Chapter 2

Combined Approaches of NMR Spectroscopy in Pharmaceutical Sciences

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Abstract

NMR spectroscopy is extensively utilized across various disciplines of pharmaceutical research. The advancement of magnetic resonance technology has enabled diverse and high-resolution analyses. The importance of data from different sources in pharmaceutical research has increased. NMR spectroscopy is an effective pharmaceutical method with easy application and routine applicability. Another advantage of this method, which allows drug research not only from the chemical but also from the biological and biophysical aspects, is that it is marker-free. Initially utilized at a single level and primarily focused on specific elements such as chemical synthesis and structure-activity elucidation, NMR methodology has evolved to produce multi-level biomolecular and clinically relevant data, including metabolomics and 3D modelling, by integrating it with other instrumental techniques used in pharmaceutical sciences. From this perspective, NMR spectroscopy has found broader applications in pharmaceutical sciences. This section discusses pharmaceutical instrumentation methods combined with NMR technology, their importance, and current publications.

1. Single-level NMR Applications in Pharmaceutical Sciences

Over the decades, Nuclear Magnetic Resonance (NMR) Spectroscopy has been used in the pharmaceutical sciences as a "gold" standard and a helpful tool in a broader range between compound characterization and clinical data (Daly and Cohen 1989, Forseth and Schroeder 2011, Breton and Reynolds 2013, Emwas, Szczepski et al. 2020). There are various advantages to the

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usage of NMR spectroscopy in pharmaceutics research, and these are the reasons why researchers choose this method in every step of the research, which will be mentioned later in this section.

One of the most powerful ways of using NMR is by utilizing various types of research material, such as liquid-state and solid-state samples (Webb 2020). Therefore, this instrumentation can efficiently employ a wide range of compounds and biomaterials related to drug activity and delivery. Another strong side of the NMR method in pharmaceutical research is the function of different types of atoms (^{1}H, ^{13}C, ^{15}N, ^{17}O, ^{31}P, etc.) (Rehman and Akash 2020) present in the drug and target molecules. This feature can help to selectively identify molecular interactions and provide information on the target-ligand approaches. One of the other advantages of the NMR approach is that it is not necessary to label[3] the target or ligand molecules (Gossert and Jahnke 2016). Therefore, the ligand and target do not need to be chemically modified, and there is no need to add an extra synthesis or characterization step. The significance of label-free spectroscopy is related to reaction monitoring using NMR in various steps. Another excellent value of this method is its adaptability when used with different magnetization and relaxation parameters. By modifying (increasing) the magnetic field strengthand probe types, the sample will be able to be investigated in depth and more accurately (Conradi 2022).

Despite its widespread use and the possibility of label-free analysis, NMR spectroscopy does not provide detailed data on functional pharmaceutical aspects with a single methodology alone. The main reason for this is that proton NMR, which is the most commonly used, reflects every proton in the sample in the spectrum. This results in a highly complex NMR spectrum, making spectrum interpretation challenging. Complexity of NMR spectrum for samples containing biological material with high molecular weight (protein, peptide, long DNA chains, cell cultures, etc.) is a disadvantage for interpreting the biomolecular NMR data (Ardenkjaer-Larsen, et al. 2015, Selenko 2019). Another issue is the type of solvent and its applicability in pharmaceutical research (Laszlo 1967). Samples containing protons must be replaced with deuterium, and this modification must be made at every stage of the research. Deuteriation is a very costly method that requires additional process optimization (Di Martino, et al. 2023). In addition, although it

3 Except in situ labeling. In this technique, the label (isotope) is not required for spectrum acquisition, but it helps to understand the interaction mechanism or reaction footprinting, by the subsequential changes on label atom resonances. For details, please see Nishida, N., Y. Ito and I. Shimada (2020). *"In situ structural biology using in-cell NMR."* *Biochim Biophys Acta Gen Subj* ***1864****(2): 129364.*

is easy to apply, the NMR methodology must be expertly designed and optimized to perform more qualified research. This optimization requires qualified experts, new protocols using different probes, and detailed information. Finally, interpreting the optimized spectrum requires complex software and a detailed interpretation protocol (Johnson and Blevins 1994, Vranken, et al. 2005, Elyashberg, et al. 2008, Ellinger, et al. 2013, Lee, et al. 2015, Maciejewski, et al. 2017).

From synthesis to in-cell NMR, from clinical sample analysis to biological imaging techniques, NMR spectroscopy is used in pharmaceutical research for a wide range of purposes. In this section, examples of this usage, single-level applications, and multiple-level methods in pharmaceutical research will be discussed (Shapiro and Wareing 1998). In addition, not only NMR spectroscopy but also the advantages of NMR techniques combined with other instrumental methods in the pharmaceutical field will be discussed and a brief discussion horizon for future studies will be presented.

