

# Investigating The Molecular Dynamics Of Caffeine In Protic And Aprotic Solvents Using $^1\text{H}$ NMR Relaxation Measurements

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**Abstract.** Coffee is renowned for its rich composition of bioactive compounds, including caffeine. While the macroscopic effects of caffeine are well documented, a comprehensive understanding of molecular dynamics at the microscopic level remains limited, particularly regarding solvent effects and spin-lattice relaxation time ( $T_1$ ), which are critical for quality control/authentication of coffee. This study addresses this gap by developing a Benchtop Nuclear Magnetic Resonance (NMR) spectroscopy method to investigate the molecular dynamics of caffeine by determining the spin-lattice relaxation time ( $T_1$ ). Our approach focuses on comparing the  $T_1$  values of pure caffeine in two distinct solvent environments, the protic heavy water ( $\text{D}_2\text{O}$ ) and the aprotic deuterated chloroform ( $\text{CDCl}_3$ ). Experimental procedures involved preparing caffeine samples in both solvents and measuring  $T_1$  values using the inversion-recovery method with relaxation delays of 3, 5.5, 11, 20, and 25 seconds. Preliminary results show that the  $T_1$  value of caffeine is influenced by both the solvent type and the relaxation delay. Specifically, the  $T_1$  values in  $\text{CDCl}_3$  were more uniform than those in  $\text{D}_2\text{O}$ , suggesting substantial differences in the molecular interactions and motional dynamics of caffeine in these two environments. These findings can contribute to understanding the molecular dynamics of complex coffee, especially its caffeine behaviour.

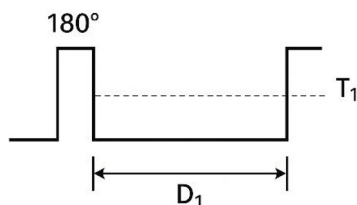
## 1 Introduction

Coffee is a globally significant commodity, valued not only for its economic impact but also for its role in health and wellness. As one of the world's largest coffee producers, Indonesia cultivates numerous varieties across its diverse archipelago. This geographical diversity yields coffees with unique flavor profiles, aromas, and a rich composition of bioactive compounds, making them a compelling subject for scientific research[1]. While not a primary source of macronutrients, coffee beans contain thousands of natural chemical constituents,

including lipids, carbohydrates, alkaloids, phenolic compounds, vitamins, and minerals. Among these, caffeine is recognized as a key compound responsible for many of coffee's physiological effects. It functions as a central nervous system stimulant, enhancing alertness and energy [2,3]. Beyond coffee, caffeine is also naturally present in tea and chocolate and is a common additive in energy drinks. In recent studies, caffeine in coffee has not been limited to molecular dynamics simulations; it has also been investigated using Nuclear Magnetic Resonance (NMR) spectroscopy to probe the real-time dynamics of components in solution.

NMR spectroscopy has been pivotal for the rapid identification and quantification of compounds with minimal sample preparation [4]. In previous limited studies, the 60 MHz benchtop measurements by Kent and Bell (2019) [5], have hinted at the value of relaxation data, a systematic link between solvent environments and caffeine dynamics is still lacking. On the other hand, caffeine in D<sub>2</sub>O has been analyzed by concentration-dependent chemical shift changes in the high-field <sup>1</sup>H NMR spectra of caffeine dissolved in D<sub>2</sub>O [6]. The study did not report on the solvent effect of caffeine solvation and T<sub>1</sub>. The present study performed an 80 MHz benchtop NMR to examine <sup>1</sup>H NMR and T<sub>1</sub>. Therefore, using low-field NMR in this study will reduce costs and be easier to maintain.

In an NMR study, solvent environments play a fundamental role in dictating caffeine's molecular mobility and intermolecular interactions, which are directly probed by NMR relaxation times (T<sub>1</sub>). Unlike simple extraction processes based on solubility differences [7]. The choice of solvent in NMR studies fundamentally alters the molecular dynamics of caffeine. Therefore, this study highlights the effect of solvent-solute interactions on molecular dynamics. Furthermore, accurate T<sub>1</sub> determination depends on optimizing experimental parameters, particularly the relaxation delay (D<sub>1</sub>) as represented in Figure 1 [14]. While general NMR protocols exist, the specific impact of varying the relaxation delay on the accuracy of caffeine's T<sub>1</sub> measurement has not been systematically established on benchtop instruments.



