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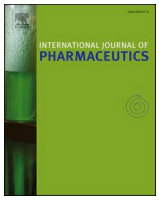

Mini review

Time domain NMR for polymorphism characterization: Current status and future perspectives

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ABSTRACT

Polymorphism is the ability of a compound to exist in multiple crystal forms while maintaining the same chemical composition. This phenomenon is reflected in different solid-state physicochemical properties due to variations in structural energy and the degree of lattice disorder. The pharmaceutical industry places significant emphasis on thoroughly characterizing polymorphism in Active Pharmaceutical Ingredients (APIs) because of its impact on the pharmacokinetic properties on the final medicine product. Standard characterization techniques are well documented in pharmacopeias and by international agencies. These techniques, whether applied individually or in combination, include crystallography (X-Ray Diffraction), thermal analysis (Differential Scanning Calorimetry), and various forms of spectroscopy, such as Near-Infrared, Raman, and solid-state Nuclear Magnetic Resonance (NMR). Analyzing NMR applications for solid-state characterization over the past five years, there has been a growing number of reports on the use of Time Domain NMR (TD-NMR) to evaluate polymorphism on APIs. Due to the increasing interest in this compelling technique, this study provides an overview of the current advancements in TD-NMR for polymorphism assessment in pharmaceutical products. Compared to high-field devices, TD-NMR has proven to be more convenient to industrial applications due to its smaller equipment size and shorter measurement times. This mini-review compares various applications of TD-NMR for API solid-state characterization and offer guidance for future research in this area.

1. Introduction

In 1998, the drug ritonavir drew the attention of the pharmaceutical industry to polymorphism in Active Pharmaceutical Ingredients (APIs). Ritonavir initially crystallized as Form I, but a few years later, a new, less soluble and more stable crystal form (Form II) emerged, causing the drug to fail dissolution tests and reducing its bioavailability. The widespread occurrence of Form II in production lines led to the withdrawal of the original formulations. Following extensive investigation and considerable expense, a new formulation was developed, submitted to the FDA, and successfully reintroduced to the market (Datta and Grant, 2004; Shi et al., 2022; Ticona Chambi et al., 2024b). This phenomenon is known as polymorphism, which is the ability of a compound to exist in multiple crystal forms while maintaining the same chemical composition (Bauer et al., 2001; Jeziorna et al., 2023).

Polymorphism results in different solid-state physicochemical properties, such as molecular packing, intermolecular interactions, refractive index, electrical conductivity, enthalpy and entropy values due to differences in structural energy and the degree of lattice disorder (Byrn et al., 1999; Li et al., 2022; Lu and Rohani, 2009). Additionally, these crystalline arrangements significantly influence the bioavailability, toxicity, and manufacturability of pharmaceutical products (Council of Europe, 2023; Dudek et al., 2021; Food and Drug Administration, 2007; Jiménez Cruz et al., 2021; Lu and Rohani, 2009; Pindelska et al., 2017). In this context, having a thorough understanding of the solid-state properties and structures of APIs is essential for fully grasping the concept of polymorphism (Bhatia et al., 2018; Shi et al., 2022).

In recent years, the scientific literature has increasingly focused on the analytical methods for characterizing the polymorphism of APIs (Cerreia Vioglio et al., 2017; Lu and Rohani, 2009). This growing attention is highlighted in a review by Pindelska and colleagues, which outlines the most common analytical techniques used over the past decade to examine solid-state drug forms (Pindelska et al., 2017). We also recommend the review paper of Chieng and coworkers that

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summarize polymorphism characterization methods on molecular, particulate and bulk levels, including those methods reported in the to a further comprehension on this matter (Chieng et al., 2011). Standard characterization procedures are also extensively documented in pharmacopeias and by international agencies, such as the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Food and Drug Administration (FDA). These guidelines are essential for ensuring robust quality control and consistency throughout the pharmaceutical pipeline, from raw materials to the final product. By adhering to these standardized practices, manufacturers can ensure the uniformity of bulk ingredients, optimize the manufacturing process, and guarantee the quality, safety, and efficacy of the final pharmaceutical products (Almeida et al., 2024; Ticona Chambi et al., 2024b).

Many different, advanced characterization techniques are employed to identify and differentiate polymorphs, each providing unique information about the solid-state properties of a compound, often used in combination (Cerreia Vioglio et al., 2017; Hao et al., 2020; Lu and Rohani, 2009; Pindelska et al., 2017). The most critical aspects of pharmaceutical characterization include solubility behavior, stability, bioavailability, and pharmacokinetic profiles (Pindelska et al., 2017). At the molecular level, APIs are typically characterized using spectroscopic methods such as Infrared (IR), Raman, and Nuclear Magnetic Resonance (NMR) spectroscopy. Solid-state properties of individual particles are often investigated using techniques like X-ray diffraction (XRD), Powder X-ray Diffraction (PXRD), Scanning Electron Microscopy (SEM), and Small Angle X-ray Scattering (SAXS). Thermoanalytical and gravimetric techniques, including Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and Hot-Stage Microscopy (HSM), provide valuable insights into the thermal and morphological behavior of compounds. For bulk-level characterization, which focuses on the properties of particle assemblies, techniques such as the Brunauer-Emmett-Teller (BET) method for surface area analysis, Karl Fischer titration for moisture content, and density measurements are commonly employed (Chieng et al., 2011). Among these techniques, the most commonly used compendial methods for characterization are X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Near-Infrared (NIR) Spectroscopy, Raman Spectroscopy, and Nuclear Magnetic Resonance (NMR) (Tian et al., 2020; Ticona Chambi et al., 2024b).

