non-equivalent. These signals depend on temperature. Through variable temperature NMR investigations, the resulting dynamic NMR spectra offer kinetic and thermodynamic data.

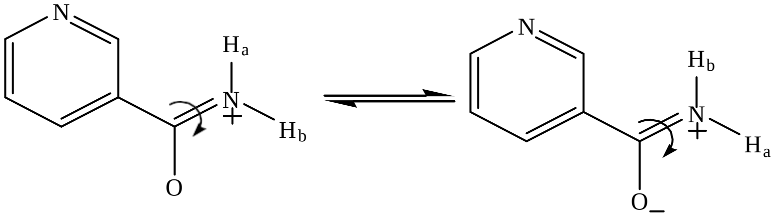


Figure 5. Rotation about the amide bond leads to the exchange of the two protons on nitrogen.

2.2. Fundamentals of spectroscopy

The quantized interaction of electromagnetic energy with matter is studied in spectroscopy. Nuclear spin orientation (NMR), electronic excitation (UV), and molecular vibration (IR) could result from the interaction [7]. A technique for examining molecules and the absorption of electromagnetic radiation is molecular spectroscopy.

A molecule's Eigen states change as a result of electromagnetic radiation absorption. The energy of the absorbed radiation determines the kind of eigenstates involved in a transition. An electromagnetic spectrum and the relative energies, wavelengths, and frequencies connected to each kind of radiation are depicted in Figure (6). Absorbing visible and ultraviolet light typically causes transitions between electronic eigenstates; absorbing infrared light causes vibrational Eigen states to change; and absorbing microwave radiation causes rotational Eigen states to shift [8].

The energy differential between the eigenstates of a molecule determines the precise wavelengths of radiation that are absorbed in each region of the electromagnetic spectrum. A spectrum's absorbed radiation can be used to determine the energy differences between a molecule's different eigenstates, but it cannot reveal the specific eigenstates that are undergoing these transitions. To study a spectrum and associate it with a particular transition in a molecule's eigenstates, one must understand quantum mechanics.

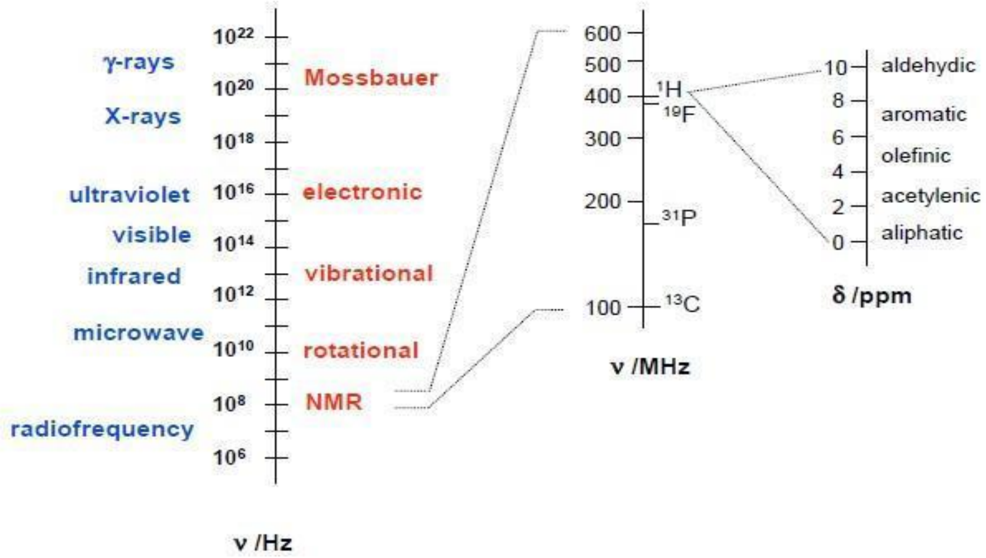


Figure 6. The electromagnetic spectrum (the scale is logarithmic)[9]

The energy of a photon of electromagnetic radiation is directly proportional to its frequency v and is inversely proportional to its wavelength v^{-1}.

$$E_{photon} = hv \quad (1)$$

2.3. The equilibrium of amide tautomerism

A thorough analysis of the tautomeric equilibrium in a series of amides—isoquinoline derivatives that differed in the acidity of the labile proton produced by adding different substituents to a phenyl ring was the goal of the current study. These amides are schematically shown in Figure.

NMR spectroscopy was applied as a precise tool to qualitatively determine the tautomeric equilibrium in heterocyclic systems. This technique provides not only information about the ratio of tautomers but also their structure, which can be further compared with the results of quantum chemical calculations (geometry, shielding, and relative energy). To confirm our experimental findings and justify the tautomer preferences in the systems containing a palette of substituents, quantum chemistry approaches were applied.

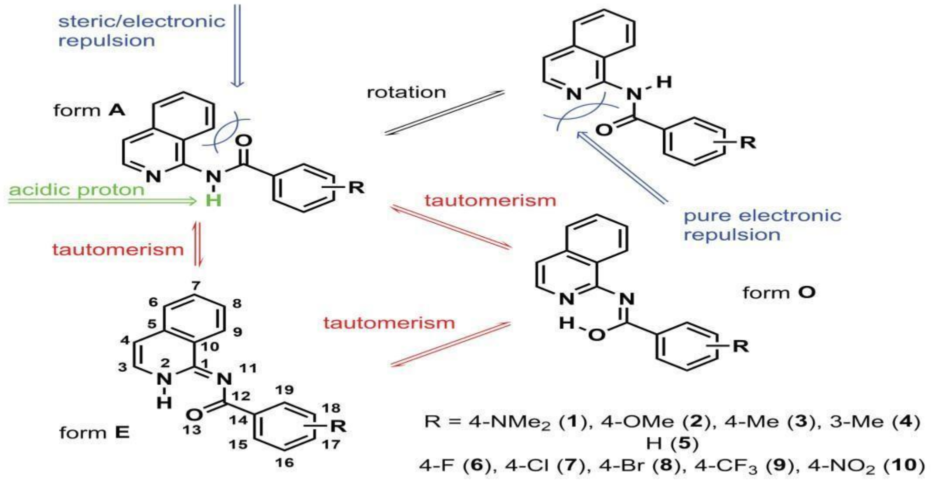


Fig.7. The tautomerism and steric/electronic repulsion in the studied series of molecules

2.4. NMR spectroscopy

NMR spectroscopy is a great tool for determining the structures of organic compounds. It is the study of the interaction of magnetic nuclei having resultant nuclear spin (1H, 13C.) with electromagnetic radiations in radiofrequency regions under the influence of an appropriate magnetic field. The study of hydrogen nuclei comes under the preview of 1H NMR spectroscopy or proton magnetic resonance (PMR) spectroscopy. Combined with 13C NMR, 1H NMR (PMR) can be used to reveal most of the structural and stereochemical features of an organic compound. By studying a compound by 1H NMR (PMR) spectroscopy, one can obtain the following types of information regarding the compound:

(i)The number of types of hydrogen atoms in a molecule.