1.1. Chemical Characterizations

1.1.1. Small-molecule synthesis characterization

Over the past several decades, more than 150,000 distinct publications[4] have established a strong correlation between NMR spectroscopy and chemical synthesis in the fields of drug and pharmaceutical discovery. NMR strategies have been confirmed to be invaluable for elucidating and characterising the structural compositions of both reactants and products, determining functional and side groups, studying reaction monitoring progress, assessing solubility, measuring pH, assessing purity, and fulfilling miscellaneous experimental requirements (Figure 1.1). Accurately determining the chemical structures of compounds synthesised as potential drug candidates is crucial for drug research, as well as for investigating structure-activity relationships and ADME (Absorption, Distribution, Metabolism, and Excretion) properties. Furthermore, the pharmaceutical industry relies on NMR as an essential tool for routine quality assurance testing in drug manufacturing workflows, in addition to its application in research and development studies.

4 According to the PubMed listing.

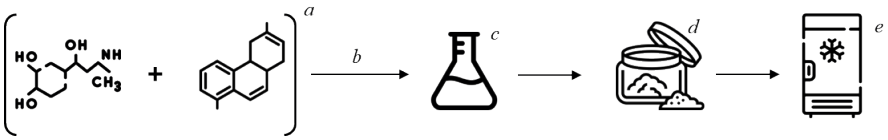

Figure 1.1. The scheme of chemical synthesis of small-molecule drug candidate/ pharmaceuticals (*a*: reactants, *b*: reaction, *c*: product (in liquid form), *d*: lyophilized powder, *e*: stability)

As illustrated in the small-molecule synthesis scheme (Figure 1.1), NMR can be utilised as the sole analytical method at various stages of pharmaceutical sciences. Most chemical syntheses in pharmaceutics begin with the acquisition of 1D-^{1}H spectra to identify impurities and confirm the intended structural composition of the target compound. Reaction monitoring is employed to determine whether a reaction has occurred (using 1D-^{1}H NMR) and to observe time-scale structural modifications through Diffusion NMR. The NMR spectra also reveal changes in the synthesis media and catalysts, referred to as reaction spectra. These reaction spectra are essential for optimising synthesis processes to enhance reaction efficiency, allowing for modifications to accommodate different reactions based on reference synthesis data.

The analysis of products can be performed using 1D-^{1}H spectra, as well as homonuclear or heteronuclear spectroscopy[5]. Product evaluation includes structural analysis, yield assessment, solubility testing, and pH measurement[6]. Once the liquid product is obtained at the conclusion of synthesis, a lyophilisation process is implemented to ensure the product's stability over time. Additionally, lyophilisation serves to standardise the concentration in the solvent (molarity) for subsequent pharmaceutical research steps. For these purposes, the lyophilised powder is tested using methodologies akin to those applied to liquid-form products. Stability studies can also be conducted using NMR spectroscopy to quantify the molecules and assess long-term structural modifications in small-molecule compounds.

5 Homonuclear spectroscopy refers to only one type of spectra that includes a single type of atom, and heteronuclear spectroscopy refers to combining resonance effects from two different types of atoms (^{1}H-^{31}P, ^{1}H-^{13}C, ^{1}H-^{15}N etc.). Therefore, NMR spectroscopy gives an opportunity to illustrate the interactions of different atom types in the drug molecule.

6 These tests are not only used in novel drug research, but also in routine pharmaceutical drug quality processes.

1.1.2. Natural products characterization

Natural-sourced drug raw materials undergo a variety of analytical processes after being extracted from plant and animal sources. NMR spectroscopy plays a crucial role at every stage of the characterization and quantification of these compounds (Figure 1.2). It is particularly effective for both qualitative and quantitative analysis of the analyte.

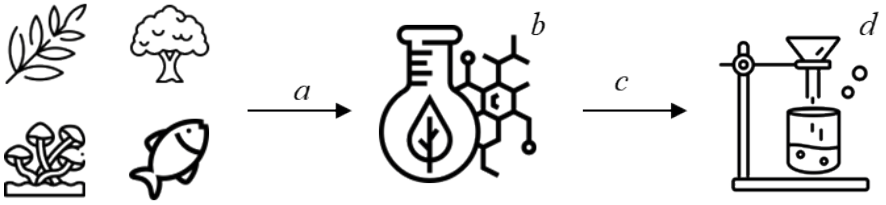

Figure 1.2. The scheme of naturally sourced drug candidate/pharmaceuticals (a: source extraction process (plant, fungi, animal, marine etc.), b: extracted material, c: purification process, d: purified compound/semi-synthesis material)

In addition to one-dimensional NMR spectroscopy, two-dimensional NMR techniques are utilized to monitor the extraction outcomes of raw materials from plant-or-animal-derived drug precursors, as well as to optimize the extraction process. Furthermore, in the semi-synthesis method employed for obtaining these drug raw materials, the characterized pre-pharmaceutical compounds sourced from plants or animals are subsequently transformed into full pharmaceutical drug candidates through semi-synthesis. NMR continues to play an essential role at this stage as well.