**Fig. 1.** Time-domain schematic of Free Induction Decay (FID) signal in NMR spectroscopy.

Several experimental parameters can influence the determination of relaxation times using NMR, including the number of scans, dummy scans, frequency offset ( $\omega_p$ ), spectral width (SW), relaxation delay, and acquisition time[5]. While the relaxation delay (D<sub>1</sub>) for optimizing T<sub>1</sub> measurements, the specific effect of varying this parameter on caffeine has not been systematically investigated. Therefore, this study will also investigate the effect of varying D<sub>1</sub> on the measured T<sub>1</sub> values of caffeine and evaluate the microscopic molecular dynamics of caffeine in protic (D<sub>2</sub>O) *versus* aprotic solvents (CDCl<sub>3</sub>) by analysing <sup>1</sup>H and T<sub>1</sub> data.

## 2 Research Methods

This research was a comparative quantitative approach to investigate the molecular dynamics of the bioactive coffee compound, caffeine. Pure caffeine samples were dissolved in two distinct solvents (protic and aprotic). The samples were analyzed using a benchtop

NMR instrument to obtain Proton NMR ( $^1\text{H}$  NMR) spectra and spin-lattice relaxation times ( $T_1$ ). The resulting data were analysed statistically and qualitatively to compare  $T_1$  values obtained with varying relaxation delay parameters within the same solvent, as well as to compare  $T_1$  values across different solvents.

The samples (51.5 mM) were prepared using the following procedure: 10 mg sample of pure caffeine (p.a., Tokyo Chemical Industry, Japan) was dissolved in 1 mL of either deuterium oxide ( $\text{D}_2\text{O}$ ) (Shandong Fan Tai Fine Chemical Biotechnology) or deuterated chloroform ( $\text{CDCl}_3$ ) (Shandong Fan Tai Fine Chemical Biotechnology). The mixtures were then ultrasonicated (Power Sonic 405) for 30 minutes to ensure complete dissolution. A 600  $\mu\text{L}$  aliquot of the supernatant from each solution was transferred into a 5 mm NMR tube for analysis.  $^1\text{H}$  NMR Spectra Measurement  $^1\text{H}$  NMR spectra were acquired using a BRUKER FOURIER 80 benchtop NMR spectrometer. Measurements were performed at 25  $^\circ\text{C}$  using the standard zg pulse program with a DQD acquisition mode, 8 scans, and 8192 data points (TD). Subsequently,  $T_1$  relaxation times were measured for caffeine in both  $\text{D}_2\text{O}$  and  $\text{CDCl}_3$  at identical concentrations. The measurements used the inversion recovery (t1ir) pulse program with a DQD acquisition mode, 2 dummy scans, and 16 scans. The relaxation delay ( $D_1$ ) varied using the following values: 3, 5.5, 11, 20, 25, and 30 seconds. Furthermore, the data was analyzed using Dynamic Center software from Bruker.

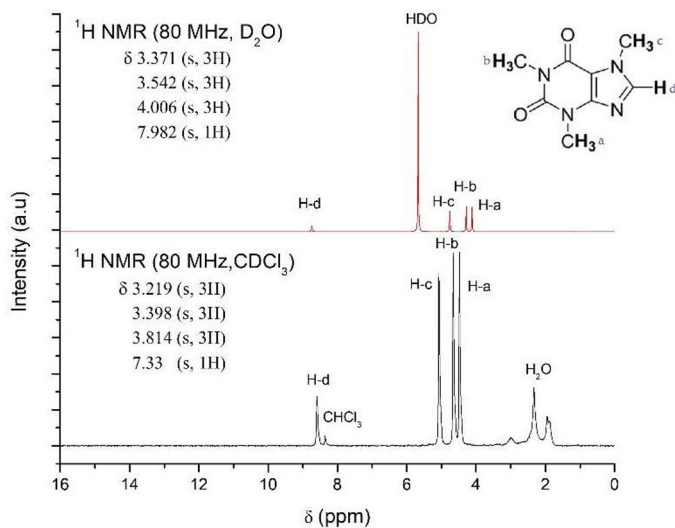
### 3 Results and Discussion

The  $^1\text{H}$ -NMR spectra of caffeine in  $\text{D}_2\text{O}$  and  $\text{CDCl}_3$  are directly compared in Figure 2, revealing four distinct proton environments corresponding to the caffeine structure: one vinylic proton on the imidazole ring (H-d) and three chemically inequivalent methyl groups (H-a, H-b, H-c). A consistent downfield shift is observed for all protons when the solvent is changed from  $\text{CDCl}_3$  to  $\text{D}_2\text{O}$ . Specifically, the aromatic proton H-d shifts significantly from  $\delta$  7.333 ppm in  $\text{CDCl}_3$  to  $\delta$  7.982 ppm in  $\text{D}_2\text{O}$ . Similarly, the methyl protons appear at higher frequencies in  $\text{D}_2\text{O}$  ( $\delta$  4.006, 3.542, and 3.371 ppm) compared to  $\text{CDCl}_3$  ( $\delta$  3.814, 3.396, and 3.219 ppm). The chemical shift differences due to the solvent effect are also found in the previous study [8].