(ii) The chemical environment of hydrogen atoms.

(iii) The number of hydrogen atoms in a given chemical environment. Their frequency is on the order of 107Hz. The energy of radiofrequency (RF) radiation can therefore be calculated by using

f=c/λ. Here, f-frequency, c= speed of light, and λ =wavelength

Eq. (2).

Radiofrequency radiation involves a very small amount of energy. It is too tiny to electronically excite a molecule or an atom or to rotate or vibrate. It is strong enough to change an atom's nuclear spin within a molecule. Thus, in a magnetic field, certain atoms' spinning nuclei within molecules can absorb radiofrequency radiation.

Radiation and shift the axis of rotation's direction. If a molecule's nucleus has a magnetic moment, then, in theory, every chemically unique atom in the molecule will have a distinct absorption frequency or resonance. NMR spectroscopy is the analytical field that makes use of such nuclei's ability to absorb radiofrequency radiation in a magnetic field to convey information about a sample [15].

By using NMR, we may examine the structure and form of molecules. Specifically, it discloses the many chemical environments that the NMR-active nuclei in a molecule are found in, allowing us to determine the molecule's structure. The spatial orientation of the atoms in a molecule can be determined via NMR. NMR can help us figure out how much of each sort of component is in the mixture if we already know what kind of compounds are there. Hence, it's a technique for qualitative as well as quantitative analysis, especially concerning organic molecules. Moreover, chemical equilibrium, reaction kinetics, molecular mobility, and intermolecular interactions are all studied with NMR [15].

2.4.1. Properties of nuclei

To comprehend the characteristics of specific nuclei in an NMR experiment, one physical characteristic of the nucleus, which is composed of protons and neutrons, is the spin quantum number, or I. Along its axis of rotation, a charged body spinning creates a magnetic moment. In an NMR experiment, a nucleus needs a magnetic dipole moment and a nonzero spin quantum number (I) to produce a signal. The quantity of magnetic quantum states or orientations is determined by the physical characteristics of the nuclei and can be expressed mathematically as $2I = 1$. This allows for spin states with integral differences varying between +I and –I. The sequence that the individual spin states fit into is +I, (I-1)... (I+1), -I.

Certain atoms, such as 12C, 16O, and 32S, have paired spins that cancel each other out, leaving the atom's nucleus without an overall spin. But the nucleus of many atoms, including 1H, 13C, 31P, 19F, etc., has an overall spin. The spin of a particular nucleus can be ascertained using the following rules:

The nucleus lacks spin if the number of protons and neutrons is equal. The nucleus has a half-integer spin if the number of protons and neutrons is odd, such as 1/2, 3/2, 5 /2, The nucleus has an integer spin, or 1, 2, 3,..., if the number of protons and neutrons is odd. As a result, the foundation of the NMR phenomenon is the fact that atoms' nuclei have magnetic characteristics, which are employed to provide chemical information. A nucleus with an I=1/2, like 1H, will align its magnetic moment either with the applied field or against it when it is exposed to an external magnetic field.

As seen in Figure 8, this produces two distinct energy levels, one of which is higher than the other. The magnetic moment is in line with the field at the lower energy level. Since the lower energy state has a greater energetic preference than the higher energy state, there will be more nuclei in the lower energy state than in the higher energy state. The strength of the external magnetic field determines how much energy differs between levels [15].

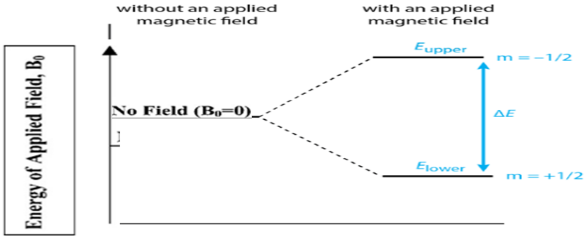


Figure 8.Energy levels for a nucleus with a spin quantum number [15].

As depicted in Figure 8, the axis of rotation likewise revolves circularly around the axis of the external magnetic field, resembling a spinning top. Precession refers to this rotation, which is the regular periodic movement of the particle. Precession is either in the applied field B_0's direction or in the opposite way.

Thus, we have two distinct energy levels for our nuclei, in this case, protons. More protons will be in the lower energy state in a large sample of nuclei. The goal of the NMR experiment is to use radiation absorption to induce a transition between these two states. Figure 8 illustrates how radiation with a frequency equal to or greater than this might cause a transition between these two energy levels.

ΔE according to the relationship $\Delta E = h\text{v}$ (3)

The applied magnetic field B0 and the nucleus's magnetic moment m determine the energy difference between its two quantum states when I = 1/2. The following formula represents the link between these energy levels and the frequency of absorbed radiation. In a magnetic field, the expression for a specific nuclear energy level is E:

$$E = -\ m\left(\frac{\mu}{I}\,\frac{h}{2\pi}\right)B_0 = -m\left(\gamma\frac{h}{2\pi}\right)B_0 \qquad (4)$$

where h is Planck's constant, I is the spin angular momentum, ɤ is the magnetogyric ratio, m is the magnetic quantum number, m is the nuclear magnetic spin, and B0 is the applied magnetic field.

The generic formula for a specific energy level for every nucleus that reacts to NMR is equation (5).

$$E=\left(\frac{\mu h}{I2\pi}\right)B_o = \text{ɤ}\frac{h}{2\pi}Bo \qquad (5)$$

Therefore, the absorption frequency that can result in a transition of ΔE is:$v = \frac{\text{ɤ}\, B_0}{2\prod}$

or $\text{ɷ} = \text{ɤ}\, B_0$,(Larmor equation), which is fundamental to NMR[15]. It indicates that for a given nucleus there is a direct relationship between the frequency ɷ of RF radiation absorbed by that nucleus and the applied magnetic field B_0.

2.4.2. Spin-spin splitting in proton NMR

Spin-spin splitting, an interaction between non-equivalent protons on neighboring carbons, frequently causes peaks to divide into several peaks. According to the "n+1 rule," there is one more peak formed by the splitting than there are Hs on the nearby carbon. [25]

A multiplet's peak separation is measured and is a constant expressed in Hz [21]; nearby nuclei may affect each other's effective magnetic fields. This impact is detectable if the distance between non-equivalent nuclei is less than or equal to three bond lengths. J coupling, or spin-spin coupling, is the term for this. The distance between the two peaks in the NMR spectrum is known as the coupling constant J, expressed in Hertz. When a molecule contains two proton nuclei, they are attracted to one another and affect one another. When two nuclei are connected, the coupling constant's magnitude remains constant and is solely dependent upon the nature of their interaction.