1.1.3. Drug delivery system characterization

Drug delivery systems are specialized pharmaceutical formulations designed to facilitate the effective delivery of active drug ingredients to targeted tissues and organs while minimizing drug toxicity. The stages of synthesis, characterization, and purification of these materials often employ NMR spectroscopy techniques. The methodologies used vary based on the chemical classification of the drug delivery system. In addition to the delivery systems themselves, drug-loaded formulations are also analyzed using NMR spectroscopy. Typically, one-dimensional methods such as DOSY and COSY, along with other NMR techniques, are applied for the characterization of drug delivery systems. Long-term stability assessments are conducted by measuring the stability of micellar nanoparticles and nano-delivery systems using quantitative NMR methods (Figure 1.3.).

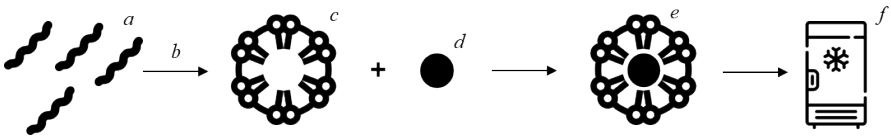

Figure 1.3. The scheme of synthesizing micellar nanoparticles (a: amphiphilic alkyl chains, b: micelle formation process, c: micelles, d: drug active ingredients, e: drug-loaded micelles, f: stability)

1.1.3. Biopharmaceutical characterization

The phrase 'biopharmaceutical' was first created to refer to therapeutic proteins generated through genetic engineering, as opposed to those obtained from typical biological sources (LeVine 2013). Over time, its definition has expanded, and the term now includes nucleic acids along with proteins, vaccines alongside therapeutic products, and even therapies based on cells (Figure 1.4.). Although these biologically based therapeutic approaches are considered to be areas where NMR methodology is not widely used due to their complex structures, it is possible to come across examples of NMR spectroscopy at different steps within the relevant methods.

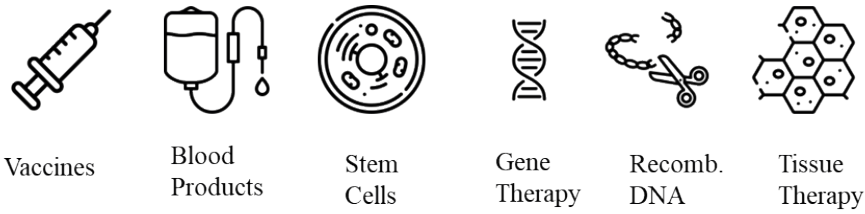

Figure 1.4. Examples of various biopharmaceuticals that are in use within pharmaceutical applications.

Structural changes (Wishart 2013, Ma, et al. 2023) and enzyme-substrate complexation (Scott 1986, Palmer 2015, Vang, et al. 2022) of biopharmaceuticals can be tracked with the NMR method. Saturation Transfer Difference spectra can be employed to identify the on/off states for protein-ligand interaction widely. Thus, it provides an idea about the therapeutic target binding mechanism. In addition, the NMR metabolomics technique can be used to show metabolic changes of biopharmaceuticals that will be mentioned later.

1.2. Structure-Activity Relationship (SAR) Applications

NMR methodology is widely used in drug discovery studies to determine structure-activity relationships. Among the basic principles of NMR, chemical shift and related principles are widely used. These principles are prominent, such as electron density, chemical anisotropy, mesomerism, and steric effects (Diehl 2008). Using these principles, pre- and post-treated structure-activity and binding mechanics are elucidated, and suitable drug candidate compounds can be effectively selected (Dias and Ciulli 2014). Binding titration and K_D values can be determined with concentration-dependent sample studies, which are informative about drug efficacy and safety from a pharmacological perspective (Dalvit, et al. 2023).

Structure-based drug design (SBDD) employs computational chemistry tools that utilize the structural information of a protein to identify or create new chemical compounds capable of binding to the target, thereby inhibiting the target protein. This method helps determine how compounds should be arranged, called the "docking pose." Scoring methods predict the most stable interactions, guiding the choice of compounds for testing against the target protein. NMR data of target-ligand interaction can be employed to support the SBDD computational docking studies to guide the binding interaction residues (Sugiki, et al. 2018). In each step, SBDD data can be checked with single-level NMR methods (Figure 1.5.).