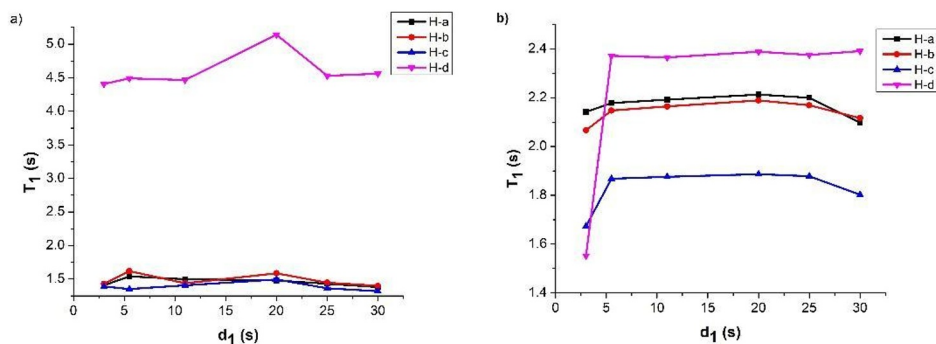
On the other hand,  $\text{D}_2\text{O}$  appears at  $\delta$  4.893 ppm, and  $\text{CDCl}_3$  appears at 1.981 and 7.111 ppm. These peaks do not overlap with other peaks. In  $\text{D}_2\text{O}$ , the formation of hydrogen bonds between water molecules and caffeine's polar carbonyl and nitrogen sites leads to a deshielding effect. In contrast,  $\text{CDCl}_3$  lacks hydrogen bond donor capability, so it is more shielded from protons. Our results obtained with an 80 MHz benchtop spectrometer are consistent with those reported in [9].

Regarding impurities, the  $\text{CDCl}_3$  spectrum in Figure 2 displays minor signals at  $\delta$  7.111 ppm and  $\delta$  1.981 ppm, attributed to residual non-deuterated chloroform ( $\text{CHCl}_3$ ) and trace water, respectively. Crucially, these impurities have no measurable impact on the  $^1\text{H}$  NMR result and the accuracy of the  $T_1$  relaxation measurements investigated in this study, because there is no signal overlapping or interfering [9].

The accuracy of spin-lattice relaxation time ( $T_1$ ) measurements is critically dependent on the relaxation delay ( $D_1$ ). As illustrated in Figure 3, measured  $T_1$  values increase with longer delays before plateauing. In both solvents, insufficient delays (e.g.,  $D_1 = 5.5$  s) result in artificially short  $T_1$  values due to incomplete magnetisation recovery [10]. For the longest  $T_1$  measured (H-d in  $\text{D}_2\text{O}$ , 5.14 s), a 20-second delay corresponds to approximately  $4 \times T_1$ , which provides a reasonable estimation of full recovery (98%), though ideal  $D_1$  recommends  $5 \times T_1$  ( $T_1$  is predicted) [10]. Consequently, the values obtained at  $D_1 = 20$  s are considered reliable within this experimental study.



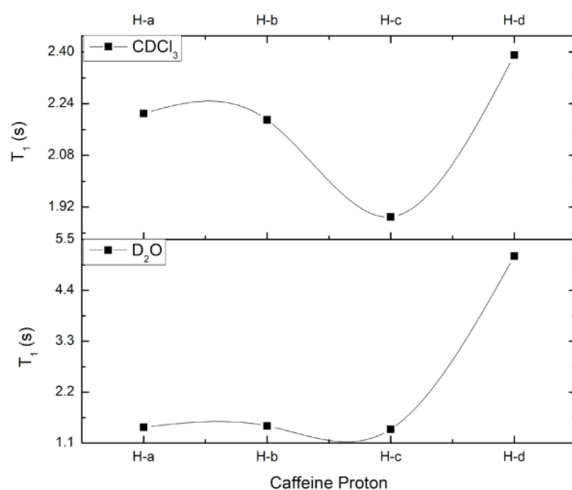
**Fig. 2.** <sup>1</sup>H-NMR results of caffeine solution in D<sub>2</sub>O and CDCl<sub>3</sub> solvent.