The XRD technique assesses a variety of solid-state characteristics such as crystalline phase, size of the sample's unit cell, hydrogen bond arrangement, morphology and porosity (Bhatia et al., 2018; Cerreia Vioglio et al., 2017). This analysis provides valuable information about individual polymorphs or mixtures of crystalline phases, serving as a fingerprint of the sample through multiple peaks that are compared to a standard reference (Pindelska et al., 2017). The quantitative analysis offers the advantage of not requiring a calibration curve, though it is subject to interference from factors such as preferred orientation of the crystalline arrangement, particle size, humidity, and potential phase transitions induced during sample preparation (Ticona Chambi et al., 2024b).

DSC is a technique that provides quantitative and qualitative solid-state characterization for measuring heat quantity (enthalpy) changes (Bhatia et al., 2018; Ministry of Food and Drug Safety, 2020). The properties evaluated include the transition temperature and/or melting points, allowing for the characterization of different polymorphs. It is recommended by several pharmacopeias as an assay for determining a sample's purity (Ticona Chambi et al., 2024b). However, this type of analysis encounters challenges in resolving overlapping thermal events occurring at the same temperature (Chieng et al., 2011).

The vibrational spectroscopy analysis, NIR, evaluates the vibrational and rotational states of the atoms in the API molecule, providing information on inter- and intramolecular C–H, N–H, O–H, and S-H bonds (Chieng et al., 2011; Council of Europe, 2023; Ticona Chambi et al., 2024b). A major limitation of vibrational methods includes low sensitivity, baseline slope issues and the need for chemometric tools for data analysis. Raman spectroscopy, on the other hand, is based on the the scattering of a monochromatic light beam on a sample, offering insights into rotational, vibrational, and low-frequency phenomena. However, some challenges in using Raman spectroscopy for solid-state characterization include local heating of the sample, interference from analytes with fluorescence and the masking of Raman scattering by other interferences (e.g. ambient light) (Bhatia et al., 2018; Ticona Chambi et al., 2024b).

High-resolution, high-field ^{13}C solid-state NMR spectroscopy enables the identification of distinct polymorphs. This technique provides information about molecular dynamics, due to anisotropic interactions of the molecules under analysis (Bhatia et al., 2018). In addition to its high sensitivity, the differences in chemical shifts, related to intermolecular packing make this analysis user-friendly and valuable for differentiating mixtures and pure forms of API (Dudek et al., 2021). However, since it primarily relies on the cross-polarization (CP) sequence, the signal areas of the polymorphs may not correspond to their concentration. For quantitative analyses, excitation directly at the ^{13}C resonance frequency can be used, although these analyses are very time-consuming compared to those using CP. An advantage of ^{13}C solid-state NMR over XRD or infrared spectroscopy is that it does not require consideration of effects related to particle size concerning the intensity of the analyzed signal (Campbell, 1998). In contrast, the literature also reports using ^{19}F in solid-state NMR spectroscopy for polymorphic characterization. Major advantages of this approach include the 100 % isotopic abundance of ^{19}F and the fact that almost no pharmaceutical excipient contains fluorine atoms in its structure. Brus and coworkers (2011) demonstrated that ^{19}F MAS NMR analysis is capable of both qualitative and quantitative polymorph analysis of atorvastatin, marking a first in this type of study. However, the authors identified limitations, including poorly resolved spectra even when data is acquired at high spinning rates (Brus et al., 2011). For a deeper understanding of solid-state ^{19}F NMR spectroscopy in pharmaceutical characterization, we recommend the review of Du and Su (Du and Su, 2022).

Nevertheless, an analysis of NMR applications for solid-state characterization over the past five years reveals an increase in reports on the use of Time Domain NMR (TD-NMR) to evaluate polymorphism in APIs. Due to its high sensitivity to molecular mobility, TD-NMR can detect dynamical changes associated with thermal transitions, the ratio between rigid and mobile segments, emulsion sizes of molecular clusters, the degree of crystallinity, and crystallite sizes in semi-crystalline systems (Dos Santos Garcia et al., 2022). The main advantages of TD-NMR listed in the literature are its low-cost instrumentation, minimal maintenance requirements, and the fact that it does not necessitate sample derivatization or result in the destruction of samples. It eliminates the need for cryogenic gasses allowing relatively fast analysis in solid and liquid matrices compared to high-field NMR as well as providing the flexibility to relocate the equipment to various locations within an industrial facility (Grunin et al., 2023; Kock and Colnago, 2016; Okada et al., 2019a; Schumacher et al., 2017).

Due to the growing interest in this promising technique, this paper presents an overview of the current advancements in TD-NMR for polymorphism assessment in pharmaceutical products. We aim to compare the various applications of TD-NMR for API solid-state characterization and provide guidance for future research in this area.

2. API polymorphism: Concerns on bioavailability

Since the 1950s, researchers have observed a correlation between the dissolution rate and the bioavailability of a drug. L. J. Edwards was one of the first researchers to establish a connection between the gastrointestinal absorption of a drug in its solid state and its dissolution rate. Decades later, in the mid-1970s, bioavailability was defined as the fraction of a dose reaching the circulatory system (Dokoumetzidis and Macheras, 2006). Therefore, a dependent relationship is observed between bioavailability and solubility, with the latter being a

characteristic that influences its dissolution.