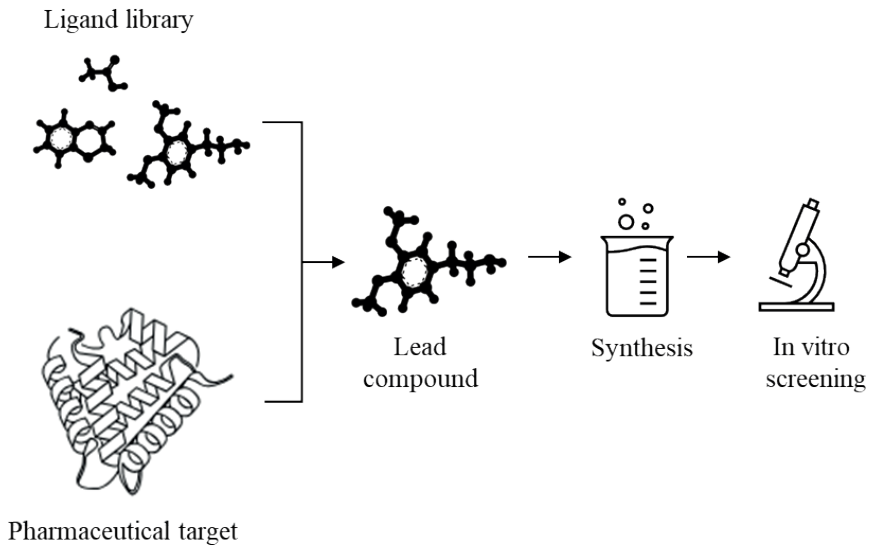

Figure 1.5. The workflow of structure-based drug design (SBDD) process in general.

At the single-level NMR spectroscopy aspect, the SBDD data is not generated adequaetly to conclude the lead compound alone. In this step, other major approaches should be employed to better understand target-ligand interaction from a ligand library source. Moreover, most of the SBDD approaches need to modify the structure, for optimizing the efficacy and safety limits of lead compounds. In this case, NMR data should be combined with other in vitro approaches (Stark and Powers 2012).

Pharmacological analogs are used as scaffolds in new generation drug design, allowing more effective and target-selective drug design through pharmacophore interactions. New drugs from the same group are designed with different modifications using the skeleton of the chemical analog. NMR spectroscopy is used in scaffold-based drug design studies using on/off binding affinity (Figure 1.6.).

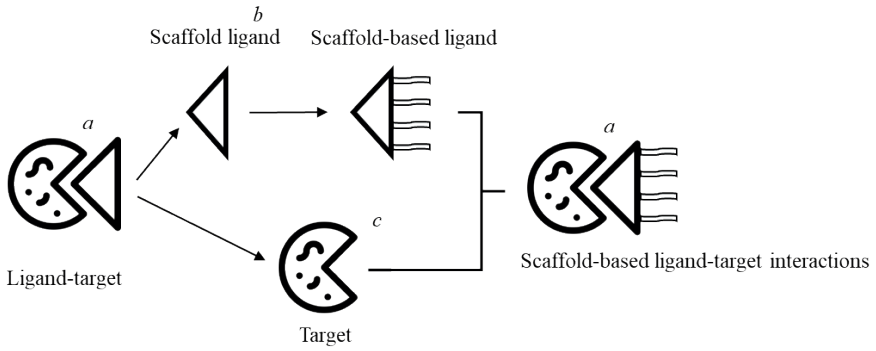

Figure 1.6. The illustration of the process for scaffold-based drug design. NMR spectroscopy is used in a) complex 2D correlation spectra, b) ligand characterization spectra and c) target identification spectra.

Not only scaffolds but also chemical fragments are effectively used in the design of new drugs (Figure 1.7.). The main difference here is that, apart from pharmacophore analogs, related chemical functional group fragments are used (Prosser, Kohlbrand et al. 2021). NMR spectroscopy is used for the structure-activity elucidation of compounds obtained by combining fragment combinations (Li and Kang 2024).

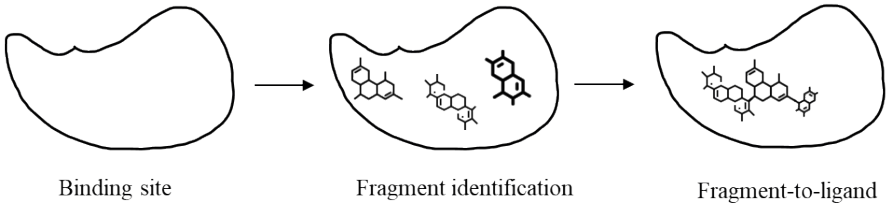

Figure 1.7. The scheme of fragment-based drug design.

The usage of alone NMR is quite common in drug design and discovery stages, which are the most essential pillars of pharmaceutical research. However, NMR data alone cannot elucidate binding and action mechanisms. When NMR data is integrated with various methods, it yields a precise structure-activity profile and serves as an effective tool for standardizing drug functionality.