2.4.3 Chemical Shift

Because of various chemical bonds and nearby atoms, protons in different chemical environments within molecules absorb at slightly different frequencies in NMR experiments[15]. This variation in absorption frequency is brought about by a slight difference in the proton's electronic environment. A given proton's absorption frequency is determined by the molecule's chemical structure. We refer to this change in absorption frequency as a "chemical shift." Therefore, the difference between the resonance frequencies of a nucleus and a standard, relative to the standard, is used to estimate the chemical shift of a nucleus. This quantity has the symbol delta and is reported in parts per million.

$$(\ \delta\ (ppm)=\frac{V-V_{ref}}{V_{ref}}\,10^{6} \qquad ---- \qquad (6)$$

Tetramethylsilane (TMS), with the chemical formula Si(CH3)4, is a widely recognized standard reference for 1H NMR spectroscopy. Since all 12 of the hydrogen nuclei in the TMS have identical chemical properties, they are all subjected to the same shielding and produce a single absorption peak [15]. For different hydrogen nuclei, the chemical shift is shown above Eq (6).

2.4.4 NMR Data Processing

Even for the same sort of nucleus, there are differences in the magnetic field that they experience based on the chemical environment. The NMR signal is detected on the xy-plane as a result of the oscillation of the net nuclear magnetization on the xy-plane, which generates a current in a coil.

Because the frequency difference from TMS is used to determine the chemical shifts of the peaks in the spectrum. A frequency-domain spectrum is the kind of spectrum that this is. Ringing is a decrease in the series of oscillations that happens after the instrument has scanned past a peak produced by a continuous-wave instrument. When the instrument's fields advance to a new place before the excited nuclei have time to return to their equilibrium condition, ringing ensues. The relaxation rate of the stimulated nuclei is slower than the scan rate. They continue to produce an oscillating, quickly declining signal as a result, which is heard as ringing.

2.4.5 The Fourier Transform (FT) NMR

When the intense but brief pulse used by the NMR equipment is stopped, the excited nuclei start to lose their excitation energy and relax, returning to their initial spin state. Every agitated nucleus releases electromagnetic radiation as it relaxes. A wide range of electromagnetic radiation frequencies are simultaneously released by the molecule due to its many nuclei. The discovered signal is referred to as a free induction decay, or FID (Figure 9a). A mathematical process known as Fourier transformation (FT) is used to turn this type of spectrum—also known as a time domain spectrum—into the more common frequency-domain spectrum (Figure 9b). Due to the signal's requirement for mathematical processing, A computer is necessary for pulsed NMR spectrometers, and in addition to executing the Fourier transform, the computer offers a practical way to store NMR data and carry out secondary data processing and analysis [16, 20].

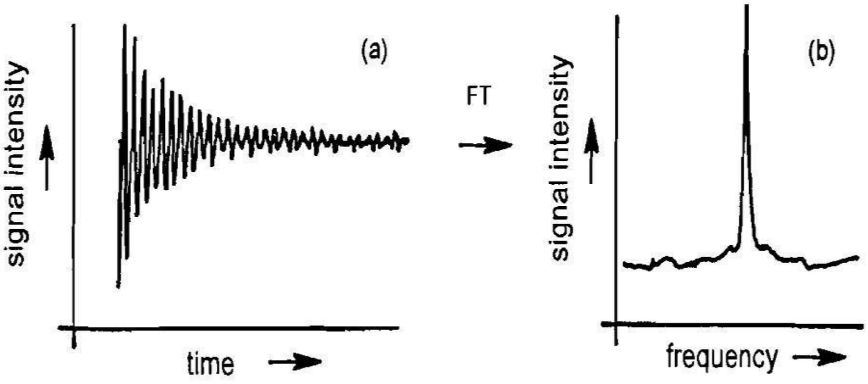


Figure 9. The free induction decay (FID) is on the left and its Fourier transform (usual frequency spectrum) is on the right of an amide

2.5 Variable temperature of the nicotinamide bond

A functional group with limited rotation around the C-N bond, the dynamic amide bond is studied using variable temperature (VT) 1 H NMR spectroscopy. We can ascertain the energy barrier (ΔG) for the restricted rotation about the C-N bond and the coalescence temperature linked to the slow equilibrium at room temperature thanks to the VT NMR data.

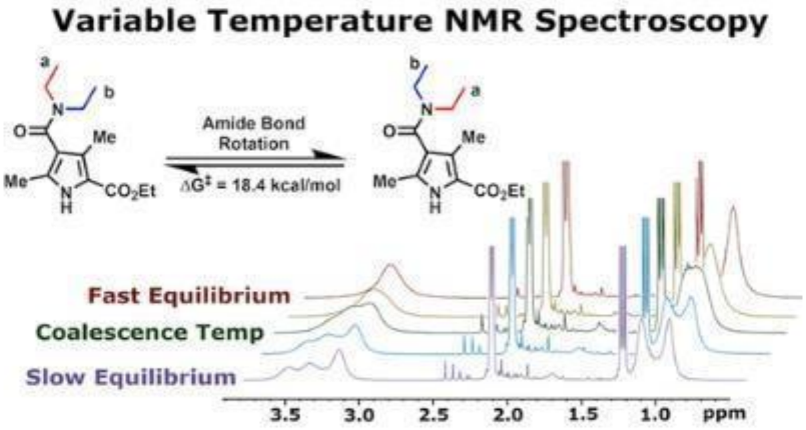


Fig.10. Variable Temperature NMR spectroscopy of amide

To use dynamic NMR spectroscopy, the NMR active nucleus must change surroundings, and depending on how quickly or slowly the process proceeds on the NMR time scale, the chemical shift will differ. Since attributes of each species individually (i.e., host, guest, and complex) can be measured during slow equilibrium, the NMR spectrum will display all of the species involved in the process.

When fast equilibrium occurs, all of the species in the solution average out to produce the observable signal. An NMR spectrum in fast equilibrium shows no evidence of a single species.

The coalescence temperature, or Tc, is the point at which a dynamic process changes from being fast to slow or vice versa. This usually leads to large peaks, making it challenging to estimate the number of peaks that vary between states.

3. Objectives

3.1. General objective

To ascertain the dynamic variable temperature NMR analysis of nicotinamide tautomerism.

3.1.1. Specific objectives

- Determine the variable temperature of the proton NMR absorption peaks of the nicotinamide.
- Determination of the magnitude of the kinetic and thermodynamic parameters for Dynamic Variable temperature tautomerism nicotinamide

4. Experimental part

4.1. Materials

Proton NMR studies using nicotinamide (98% Alfa Aldrich Japan), solvent-deuterated benzene (99.5%, Fluca-Garantie, Switzerland), solvent ACN (99.9%, Sigma-Aldrich, Switzerland), and carbon tetrachloride (CCl4) in 2% tetramethylsilane (TMS) were conducted using nicotinamide (BDH), an English laboratory reagent. Distilled water and 99.9% Acetone (Sigma-Aldrich, Switzerland) were used as cleaning agents. All of the previously described compounds were investigated without further purification.