Amidon and co-workers (1995) proposed a biopharmaceutical classification system model (Biopharmaceutics Classification System – BCS) that relates the solubility and permeability of a drug in vivo (Fig. 1) (Amidon et al., 1995). In this system, four classes are represented by Roman numerals to estimate the in vitro-in vivo (IVIV) correlation. Class I refers to drugs with high solubility and high permeability. The only limitation to bioavailability in this case would be rapid gastric emptying, provided the dissolution rate is fast. For Class II, drug dissolution controls the absorption (permeability) rate, unless the dose is very high. In Class III, absorption determines the rate, and there is limited IVIV correlation with the dissolution rate. In Class IV, the IVIV correlation is either limited or not expected (Amidon et al., 1995). Currently, the FDA considers substances with high solubility to be those that dissolve in $\leq$ 250 mL of aqueous medium with a pH between 1 and 6.8 at 37 °C $\pm$ 1 °C. High permeability substances are those whose absorption is 85 % or more of the administered dose, based on mass balance or compared with the corresponding intravenous dose (Charalabidis et al., 2019).

For a drug whose absorption is limited only by its dissolution, as in classes II and IV, large differences in the apparent solubilities of various polymorphic forms are likely to affect the bioavailability of an API. Furthermore, when the apparent solubilities of the polymorphic forms are sufficiently high and drug dissolution is rapid compared to gastric emptying, it is unlikely that discrepancies in solubility will significantly impact bioavailability (Food and Drug Administration, 2014).

It is estimated that 50 % of crystalline API exhibit polymorphism (Jiménez Cruz et al., 2021; Lin, 2014). The International Pharmacopoeia lists 35 APIs that present polymorphism, as described in the "Pharmaceutical Substances" subsection of its monograph (World Health Organization, 2022). A major concern regarding polymorphic forms in pharmaceutical products is the potential loss of bioavailability and efficacy due to specific non-active polymorphic forms. For example, the anthelmintic drug Mebendazole (MBZ; methyl N-(6-benzoyl-1H-benzimidazol-2-yl)carbamate), an essential medicine by the World Health Organization (WHO) for treating worm infections (World Health Organization, 2022), has three polymorphic forms. These polymorphs vary in solubility, with form B being the most soluble, followed by form C, and then form A. However, the higher solubility of form B also results in increased toxicity, making form C the preferred choice in clinical settings, as it offers sufficient solubility for optimal bioavailability. This preference is particularly important because polymorph A lacks anthelmintic activity on its own or when it constitutes more than 30 % of polymorphic mixtures (Aboul-Enein et al., 2002; Brits et al., 2010; Brusau et al., 2008; Kumar et al., 2008) (Fig. 2). Studies show that form B tends to transform quickly into form C, but form C takes longer to form A (Brits et al., 2010). This transition to the most stable form results in the loss of API activity on biological systems (Brits et al., 2010; Rodriguez-Caabeiro et al., 1987).

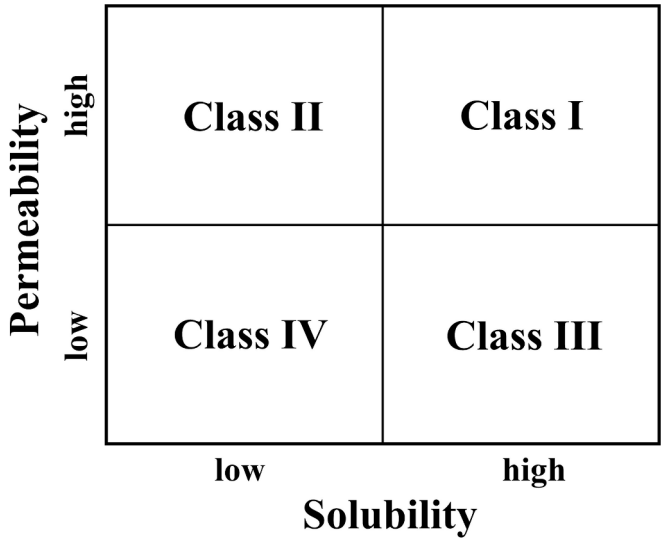

Fig. 1. Biopharmaceutical Classification System illustrating the different drug classes based on their permeability and solubility.
Adapted from Charalabidis et al. (2019).

The findings of Chambi (2024) indicate that commercialized samples of medicines containing MBZ, even within the expiration date, contained the API in the inactive form (MBZ A). Out of eight samples analyzed, seven presented a mixture of polymorphic forms A and C, while one did not present either form (Ticona Chambi et al., 2024a). The presence of Form A in commercially available products highlights the ongoing issue of polymorphisms in APIs within the pharmaceutical industry. Considering this study, two main causes can be identified for the identification of MBZ A in commercial tablets: similar to Ritonavir case, there may have been cross-contamination of the metastable form with the more stable form, with the most stable form potentially acting as a seed or accelerator in the transformation process between polymorphic forms (Byrn et al., 1999; Shi et al., 2022).