**Fig. 3.** (a) Variation of D<sub>1</sub> Value against T<sub>1</sub> value of caffeine in D<sub>2</sub>O, and (b) Variation of D<sub>1</sub> Value against T<sub>1</sub> value of caffeine in CDCl<sub>3</sub>.

The longitudinal relaxation times ( $T_1$ ) of caffeine exhibit clear differences between the N-CH<sub>3</sub> protons (H-a, H-b, and H-c) and the aromatic proton (H-d) when measured in D<sub>2</sub>O (higher viscosity, higher polarity, and stronger hydrogen-bonding capability) and CDCl<sub>3</sub>. The  $T_1$  values of H-a, H-b, and H-c are higher in CDCl<sub>3</sub>, whereas the  $T_1$  of H-d is higher in D<sub>2</sub>O. For the N-CH<sub>3</sub> protons (H-a, H-b, and H-c),  $T_1$  values are predominantly influenced by solvent viscosity (D<sub>2</sub>O > CDCl<sub>3</sub>); higher viscosity leads to shorter  $T_1$  values [11].

In contrast, the  $T_1$  value of proton H-d is mainly governed by solvent properties such as polarity and hydrogen bonding. Proton H-d is more polar and therefore more susceptible to dipole-dipole interactions with D<sub>2</sub>O, resulting in a higher  $T_1$  value in this solvent. Furthermore, hydrogen bonding involving H-d forms more readily with D<sub>2</sub>O, facilitating the formation of a hydration-like shell around the aromatic moiety and further increasing the  $T_1$  of H-d in D<sub>2</sub>O. In addition, proton H-d can undergo isotopic exchange with D<sub>2</sub>O more readily than with CDCl<sub>3</sub>. These observations confirm that solvent-induced differences in  $T_1$  values among caffeine protons strongly depend on the local structural environment of each proton and their specific interactions with the solvent.



**Fig. 4.**  $T_1$  of Caffeine in different solvents at optimized  $D_1$ .

Within the caffeine molecule, the  $T_1$  values of the four protons are also influenced by polarity effects. In  $D_2O$ , the polarity order of the protons is  $H-d > H-b > H-a > H-c$ . However, the corresponding  $T_1$  values follow the sequence  $H-d > H-a \approx H-b > H-c$  [12]. For protons H-a and H-b, the  $T_1$  values differ between solvents: in  $D_2O$ ,  $T_1(H-b) > T_1(H-a)$ , whereas in  $CDCl_3$ ,  $T_1(H-a) > T_1(H-b)$ . This behavior arises from differences in intrinsic proton polarity within the caffeine molecule, combined with the physicochemical properties of the solvent. Consequently,  $CDCl_3$  attenuates solvent effects on caffeine, thereby reducing the influence of the solvent environment on the determination of longitudinal relaxation times ( $T_1$ ). On the other hand, the results of  $T_1$  relaxation time were relevant to the previous study [5]. Furthermore, previous studies investigating the molecular dynamics of caffeine predominantly utilize  $^1H$  NMR spectroscopy demonstrated that in protic solvents (like water or  $D_2O$ ), the hydrophobic effect drives the formation of caffeine aggregates via  $\pi$ -stacking [13].

## 4 Conclusion

This study demonstrates that both the solvent and experimental parameters significantly influence the analysis of caffeine's molecular dynamics using  $^1H$ -NMR. The observed downfield shift of caffeine protons in  $D_2O$  compared to  $CDCl_3$  indicates distinct solvent-dependent interactions due to the chemical shift differences. Furthermore, the research confirms that a sufficiently long relaxation delay ( $D_1$ ) of 20 seconds is crucial for obtaining accurate spin-lattice relaxation times ( $T_1$ ), as shorter delays result in artificially low values due to incomplete magnetization recovery.

The molecular dynamics of caffeine were found to be highly dependent on the solvent environment. In  $CDCl_3$ , caffeine exhibits more uniform molecular motion, reflected in consistent  $T_1$  values across its protons. In contrast, the higher viscosity of  $D_2O$  and its capacity for hydrogen bonding restrict molecular movement, leading to a more efficient relaxation process and, consequently, shorter and more variable  $T_1$  values (H-a, H-b & H-c). Therefore, proton mobility in caffeine is not uniform; it is a specific interplay between the proton's structural position and the intermolecular forces (like aggregation) dictated by the solvent. This study used only pure caffeine. The conditions of caffeine within the actual coffee matrix are far more complex, as it interacts with numerous other compounds that can alter its molecular dynamics. Overall, the study validates NMR relaxation as an effective

method for investigating molecular dynamics and establishes it as the preferred approach for future analyses of compound interactions in complex coffee, particularly for quality control/authentication.

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