4.2. Methods and procedures

4.2.1. Proton NMR measurements

Using a Bruker Avance 400 MHz spectrophotometer, proton NMR spectra of amide solutions were captured. The following were the typical spectral conditions: Four scans per spectrum and a two-second acquisition period. Using a Bruker Avance digital variable-temperature control unit, the spectra were obtained across a range of 298 K– 333 K for nicotinamide, 296 K–336 K for nicotinamide in ACN solvent and 296 K–338 K in C6D6. A tiny quantity of amide was collected and dissolved in an ACN solvent. Tiny amounts of nicotinamide were also dissolved in ACN and deuterated benzene using separate beakers.

The samples are moved to an NMR tube that has been dried, cleaned, and sealed permanently. Paramagnetic oxygen must be eliminated during degassing; otherwise, there would be an extra unknown line-broadening factor. These solutions' spectra were captured in carbon tetrachloride with 2% TMS.

The spectrum measurements can be performed with any NMR spectrometer that has a variable temperature probe attached. Spectra are taken at each temperature in turn until no more changes in the spectral properties (line width and peak separation) are noticed. At every temperature, the methyl resonance of TMS should be recorded to verify the instrument's resolution. Three kinds of data are identified by coalescence temperature, fast exchange, and slow and intermediate exchange.

.

Following the NMR data, the Arrhenius plot (ln k Vs 1/T) and the exchange rate are calculated by extracting the line widths and exact line position using the MestReC software to convert Fourier data into an ASCII file.

To determine the activation energy, and the enthalpy and entropy changes are determined using the Eyring plot (ln (k/T) Vs 1/T). and using a plot of frequency against temperature can detect the pattern in the amide's 1H NMR spectra.

5. Results and Discussion

5.2.^{1}H NMR Result nicotinamide

The two protons of the amide's amine group were found to be on distinct chemical shifts in the 1H NMR spectra, indicating that the resonance structure is what creates the C-N partial double bond of first-order amides. Rotating around the C-N partial double bond of amides requires energy. The rotating barrier's effect is temperature dependent; that is, at low temperatures, the two protons on the amide's nitrogen have different magnetic surroundings, which results in two peaks of equal strength and sluggish rotation around the amide bond. At high temperatures, these peaks disappear. A broadening peak is observed at the temperature of coalescence, indicating a broad overlap of the two protons on the amide's amine.

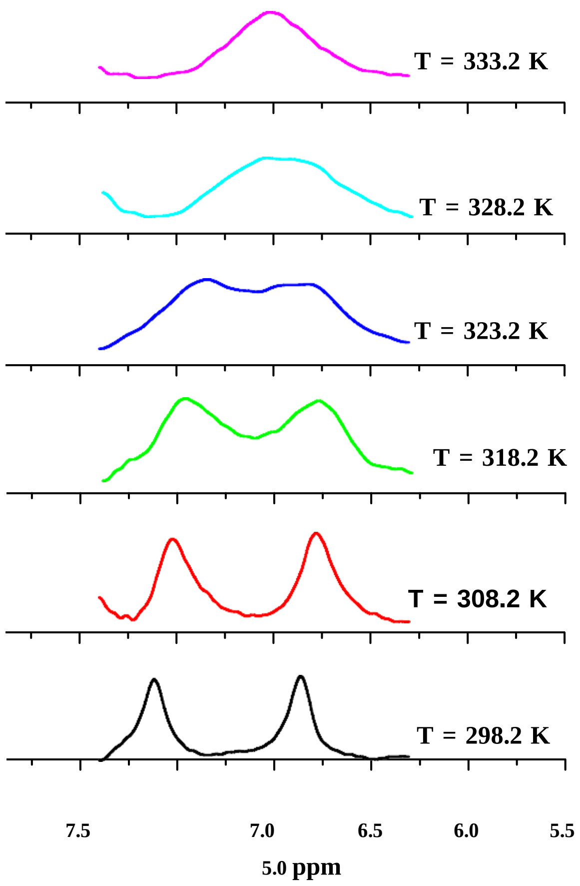


Figure 11. Effect of temperature on line shape for the two protons on nitrogen amide in ACN.

The above image shows the proton NMR spectra of the amide at different temperatures; the coalescence temperature was determined to be 328 K. Few molecules have enough energy below the coalescence temperature to overcome the rotational barrier. Rotation, however, takes place at temperatures higher than the coalescence temperature because numerous molecules have enough thermal energy to get through the barrier at those temperatures [19]. The two protons on the nitrogen of the amide peaks collapse to a single peak when the amide bond rotates quickly at high temperatures, above the coalescence temperature.

In both rotamers, the protons' surroundings on the amide's nitrogen are the same. The single line characteristic of free rotation around a single bond is, thus, the spectrum. The line narrows as the temperature rises further.

5.3. Determination of Kinetic Parameter for amide

5.3.1. Rate exchange constant (k)

The two protons' NMR spectra on the amide's nitrogen can be used to compute the exchange rate constant by comparing their peak separation and peak width changes. These values are shown in the table below.

Table 1: Changes in peak widths and separations at half height with different amide temperatures

Temperature(K)	Peak separation(ΔV)/Hz
298.2	0.755
308.2	0.741
318.2	0.684
323.2	0.456
328.2	0
333.2	ΔV_e $_{1/2}$= 0.46HZ
	$\Delta v_{0)1/2}$ =0.137HZ

At a temperature of 308.2 K, the rate constant which is k $=(\frac{\pi}{\sqrt{2}} (\Delta v_0^2 - \Delta v_e^2)^{1/2}$

$$K= \frac{\pi}{\sqrt{2}}((0.755HZ)^2_- ((0.741HZ)^2)^{1/2}$$

=0.321HZ

At a temperature of 318.2K,

$$k=\frac{\pi}{\sqrt{2}}((0.755HZ)^2_- (0.684\text{HZ})^2)^{1/2}$$

=0.710HZ

At a temperature of 323.2k

$$=\frac{\pi}{\sqrt{2}}((0.755\text{HZ})^2 -(0.456\text{HZ})^2)^{1/2}$$

=1.337HZ

At coalescence temperature which is 328.2 K, the rate constant (k) is determined by

$$K = \frac{\pi \Delta Vo}{\sqrt{2}} \text{-------} \qquad (7)$$

$$K = \frac{\pi(0.755Hz)}{\sqrt{2}} = 1.677\text{Hz}$$

For fast exchange at a temperature of 333.2 K, the rate constant (k) is determined by

$$K=\frac{\pi \Delta V2_0}{2}[(\Delta v_{e)1/2}\text{-}(\Delta v_0)1/2]^{-1} \text{----} \qquad (8)$$

$$K=\pi\frac{(0.755\text{HZ})}{2}[0.461\text{-}0.137\text{HZ})^{-1}=2.76\text{HZ}$$

Table 2. Typical value for k-$_{\text{rate}}$ for amide

Temperature K	K-rate (s^{-1})
298.2	-
308.2	0.321
318.2	0.710
323.2	1.337
328.2	1.677
333.2	2.764

As the temperature rises, so does the rate of exchange between the two magnetic environments.