API polymorphs can also suffer the influence of the formulation components, process, and storage conditions. Thermally-induced transformations were previously investigated and associated with the dynamics of solvent molecule release from within the crystal lattice (Du et al., 2015; Terra and Poppi, 2014; Urbanova et al., 2016). Solvent interactions during the crystallization process (such as hydrogen bonding, π–π stacking, and ionic interactions) are also critical factors in API polymorphism, as observed in the manufacturing processes of diflunisal, dipyridodiazepinone, and erythromycin (An et al., 2012; Thakral et al., 2019). Muselik and colleagues (2021) investigated anticoagulant API formulations (containing amorphous and crystalline warfarin) and suggested that excipients could induce ion exchange within crystal lattice structures. This type of polymorphic transformation may occur not only during formulation or manufacturing stages but also during storage, affecting warfarin tablet stability and dissolution rate (Muselík et al., 2021; Tian et al., 2020). Industrial processes involving physical stress (e.g. granulation, drying, grinding, milling, micronization, and compression) may also be listed as causes of polymorph transformation (Chatziadi et al., 2020; Gorkovenko et al., 2015; Lin, 2014). Examples regarding compression-induced polymorphic changes include the partial amorphization in tablets of theophylline, nitrofurantoin, and amlodipine besylate, meanwhile, compression of indomethacin, sucrose, celecoxib induced its crystallization, in all cases affecting API's bioavailability (Thakral et al., 2019).

3. Time-Domain Nuclear Magnetic Resonance fundamentals

NMR spectroscopy can be divided into two branches of applications: high-resolution NMR using high-field devices and low-resolution NMR using low-field devices. High-resolution equipment ($B_0 > 4.7$ T) with high homogeneity ($\Delta B_0 < 0.01$ ppm for a sample of approximately 500 μL) uses superconducting magnets(Colnago et al., 2014; Monaretto et al., 2020). Research using this type of NMR can be further subdivided into two categories: solution NMR and solid-state NMR. Solution NMR analyses are widely used in various fields, including organic chemistry for structure elucidation, natural products, inorganic chemistry, physical chemistry, environmental chemistry, biochemistry, omic sciences, and the food and petroleum industries (Anaraki et al., 2021; Facchinatto et al., 2021). Recent advances have introduced medium and low-resolution benchtop NMR spectrometers, ranging from 1 to 2.3 T. These benchtop instruments have been used in some applications similar to those of high-resolution NMR (Downey et al., 2024; Jenne et al., 2024). In contrast, solid-state NMR, typically performed at high fields ($B_0 > 4.7$ T), provides information about the molecular structure and dynamics in solid-state materials. It is applied to the study of synthetic and natural polymers, catalysts, and other products (Nikolskaya and

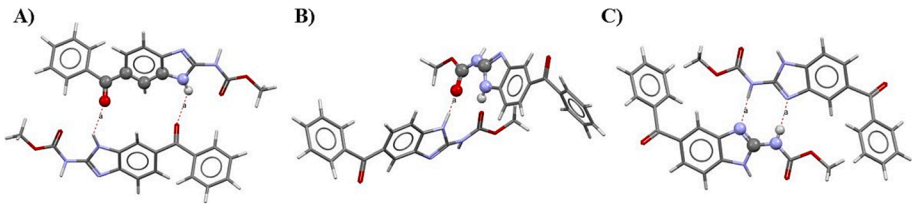

Fig. 2. Different polymorphic forms of MBZ. A) Form A, less soluble and more stable form (CSD code TUXPEJ); B) Form B, most unstable form (CSD code YULGIW02); C) Form C, metastable form with greater therapeutic activity (CSD code YULGIW).

Hiltunen, 2020).

On the other hand, TD-NMR or NMR relaxometry, often described as low-field ($B_0 < 0.7$ T), low-resolution ($B_0 > 100$ ppm), or benchtop NMR, relies on permanent magnet instrumentation (Fig. 3) (Almeida et al., 2024). For a detailed understanding of both methodologies, we recommend the comprehensive review of NMR spectroscopy and relaxometry fundamentals by Colnago et al. (Colnago et al., 2021, 2014). Compared to high-resolution devices, TD-NMR has proven to be more convenient for industrial applications due to its smaller equipment size and shorter measurement time. The liquid and solid-state characteristics measured by TD-NMR arise from different relaxation times – the irreversible evolution of a nuclear spin system toward thermal equilibrium and the decay of the NMR signal due to coherence loss (Goldman, 2001; Nagel et al., 2021). Therefore, the investigation of amorphous and crystalline systems is feasible due to the differing molecular spin dynamics. As an example, in dosage formulation, the T_1 may be reduced for smaller crystallites due to ^1H–^1H spin diffusion processes from surrounding excipients. Moreover, it is noteworthy to mention that particle size can also affect the behavior of ^1H T_1, as a result of interactions with surface oxygen present in the atmosphere (Dempah et al., 2017; Maus et al., 2006; Policianova et al., 2014).

Longitudinal relaxation (T_1), or spin–lattice relaxation, is characterized by the return of magnetization to the thermal equilibrium state given by Boltzmann distribution. Meanwhile, transverse relaxation (T_2), or spin–spin relaxation, is characterized by the loss of magnetization coherence on the xy' plane (Colnago et al., 2021; Goldman, 2001; Nagel et al., 2021). Furthermore, longitudinal relaxation in the rotating or rotating frame ($T_{1\rho}$) refers to the relaxation of spins in the presence of an oscillating magnetic field B_1 (Bugay, 1993; Stueber and Jehle, 2017). Unlike T_1 and T_2, where sensitivity to molecular mobility is dictated by local spin interactions, $T_{1\rho}$ also depends on the intensity of the oscillating field B_1 which can be adjusted to suit the changing driving frequency window to which $T_{1\rho}$ is most sensitive (Almeida et al., 2024). For a detailed *ab initio* understanding on the mentioned relaxation process we recommend the work of Goldman (Goldman, 2001). Moreover, the relaxation rates of T_1, T_2 and $T_{1\rho}$, the magnitudes relaxational signals, and the pulsed field gradient (PFG) diffusion coefficients are information that could be extracted from TD-NMR data and are vastly reported on crude oils research (Nikolskaya and Hiltunen, 2020). Once the appropriate experiment is chosen, it is important to optimize the acquisition parameters. Performing an initial screening to determine an approximate value of T_1 guarantees an estimative determination of the time required to the magnetic momenta return to thermal equilibrium magnetization (M_0) (Colnago et al., 2014). Furthermore, determining the optimal number of scans and number of points relates directly with the experiment signal to noise ratio (SNR). In general, increasing the number of scans per number of points has a better impact on accuracy, and therefore a better SNR, when compared to just increasing the number of points (Stueber and Jehle, 2017).