1.3. Drug Metabolism Monitoring

The metabolism of drugs is crucial in establishing the boundaries of efficacy and safety within pharmaceutical research. These investigations are also employed for optimizing drugs and updating clinical practices. Metabolomics is the scientific field that explores metabolism-related data in an organism and its environment (Idle and Gonzalez 2007). Generally, metabolomics data can be monitored from cells, tissues, and organisms following drug activity. This monitoring allows for an examination of metabolism pathways and drug toxicity at the cellular level.

NMR spectroscopy is commonly utilized in studies involving metabolomics (Nagana Gowda and Raftery 2023). While it is typically applied in titration and time-dependent multiple spectra analysis, NMR correlation spectroscopy, which allows for the tracking of metabolites in two dimensions, is also practically applicable (Figure 1.8.). This technique isolates the metabolite of interest from the spectrum by sampling from the extracellular fluid. Consequently, both known and unknown metabolites can be structurally identified (Nagana Gowda and Raftery 2021). In addition to extracellular fluid, some methods focus on directly observing active cellular metabolism. These techniques fall under the category of in-cell NMR. This approach aims to obtain an instantaneous NMR spectrum from the cellular environment in real time through an automated system. Thus, it enables the monitoring of pathways where the drug exerts its effects. A significant challenge associated with this technique is the complexity of the spectrum, which requires a strong magnetic field; otherwise, high-resolution resonance monitoring becomes quite challenging (Singh, et al. 2023).

Another method to analyze metabolites is through ADMET studies. Data from metabolomics provides insight into the drug's ADMET profile and pharmacokinetic effects (Phapale 2021, Nath and Chetia 2023). This information helps in anticipating potential issues that may arise during clinical applications of the drug. Additionally, it supports the development of drug candidates that are metabolized into less toxic byproducts in the context of new drug development.

Besides in vitro metabolomics techniques, NMR spectroscopy is also applied in vivo within metabolomics studies. Pharmacodynamic and pharmacokinetic profiling can be conducted by analyzing samples taken from living subjects using various NMR methodologies.

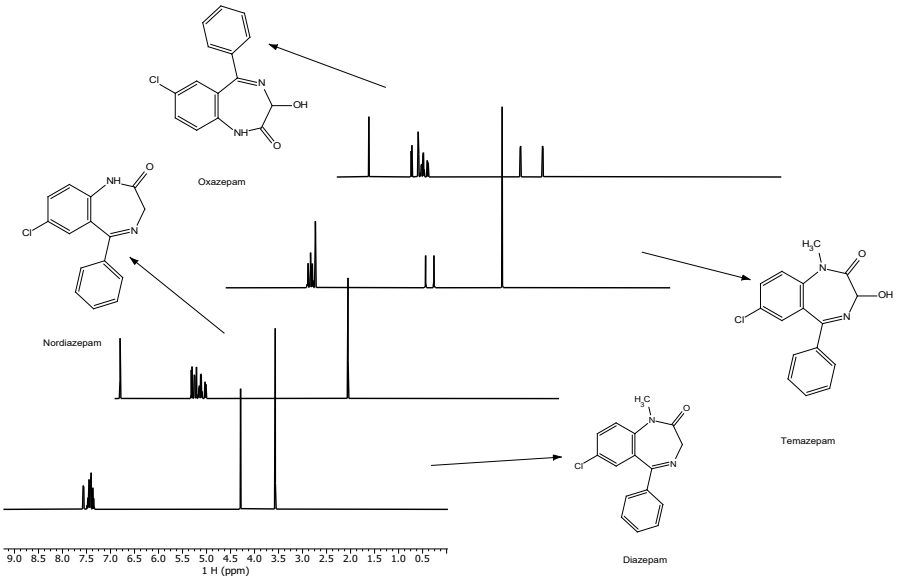

Figure 1.8. The predicted NMR spectra (MestreNova™) of different metabolites of diazepam (Langman and Jannetto 2020).

1.4. Drug Formulation and Delivery Research

Drug formulations and delivery can be effectively analyzed using a range of NMR techniques, along with various other instrumental analysis methods (Li, et al. 2021, Mousa, et al. 2023). One fundamental application is the determination of drug titrations collected from intracellular or extracellular fluids before and after drug delivery. NMR can also be utilized to characterize different drug delivery systems, while routinely assessing

the structural integrity and stability of these systems. Furthermore, NMR technology plays a key role in optimizing delivery systems by elucidating the pharmacological activities of these systems at the molecular level through techniques such as 2D homonuclear or heteronuclear correlations (Wang, et al. 2022, Zhao, et al. 2022).

The physicochemical properties of drug formulations can be clarified using NMR methodology. This technique allows for a detailed examination of the interactions between the carrier system and the active pharmaceutical ingredients through various NMR spectra. Consequently, NMR methodology is widely employed in the optimization of drug carrier formulations and in general preclinical formulation processes.