5.3.2. Activation energy

We can compute the activation energy, which connects the rate constant at various temperatures and the activation energy, once the temperature dependence of the rate constants is determined.

$$\ln k = -\frac{Ea}{R}\left(\frac{1}{T}\right) + \ln A \qquad (9)$$

So ln k Vs 1/k can be plotted and given a straight line with the slop = $-\frac{E^{a}}{R}$

(where R= 1.987cal mol^{-1} K^{-1})

So in this experiment, the activation energy is found to be 17.948Kcal $mol^{-1}K^{-1}$(see appendix A)

5.4. Determination of Thermodynamic Parameters for amide

5.4.1 Enthalpy change, entropy change and free energy change

ΔH, ΔS, and ΔG can be found using the Eyring equation (Eq.9), which is derived from the rate exchange constant at various temperatures.

$$K=\left(-\frac{KbT}{h}\right)e^{\frac{\Delta G*}{RT}} \qquad (10)$$

So an Eyring plot of ln $\left(k/T\right)$ versus 1/T gives a straight line (see Appendix B)

For which, ΔH^{*} =(slope) R, and y-intercept is

$$\frac{\Delta S}{R}+\ln {}^{kb}/_{h}=\frac{\Delta S*}{R}+23.76) \qquad (11)$$

and it is used to determine ΔS^{*}, At the coalescence temperature ΔS^{*} =6.111*cal.mol* $^{-1}$

but often a significant extrapolation based on the experimental findings.
Thus, the error in ΔS *may be rather substantial if the linear fit of ln Δk/ T vs 1/T is not very good.
The formula below can be used to obtain ΔG* directly at the coalescence temperature.
To figure out ΔG* at every temperature:
The meaning behind ΔS* a less organized transition state is indicated by a significant positive number.

A more orderly transition stage is indicated by a significant negative number. Here, the little negative entropy value shifts to the transition state that is shown as being somewhat organized. At coalescence temperature, ΔG* can be computed using the Eyring equation. (Note that the temperature dependency of ΔG* is typically unnoticeable within error, as ΔS is typically<ΔH.)

ΔG*=a T [10.319+log (T/K rate)]

ΔG*308.2=4.57*10^{-3} (308.2)

= [10.319+ logs (308.2/0.321)]

=18.755kcal/mole

Table 3. Comparison of Experimental energies for the rotational barrier of amide

Sample		ΔH (Kcal mol^{-1}	ΔS (cal mol^{-1})	ΔG $_K$ (Kcal mol^{-1})	Ref.
amide	Experimental	16.9	-6.1	18.7	This work
	From computational$^{(a)}$	12.9±0.3	-7.7±0.9	15.3	23

(a) The HF/6-31G(d.p.) optimized structures. Corrected in nitrobenzene solvent with zero point energies at HF/6-31G(d.p).

The theoretical values and the experimental results achieved in this work agreed well.

5.5. The ^{1}H NMR Result for nicotinamide

The influence of the two magnetically non-equivalent protons on the amide's nitrogen is seen in Figure 12's 1H NMR analysis of nicotinamide in ACN and benzene solvents at varying temperatures. Asterisks indicate the two protons on the amide's nitrogen.

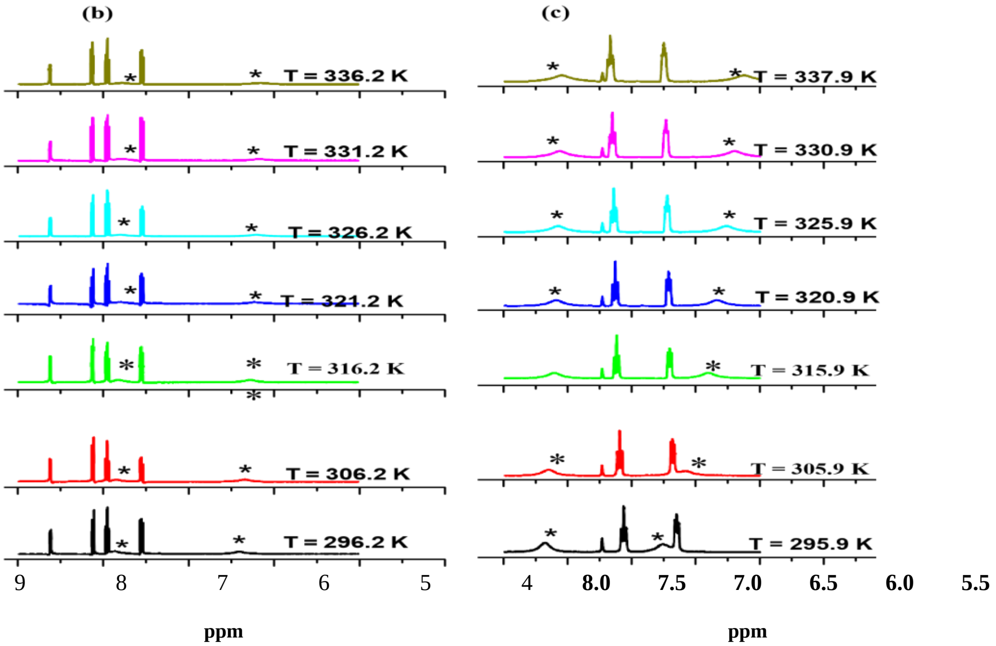


Figure 12 shows the temperature-dependent proton NMR spectra of amides (b) in acetonitrile and (c) in benzene solvents

A straight line is produced when the chemical shift of peak separation is plotted against temperature (see pictures 12, 13, and 14).

Table. 4. Properties of Some Deuterated NMR Solvents

Solvent	B.P. °C	Residual ^{1}H signal (δ)	Residual ^{13}C signal (δ)
acetone-d_6	55.5	2.05 ppm	206 & 29.8 ppm
acetonitrile-d_3	80.7	1.95 ppm	118 & 1.3 ppm
benzene-d_6	79.1	7.16 ppm	128 ppm
chloroform-d	60.9	7.27 ppm	77.2 ppm

Source [24]

To verify the product's structure, each pair will have its 1H and 13C NMR spectra examined inCDCl3at room temperature.