In the context of TD-NMR data processing, it is important to note that it differs from high-field NMR experiments because there is no need for Fourier Transform (FT) to convert data to the frequency domain (Fig. 4) (Garcia et al., 2019). As a result, extracting relaxation values involves using fitting algorithms such as exponential fitting, multiexponential fitting, Levenberg-Marquardt, or Laplace Inverse Transform (Colnago et al., 2021; Moraes, 2021; Nikolskaya and Hiltunen, 2020). Therefore, choosing the best data processing is an important step to extract the most valuable chemical information from the dataset. Additionally, depending on the study, data may be analyzed using univariate or multivariate chemometric tests for regression, exploratory, or classification analysis.

In the industrial sector, TD-NMR has validated methodologies for analyzing oil content in seeds, solid fat content in lipids, hydrogen content in gasoline, and fluorine content in toothpaste, among others. This demonstrates its potential as an inline/atline method for quality control (Colnago et al., 2014). In the pharmaceutical industry, TD-NMR has been used to characterize multi-component systems in medicinal plants, ensure quality control, detect counterfeit medicines and illegal drugs, and perform characterization studies on pharmaceutical formulations (Adels et al., 2023; Danoun et al., 2023; Fanesi et al., 2018; Pagès et al., 2014; Preto et al., 2013; van Beek, 2021). Additionally, in TD-NMR investigations on the ^{19}F nuclei, Elipe and coworkers developed and validated a quantitative methodology for determining ^{19}F in medicines that contain fluorine in the API (Elipe et al., 2017; Silva Elipe et al., 2016).

4. TD-NMR applications to polymorphism characterization reported on literature

Table 1 provides a chronological overview of studies assessing API polymorphism through the use of TD-NMR. The table highlights the APIs that were evaluated and the corresponding relaxation processes that were measured.

5. TD-NMR for polymorphism analysis on API

From 2017 onwards, the ^1H nucleus began to be investigated to determine relaxation curves of hydrated/anhydrous compounds. This study was the first to analyze the hydration of pharmaceutical products by TD-NMR and proposed a quantification model for binary mixtures of hydrates and anhydrates (Schumacher et al., 2017). In the same year, Dirk Stueber and Stefan Jehle (2017) used a Saturation-Recovery (SR) sequence to quantify fractions of polymorphs in APIs. Their proposed methodology, patented by Bruker (Stueber and Jehle, 2017), introduced a TD-NMR protocol for evaluating pharmaceutical products. They demonstrated that the T_1 and T_2 relaxation processes are involved with the molecular dynamics associated with different crystalline arrangements in samples of Ibuprofen, Indomethacin and Itraconazole APIs. SR curves for both pure components and mixtures were measured, with the relative amounts of mixture components determined by fitting the SR curve of the mixture to a linear combination of the weighted SR curves of the pure components (Stueber and Jehle, 2017). To date, this is the only study documented in the literature that employs a validated protocol for distinguishing API polymorphism using TD-NMR. However, the results presented are not suitable for a pharmacopeial quantification methodology due to insufficient investigation into the precision and quantitation limits (United States Pharmacopoeia, 2024).