In addition to enhancing the transport system within the body, NMR can also shed light on cellular uptake mechanisms, as well as on drug efficacy and resistance mechanisms (Figure 1.9.). Moreover, intracellular uptake models developed with isolated cellular carrier components can be quantitatively assessed using NMR through concentration gradient testing (Diffusion NMR) (Nicolay, et al. 2001, Claridge 2009).

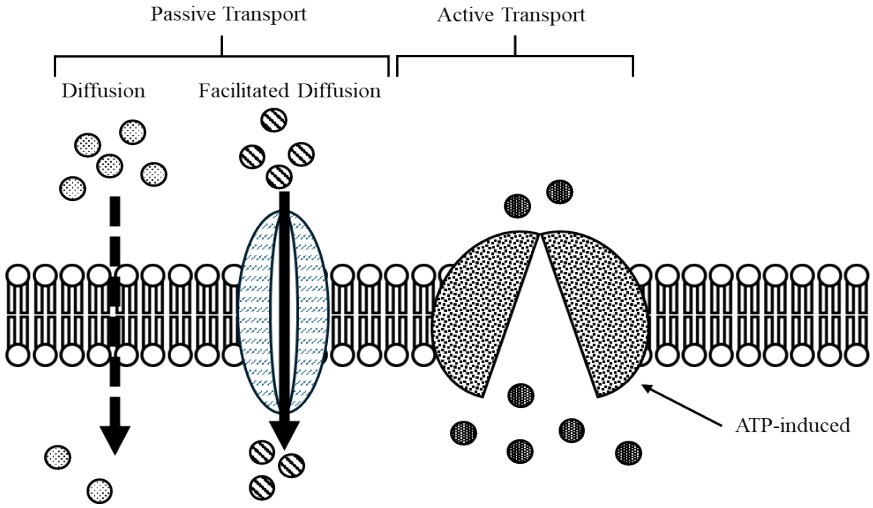

Figure 1.9. The illustration of cellular membrane and key mechanism of cellular transport.

1.5. Clinical Pharmacology

Clinical pharmacology applies various biology-based assays to evaluate the effects of drug activity on living organisms (Huang, Lertora et al. 2012). In clinical studies, the efficacy and reliability of a drug are assessed through the profiling of its metabolism in specific body fluid samples, along with

the biochemical changes observed in these samples. NMR methodology, similar to the NMR metabolomics approach, serves as an auxiliary tool for monitoring clinical phase studies and determining both the activity and dosage limits of a drug, whether qualitatively or quantitatively (Bydder, et al. 1982, Buonanno, et al. 1983, Garcia, et al. 2017).

Another notable application of NMR methods is in the analysis of clinical samples during repurposing studies. These studies enable the identification of new indications for approved drugs. In this context, NMR is utilized to investigate structure-activity relationships and to conduct drug-target binding titrations in clinical samples (Khan, et al. 2023, Rahman, et al. 2023, Atif, et al. 2024).

2. Joint (Combined) NMR Spectroscopy Data on Multi-method Pharmaceutical Research

The NMR method is commonly used in pharmaceutical research because it provides high-quality data from a single viewpoint. Improvements in NMR technology and the creation of new probes make it even more effective. However, using only NMR spectra does not provide a complete picture for pharmaceutical research. In fact, as highlighted in the previous section, more sophisticated and detailed chemical and biological testing setups are required across various research areas. Consequently, it becomes evident that multi-method approaches in NMR are more advantageous than single-method techniques, as they improve the validation of NMR data and facilitate a meaningful assessment of biological responses in model organisms or cells (Dijkstra, et al. 1989, Wang and Jardetzky 2002, Paul, et al. 2018).

Particularly for target biomolecules with high molecular weights, NMR spectra can yield complex results. To obtain clearer insights, diverse spectroscopic and chromatographic methods are employed, especially in studies focusing on drug structure-activity relationships. The challenge of molecular weight also brings forth issues related to the homogeneity and solubility of liquid-phase samples, which are addressed using various physicochemical techniques. Furthermore, the necessity for using multiple methods arises in relation to cellular function. In research involving chemotherapeutic approaches focused on targeted cellular mechanism, it is essential to relate cellular metabolite information with biological indicators like cell viability and growth. Moreover, assessing the drug's effects directly on the organism through in vivo strategies is essential for comprehensive evaluation.

Currently, numerous drug studies have effectively integrated NMR methods into their research frameworks. As a result, NMR has established itself in the field of pharmaceutical sciences not only as a "gold standard" but also as a "complementary methodology" applicable at nearly every stage of drug development and in various innovative treatments (Kotar, et al. 2020). In many advanced studies, NMR data has been successfully combined with other foundational methods that underpin the research (Figure 1.10).