All NMR measurements were performed with 15 mg of 1 in 1 mL of CDCl3 under standard conditions on a BrukerAvance 400 MHz spectrometer. Students measure five 1H NMR spectra at 5° increments across 300−320 K once the structure has been confirmed.

The data plot displays the expansions of the most relevant spectral areas, 0.5-4.0 ppm. It is important to treat chloroform-d (CDCl3) carefully as it has the potential to cause cancer.

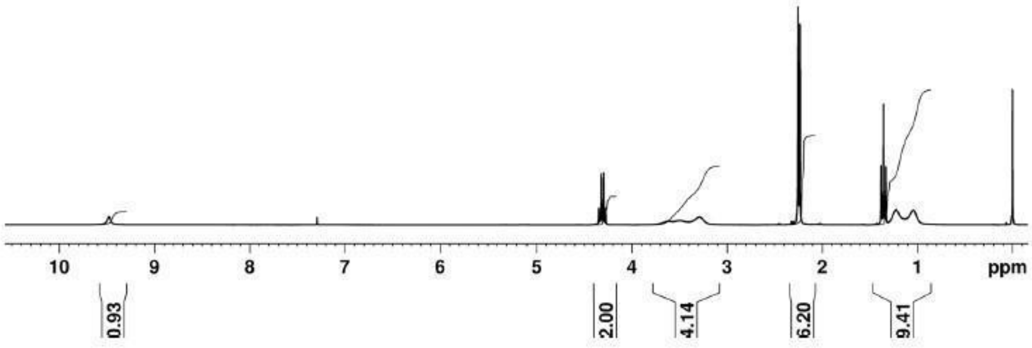
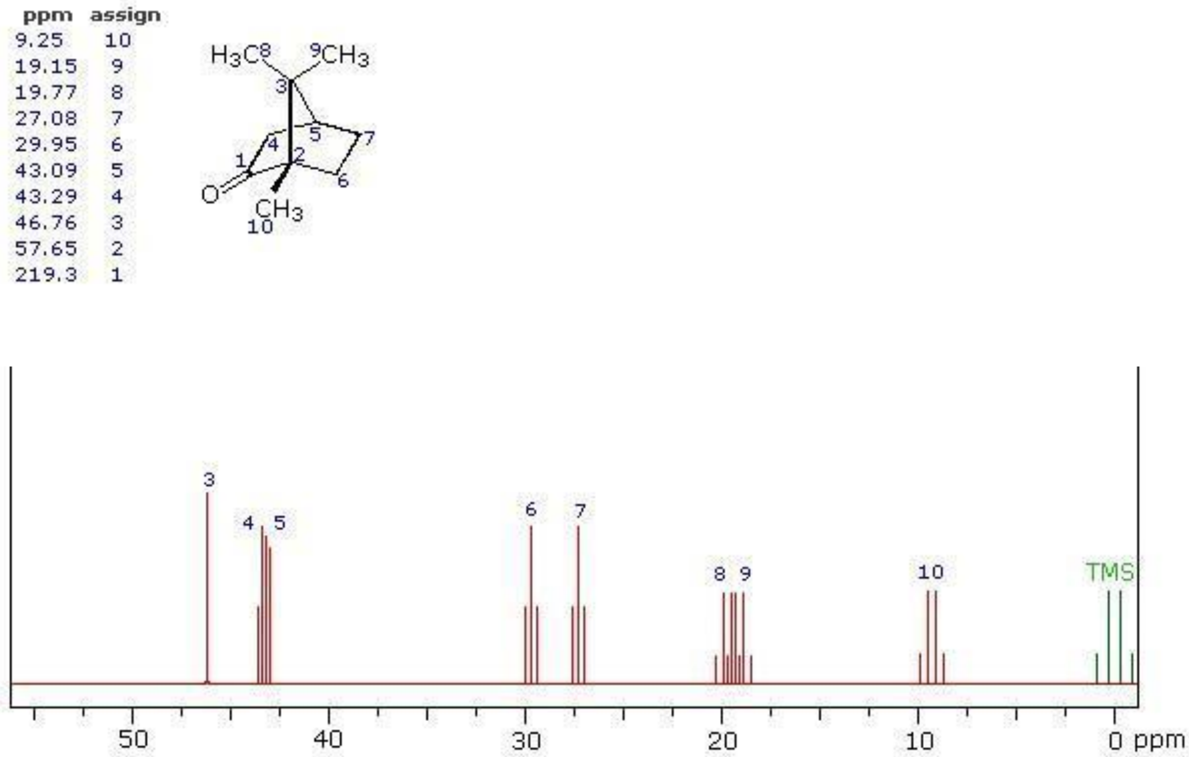


Figure 13: Room temperature (298 K or 25 °C) representative student 1H NMR spectra of 1 in CDCl3.



ppm	assign
9.25	10
19.15	9
19.77	8
27.08	7
29.95	6
43.09	5
43.29	4
46.76	3
57.65	2
219.3	1

Figure14 - : 13C NMR spectrum of in CDCl3 (125 MHz)

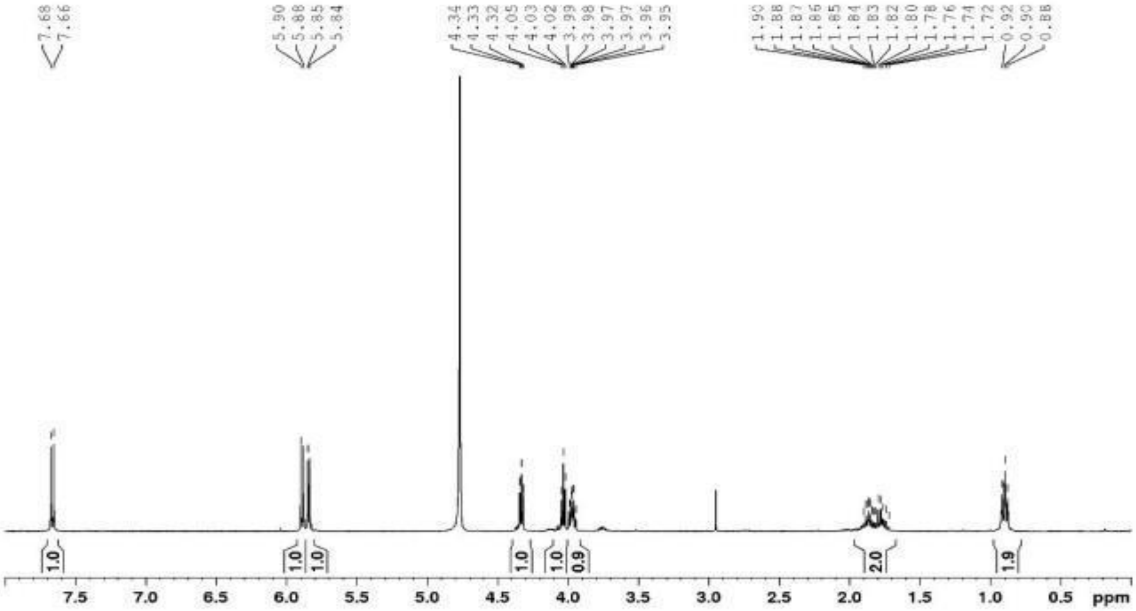


Figure15 -: 1H NMR spectrum of amide in D2O (pD 6.5, 400 MHz, 37 mM)

Since deuterium atoms lack nuclear spin and hence do not interact with the external magnetic field employed in NMR spectroscopy, the 13C NMR spectrum of D2O atomic mass is an even number. Deuterium atom absorptions won't be noticed and won't result in a peak.