Okada and co-workers (2019) found that the transverse relaxation

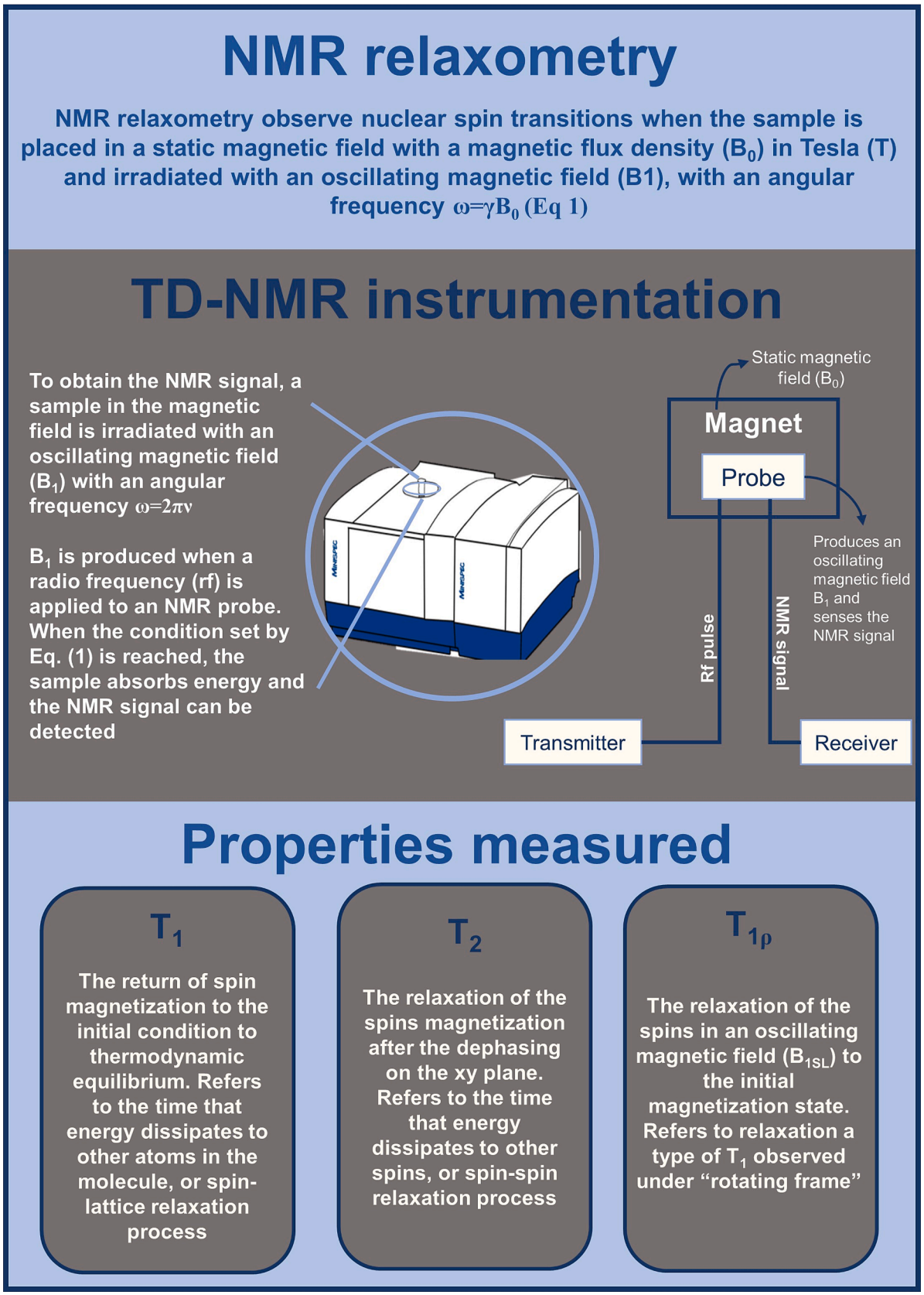

Fig. 3. Diagram illustrating the principles of NMR relaxometry, including a schematic of TD-NMR instrumentation for benchtop equipment. The figure also depicts the relaxation processes that can be evaluated using this technique.

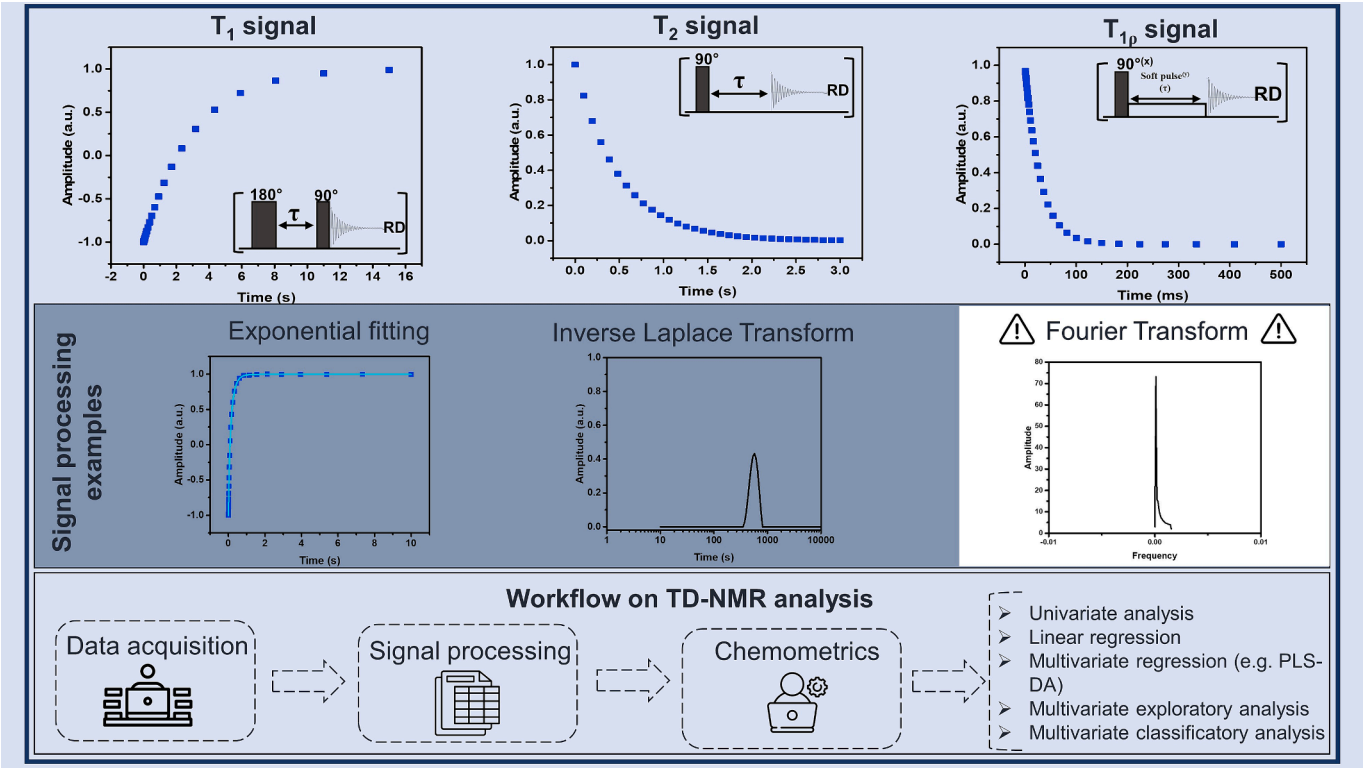

Fig. 4. Signal profile on different TD-NMR experiments. The pulse sequence for the T1 signal refers to the Inversion-Recovery experiment. The pulse sequence for the T2 signal refers to the Bloch-Decay experiment. The pulse sequence for the T1ρ signal refers to the Spin-Lock experiment. Below, examples of data processing of T1 relaxation signal for a Tylenol emulsion sample. Proposition of a general workflow for TD-NMR analysis.