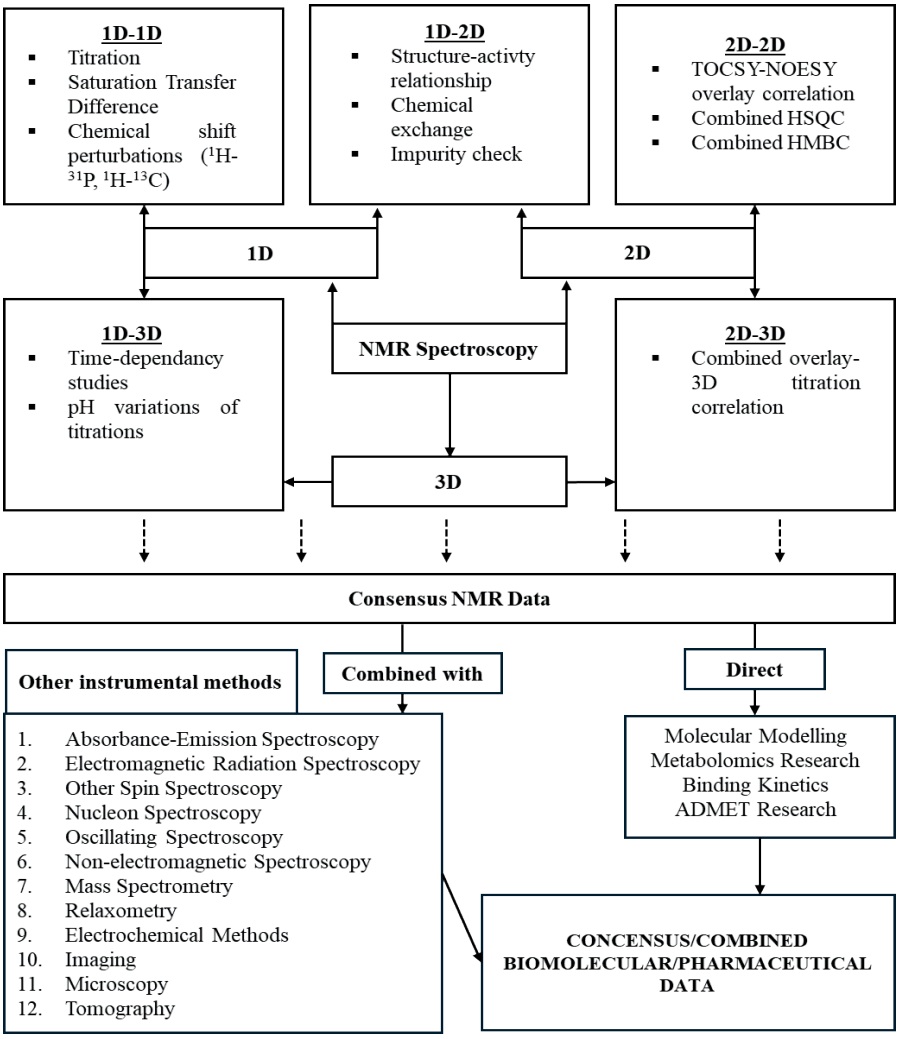

Figure 1.10. The schematic illustration of combined methologies of NMR. The data can be combined from various NMR techniques, and also combined with other external experimental methdologies (Topcu and Ulubelen 2007, Malet-Martino and Holzgrabe 2011, Farag, et al. 2012, Guennec, et al. 2014, Maggio, et al. 2014, Thamizhanban, et al. 2016, Lansdown, et al. 2019)

2.1. NMR combined with other spectroscopic approaches

Concentration-based pharmaceutical studies are primarily conducted using absorbance-emission spectroscopy. A key aspect of these studies is the validation of quantitative drug values obtained by integrating the absorbance spectrum with the quantitative NMR (qNMR) method. Additionally, diffusion-based membrane permeation studies for drugs are performed utilizing diffusion NMR spectroscopy. In this context, qNMR data is combined with the findings from these diffusion NMR studies.

Infrared spectroscopy (IR) has been a key technique for characterizing chemical structures and examining various chemical processes for quite some time (Günzler and Gremlich 2002). It is often used in conjunction with other methods. This technique is primarily utilized for the determination, detection, and analysis of small molecule drug formulations. For many years, it has played a crucial role in structure characterization and chemical structure determination when coupled with NMR spectroscopy. Today, IR spectroscopy remains widely utilized in pharmaceutical sciences across various applications wherever IR technology is accessible, frequently in tandem with NMR technology (Brittain, et al. 1993). The main reason is that both methods are very effective, especially in determining functional groups found in small-molecule drugs. The other is that IR spectroscopy, like one-dimensional NMR, is accessible and easy to apply.