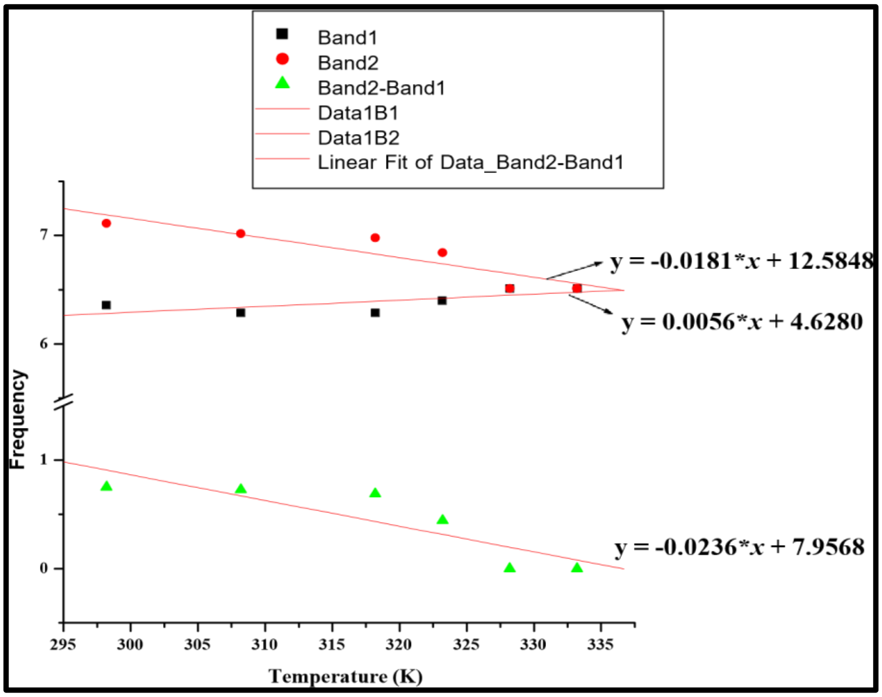


Figure 13. Plot of frequency versus temperature of nicotinamide in acetonitrile

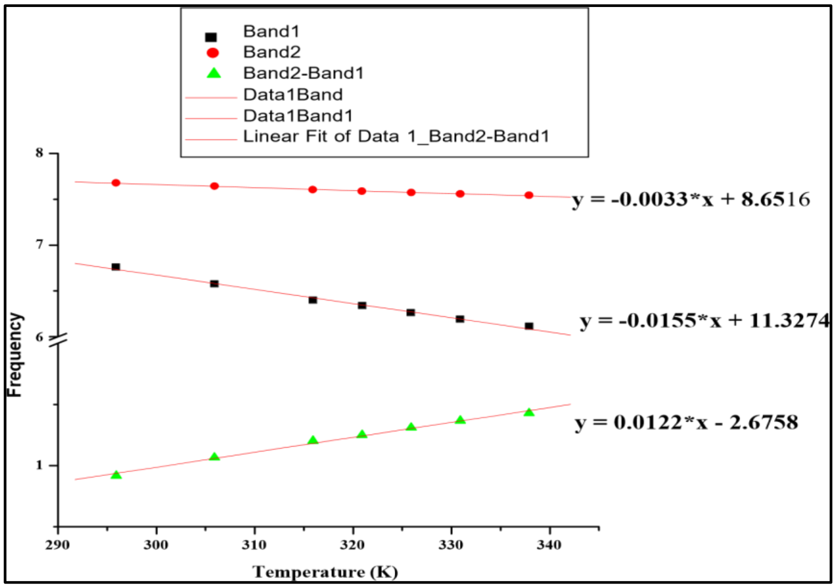
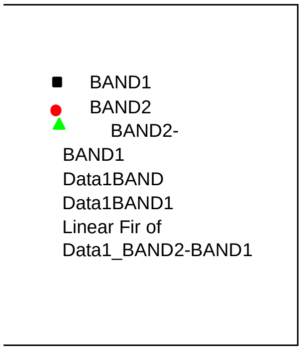


Figure 14. Plot of nicotinamide benzene frequency against temperature



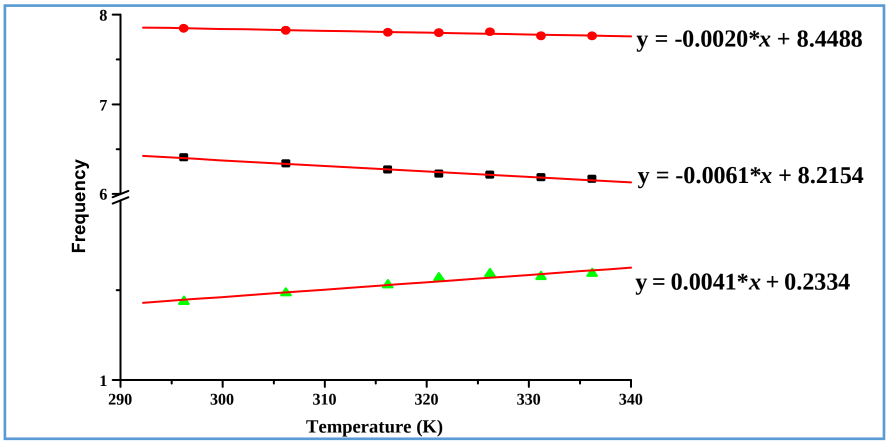
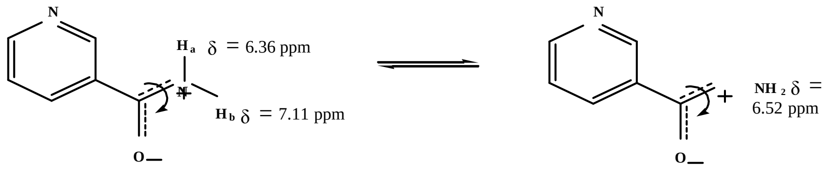