time (T_2) of the liquid component (water) of indomethacin suspensions could differentiate between the amorphous and crystalline states of the API. This change in relaxation time is attributed to variations in the surface area of the suspended solids. T_2 of water molecules depends on the time and number of molecules interacting with the surface of the suspended particles. Thus, a smaller surface area of the suspended solid results in a higher T_2 (Okada et al., 2019c). In a 2021 study, Okada and co-workers also used T_2 values to study agglomeration in Indomethacin suspensions. Larger agglomerates result in a decreased surface area of the solids and consequently a higher T_2 (Ohgi et al., 2021; Okada et al., 2019b). In a recent study, Chiba and coworkers demonstrated the utility of partial least squares (PLS) regression with T_2 TD-NMR relaxation data. They used parameters such as the coefficient of determination (r^2), root mean square error of prediction (RMSEP) and variable importance plots (VIP) parameters to evaluate the regression performance. They found that PLS regression on T_2 data is particularly useful for assessing the presence of of additional excipients in tablets. However, its applicability is limited to situations involving only two components, such as altering the solid form of the API without affecting the crystalline form of the excipient (Chiba et al., 2023).

Additionally, Pajzderska and Wąsicki (2021) tested the SR pulse sequence proposed by Steuber and Jehle, as well as a solid echo sequence. They analyzed systems containing amorphous solid dispersions or solid mixtures of three or four components, such as felodipine, carbamazepine, and indomethacin, confirming that T_1 is an appropriate parameter for analyzing three- and four-phase mixtures and can be extended to dispersions containing amorphous solids (Pajzderska and Wąsicki, 2021). Meanwhile, Sinyavsky and Mershiev (2023) evaluated the molecular dynamics of T_1 and T_2 in paracetamol and sulfanilamide polymorphs (Sinyavsky and Mershiev, 2022).

Furthermore, Almeida and coworkers (2024) explored a novel approach to differentiate polymorphic forms by investigating ^1H $T_{1\rho}$ relaxation profiles of different MBZ forms. Their study demonstrated that $T_{1\rho}$ measurements with low spin-locking field strengths offer significant advantages over T_1 measurements for solid-state characterization of API polymorphism, due to their dependence on both $^1H{-}^1H$ dipolar coupling and spin-locking field strengths, rather than solely molecular mobility at a specific frequency scale as in T_1 measurements (Almeida et al., 2024). They also tested samples at different ν_1 resonance frequencies and in mixture of solid forms to evaluate the Limit of Detection (LOD) for these measurements. The reported value of 9 % w/w falls within the LOD range established by official methods such as vibrational spectroscopy and solid-state NMR (Ticona Chambi et al., 2024b). However, achieving a lower LOD would enhance the competitiveness of the proposed method compared to existing validated techniques.

TD-NMR relaxation processes evaluated so far were able to determine in a molecular level differences on the crystal lattice structure of API. That occurs due to the ability of this technique to investigate molecular process related to the enthalpy (through T_1 or $T_{1\rho}$) or entropy (through T_2) of an API spin system (Dos Santos Garcia et al., 2022). Suspension systems are specifically well-defined by T_2, as it reflects the interactions of water with bulk API or API formulations during the relaxation process (Ohgi et al., 2021; Okada et al., 2021, 2019b; Tsuji et al., 2023). Based on the papers reviewed, there are several advantages to utilizing TD-NMR as an industrial inline method for characterizing API polymorphism. These advantages include a reduced analysis time in comparison to solid-state NMR, it allows identification of crystalline/amorphous or hydrates/anhydrous components, the ability to evaluate the bulk API or the final product (such as tablets or suspensions), the straightforward equipment instrumentation, low-cost equipment and the possibility to move it to different facilities on an industrial production line. On the other hand, we could list as disadvantages the lack of representative validation studies, which may pose a significant barrier to the broader adoption of this method in pharmaceutical industrial applications. To address this challenge, it is essential to validate

Table 1
Description of studies investigating TD-NMR relaxation process in pharmaceutical products.