Raman spectroscopy applications, particularly concerning Raman-tagged drug candidates synthesized through specific tagging techniques, are valuable in pharmaceutical sciences (Vankeirsbilck, et al. 2002). This approach evaluates drug efficacy, distribution, and solubility using Raman spectroscopy. Unlike NMR, which does not require an alkyne tag, Raman spectroscopy is instrumental in studying drug interactions and cellular entry. The necessity of using an alkyne tag can present challenges in analyzing NMR resonances that include alkyne groups. Nevertheless, the in vitro applications of both Raman and NMR spectroscopies are highly compatible, especially when the two techniques are employed in conjunction (Trefi, et al. 2008).

Mass spectrometry is one of the most effective methods used with NMR in determining the profile of target-ligand interactions (Loos, et al. 2016). NMR and mass spectrometry provide practical information at the molecular level in determining chemical and structural changes. Mass spectrometry, which is used in the pharmaceutical field by mapping drug and target complexes in detail and revealing mass changes of possible interactions, is also used to utilise synthesis monitoring in drug chemistry effectively. When

the NMR and MS methods are performed using similar samples, the results are compared on the same ground; in this case, the data can be correlated. This combination data is later used in drug optimisations and in determining structure-activity analyses. Different types of mass spectrum methods (such as MALDI-TOF and ESI-MS) can be combined with advanced 2D NMR correlation techniques to obtain detailed results about the mechanism of drug activity (Letertre, et al. 2020).

2.2. NMR combined with chromatographic approaches

Chromatographic methods are frequently used in pharmaceutical research to ensure the synthesis of drug molecules, assess their purity, and observe chemical reactions (Albert 1999, Hoffman, et al. 2008). Although multiple chromatographic techniques are available, the core principle revolves around separating components in the sample according to their molecular weight. NMR spectroscopy is often used alongside techniques such as TLC, HPLC, and GC in developing small molecule drug candidates, particularly for structure elucidation and reaction monitoring. Furthermore, NMR is selected using chromatographic methods for data validation in routine quality analysis and stability studies.

Combined HPLC-NMR methods can also examine the type of salt and crystal structure of a mixture sample alongside a defined molecular weight range identified through HPLC (Lindon, et al. 2000). This technique offers important consensus data during pre-formulation research. Additionally, while HPLC is effective, it may not suffice for conformational structure analysis, making NMR spectra essential for comprehensive evaluation.

2.3. NMR combined with chemical approaches

Chemical methods have long been employed in drug research for various purposes. While these methods typically evaluate samples containing drugs from a single perspective, they are particularly effective in determining physicochemical properties. Techniques such as electrophoresis are utilized not only for the analysis of small molecule active drug substances but also for assessing large molecular weight protein-like drug targets. Key methodologies including Potentiometry, Relaxometry, Polarimetry, and Refractometry (Kucherenko and Moiseev 1999, Valckenborg, et al. 2002, Šala, et al. 2011, Fedick, et al. 2017) continue to play important roles in drug research. Although NMR methodology does not directly yield data on chirality, conformational structure, and polarity, it provides more meaningful and sustainable insights when combined with the aforementioned techniques. Electrophoresis, for instance, is employed to elucidate target-

ligand interactions by focusing on electronegativity-based properties rather than solely on chemical properties, and is often used alongside biomolecular NMR techniques.

2.4. NMR combined with molecular biology approaches

NMR is integrated with various molecular biology methods to facilitate structural analysis of biological reactions triggered by drug activity. This approach employs metabolite PCR analysis, comprehensive protein NMR examinations, and blotting methods. The shared aspect of these combined techniques is their emphasis on metabolomics research. Furthermore, ^{31}P NMR is commonly employed to monitor changes in metabolism. Additionally, quantitative NMR results complement microscopic methods, allowing quantitative NMR assessment to reinforce qualitative findings. NMR chemical fingerprint studies help generate and validate genomics and proteomics data. The primary approach is based on NMR metabolomics to identify the presence of genome and proteome species. NMR techniques can be utilized to distinguish between Genetically Modified Organisms (GMO) and non-GMO plants, animals, and their resulting by-products (Kesanakurti, et al. 2020). The genomic modifications can be traced with 1D NMR titration spectra. Also, 3D folding features of protein structures can be illuminated with 1D and 2D NMR spectra. Thus, the protein conformations and the retrospective protein synthesis on the organism can be identified (Weininger, et al. 2025).

2.5. NMR combined with clinical approaches

Magnetic Resonance Imaging (MRI) is a fundamental tool in medical imaging, commonly used in both clinical settings and research, generating large datasets across diverse institutions worldwide. NMR techniques offer additional advantages that improve MRI techniques, as both methods are based on the same fundamental principles of magnetic resonance. In translational medicine research, many clinical samples are correlated with patients' medical images, leading to advancements in therapeutic and diagnostic strategies.

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