Figure15.Nicotinamide in acetonitrile: frequency against temperature plotted

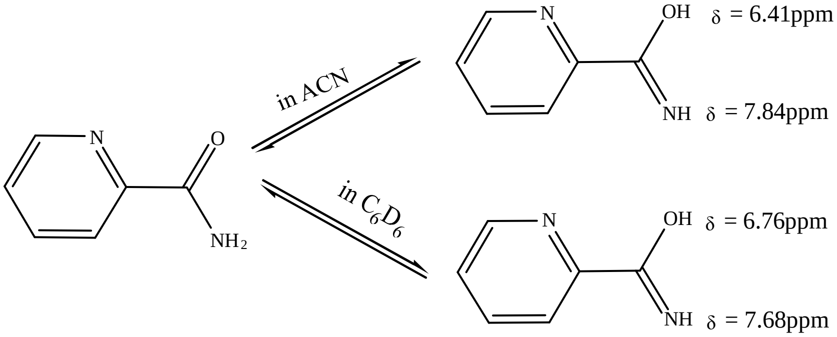
The slope of the peak separation for amide (figure 13) is negative, indicating that band 1 and band 2 converge, or that the two bands eventually fuse. This suggests that there is rotation on the amide bond. While the slope of peak separation in the amide is positive in both solvents (Figures 14 and 15), meaning that as the temperature rises, the peak separation increases and the band1 and band2 do not converge, this suggests that there is no rotation about the amide's C-N bond. The recommendations are as follows: At ambient temperature, the amide molecule exhibited two separate peaks that corresponded to the two magnetically nonequivalent protons of the amine group (figure 16).



nicotinamide at room temperature and nicotinamide at high temperature

Figure 16. The chemical shifts of nicotinamide at different temperatures.

Proton magnetic non-equivalence vanishes at high temperatures due to a fast rotation around the amide's C-N bond; as a result, the proton NMR spectra only show one peak (figure 16) O–H and ═N–H are proposed as the two bands' amides at high temperatures because imidic acid tautomers arise there and are advantageous. As a result, as Figure 17 illustrates, the peaks of the two protons are chemically distinct.



The tautomeric forms of nicotinamide's chemical changes are shown in Figure 17.

6.Conclusion

The study focused on analyzing tautomerism in nicotinamides using dynamic variable temperature NMR.

Two different peaks that separated from the protons of the amide molecules' amine groups in nicotinamide reduced in separation with increasing temperature, suggesting the presence of a rotating barrier around the nicotinamide bond.

This study involves analyzing the behavior of nicotinamide molecules at different temperatures and in various solvents using 1H NMR spectroscopy.

At 328 K, the two peaks were seen to combine. A rotational barrier of 17.9 kcal mol-1 was determined using the temperature-dependent spectra through-line shape analysis. On the other hand, two other peaks are proposed to represent the protons of the imidic acid tautomeric form of the amide at high temperatures, namely O–H and =N–H. The fact that these two peaks do not converge with temperature suggests no rotation around the nicotinamide bond.

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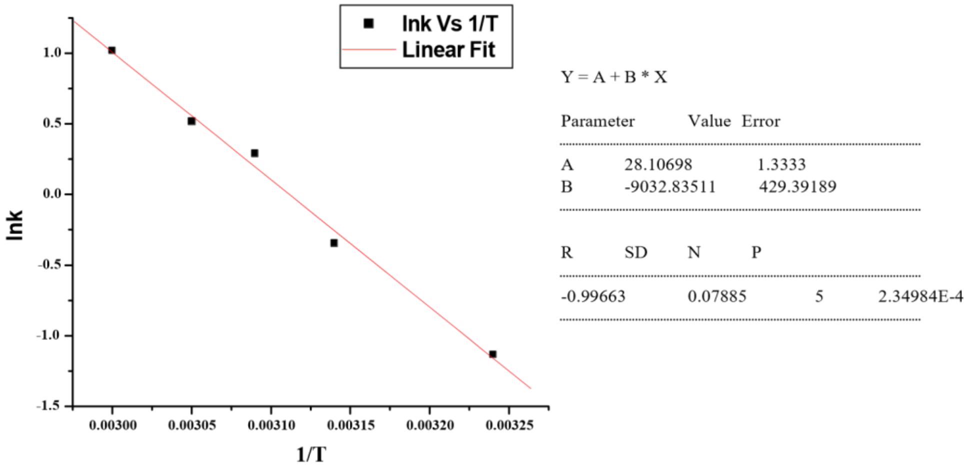
8. Appendices

Appendix A.



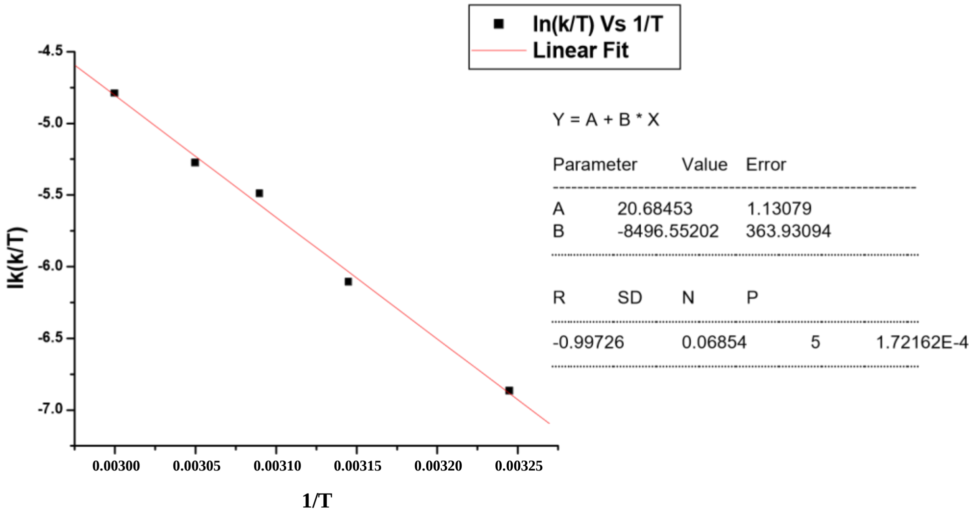
Y = A + B * X

Parameter	Value	Error
A	28.10698	1.3333
B	-9032.83511	429.39189

R	SD	N	P
-0.99663	0.07885	5	2.34984E-4

Figure19. ln k Vs 1/T for nicotinamide

Appendix B.



Y = A + B * X

Parameter	Value	Error
A	20.68453	1.13079
B	-8496.55202	363.93094

R	SD	N	P
-0.99726	0.06854	5	1.72162E-4

Figure 20. ln(k/T) Vs 1/T for nicotinamide.