Year	Title	Reference	Sample	Objective	Relaxation process observed	Outcomes and insights for pharmaceutical practices
2017	Time domain NMR as a new process monitoring method for characterization of pharmaceutical hydrates	(Schumacher et al., 2017)	Theophylline and caffeine (as hydration carriers)	Obtain a distinct relaxation signal from the hydration water within the sample; quantitatively determine the amount of hydrated water in binary mixtures of hydrates in anhydrous samples	^{1}H T_1 and T_2	Analysis of hydrates and the generation of relaxation curves for hydrate/anhydrous transitions to assess pharmaceutical products quality control
2019	^{1}H NMR Relaxation Study to Evaluate the Crystalline State of Active Pharmaceutical Ingredients Containing Solid Dosage Forms Using Time Domain NMR	(Okada et al., 2019c)	Carbamazepine and Indomethacin	Determine whether TD-NMR could identify the crystalline and amorphous forms of powdered API; determine whether the ability to identify the crystalline and amorphous forms remained effective even if/when APIs were incorporated into solid oral dosage forms	^{1}H T_1	TD-NMR presented the ability to differentiate between crystalline and amorphous states of API, even when the API are present in solid pharmaceutical formulations
2019	T_2 relaxation study to evaluate the crystalline state of indomethacin containing solid dispersions using time-domain NMR	(Okada et al., 2019b)	Indomethacin and polyvinylpirrolidone (PVP)	Evaluate the API crystalline state T_2 in solid pharmaceutical forms, including solid dispersions, in order to quantify the API content in samples; determine whether T_2 is able to differentiate samples as amorphous forms and crystalline forms	^{1}H T_2	The T_2 relaxation processes have demonstrated the ability to distinguish between the amorphous and crystalline forms of indomethacin. Furthermore, they have proven to be effective in monitoring the transformation from amorphous to crystalline during thermal stress testing
2020	Quantitative Evaluation of the Crystallinity of Indomethacin Using ^{1}H T_2 Relaxation Behaviors Measured by Time Domain NMR	(Okada et al., 2020)	Amorphous and crystalline binary mixtures of indomethacin with microcrystalline cellulose, crospovidone and magnesium stearate	Demonstrate the usefulness of T_2 relaxation as a quantitative assessment for API crystallinity with small differences in T_2 values of its amorphous crystalline forms	^{1}H T_2	Based on the T_2 relaxation values obtained, authors were able to accurately assess the sample's amorphous content, demonstrating great precision on quantitative measurements
2021	Nondestructive Investigation of the Agglomeration Process for Nanosuspensions via NMR Relaxation of Water Molecules	(Okada et al., 2021)	Indomethacin nanosuspension with PVP and poloxamer 407	Investigate whether TD-NMR is capable of assessing the nanosuspension agglomeration process	^{1}H T_2	T2 relaxation of water molecules was capable of evaluating the agglomeration process of the indomethacin-PVP nanoparticle suspension
2021	Time-domain NMR analysis for the determination of water content in pharmaceutical ingredients and wet granules	(Ohgi et al., 2021)	Acetaminophen in different solid mixtures with excipients	Assess water content in solid drug preparations	^{1}H T_2	T_2 values showed accuracy in measuring the moisture content in granules
2022	Low-Field NMR to Characterize the Crystalline State of Ibuprofen Confined in Ordered or Nonordered Mesoporous Silica	(Okada et al., 2022)	Ibuprofen	Characterize the crystalline state of a drug confined in ordered mesoporous to generate a supersaturated profile using low-field TD-NMR.	^{1}H T_1	The T_1 relaxation measurements of drug-loaded silica may characterize samples with different dissolution profiles
2023	Continuous Monitoring of the Hydration Behavior of Hydrophilic Matrix Tablets Using Time-Domain NMR	(Tsuji et al., 2023)	Diltiazem incorporated into hydrophilic matrices	Establish a TD-NMR procedure to monitor the hydration behavior of hydrophilic matrix tablets	^{1}H T_2	It was possible to monitor the hydration behavior of hydrophilic matrix tablets in a non-destructive, continuous and precise way
2023	Usefulness of Applying Partial Least Squares Regression to T_2 Relaxation Curves for Predicting the Solid form Content in Binary Physical Mixtures	(Chiba et al., 2023)	Mixtures of Indomethacin and PVP	Apply partial least squares (PLS) regression to TD-NMR data to quantify moisture-absorbed samples	^{1}H T_2	The authors were able to build a PLS with T_2 TD-NMR relaxation curves. The results demonstrate a promising tool for predicting API mixtures
2024	NMR longitudinal rotating frame relaxation time (T1rho) with week spin locking field as an approach to characterize solid-state active pharmaceutical ingredients: proof of concept	(Almeida et al., 2024)	Mebendazole solid forms A and C	Demonstrate that $T_{1\rho}$ measurements, in weak spinlock B_1 fields, could be effectively used to differentiate API	^{1}H T_1, T_2 and $T_{1\rho}$	$T_{1\rho}$ measurements were able to characterize API solid-state properties. Besides, authors reported a limit of detection of 18.07 mg in 200 mg from a polymorph mixture of Mebendazole forms

protocols based on the literature reports on API relaxation process to polymorphic characterization. We recommend that future studies concentrate primarily on optimizing analytical conditions and data processing methods to enhance performance indicators, as well as evaluating the variance of relaxation under various conditions, such as B_0, B_1 and temperature.

6. Future perspectives

This review underscores the potential of TD-NMR for evaluating polymorphism in pharmaceutical products, positioning it as a promising tool for routine application in quality control within laboratories and industry. To solidify TD-NMR as a reliable method in these settings, further development and rigorous validation of these methodologies are essential, particularly to address current limitations such as sensitivity to certain polymorphs and potential susceptibility to interpretation errors. Key areas that require careful attention include ensuring robust calibration for different polymorphic forms, optimizing experimental parameters for accurate detection, and standardizing protocols to minimize inconsistencies in data interpretation. Refining this approach, along with conducting extensive comparative studies, could broaden its applicability. Future research should focus on addressing these challenges, enabling a sustainable, cost-effective alternative for polymorphic analysis in pharmaceutical manufacturing.

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CRediT authorship contribution statement

Luisa Souza Almeida: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Jaqueline Carneiro:** Writing – review & editing, Writing – original draft. **Luiz Alberto Colnago:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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