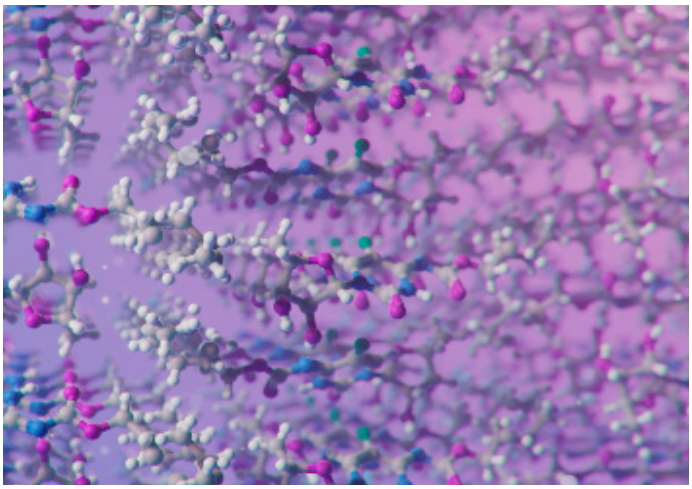
# Solid-State NMR Spectroscopy of Drug Substances and Drug Products

Eric Munson, PhD, Professor and Head of the Department of Industrial and Physical Pharmacy at Purdue University, and partial owner of Kansas Analytical Services.



Characterization of drug substances and drug products is a critical need in the pharmaceutical industry. Solid-state NMR spectroscopy (SSNMR) is an excellent analytical technique to characterize the solid forms of a drug substance to identify different crystalline and/or forms, monitor form conversion during API scale up, detect low amounts of other forms (e.g. crystalline in amorphous), measure relaxation times for prediction of physicochemical stability, and guard intellectual property. Similarly, drug product is uniquely suited to characterization by SSNMR because excipients typically do not significantly interfere with the analysis, even at low levels of drug substance in the drug product.

## The Need for the Characterization of Drug Forms, Including Polymorphs

The physical state of the drug substance and the potential interactions of the drug substance with components in a drug product play a critical role in the ability to deliver a drug to a patient. For example, different solid forms, e.g. polymorphs, solvates, and amorphous forms, can dramatically impact the amount of drug available to the body (bioavailability) and the ability to successfully make a drug product (manufacturability) by altering physicochemical properties such as solubility and morphology. In many cases, the ability to identify the most stable crystalline form in an active pharmaceutical ingredient in a drug product is critical to ensure that there will not be a conversion from one crystalline form to another that could impact the performance of the drug product. Most pharmaceutical companies do a series of screening tests to determine both the most stable crystalline form, as well as any potential polymorphic or solvated forms of the active pharmaceutical ingredient. In many cases, it can be quite difficult to isolate the individual forms that may be found in both the active pharmaceutical ingredient, particularly in the drug product.

Regulatory agencies have issued guidance on polymorphism for the development of both new and generic drugs. The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) has adopted guideline Q6A for identifying when polymorphism should be controlled and monitored. In addition, the FDA has issued guidance describing when polymorphism must be addressed in drug substances and drug products in abbreviated new drug applications (ANDAs). This is particularly important when a change of form can impact the bioavailability of the drug product. A classic example of this is Ritonavir.[1] In Ritonavir, the drug product was composed of a suspension containing a saturated form of the crystalline drug. Ritonavir was on the market for two years before a second, more stable crystalline form was discovered in the drug product. This form eventually spread to all of the drug product, resulting in a form of the drug that had substantially reduced bioavailability. This forced the withdrawal of the product from the market, with the requirement that it had to be reformulated before it could be put back onto the market. Because Ritonavir is used for the treatment of Acquired Immunodeficiency Syndrome (AIDS) the removal of this product had a serious impact on people whose treatment included this drug. In both the ICH and FDA guidance documents, SSNMR spectroscopy is highlighted as a technique for determining if multiple polymorphic forms are present. The motivation for using SSNMR spectroscopy for investigating polymorphism is provided below.

## Why SSNMR Spectroscopy?

SSNMR spectroscopy is a particularly powerful technique for the investigation of multiple crystalline forms in both an active pharmaceutical ingredient and in the drug product. Several of the benefits usually found in SSNMR spectroscopy are highlighted below:

- It is complimentary to powder X-ray diffraction for identifying different crystalline forms
- It is the only analytical technique that can quantify the amount of each form without the need for a standard
- It can identify and quantify the amount of crystalline and amorphous content in an API
- It can identify the amount of crystalline and amorphous content for each component in a formulated product
- Multiple nuclei can be analyzed, providing several unique spectra for each form
- Relaxation times can be used to identify peaks for different crystalline forms, as each relaxation time for a form is usually unique
- Relaxation times can be used to selectively enhance or remove different forms in a mixture of components, which can be useful for form identification and showing intellectual property infringement
- Selective labeling of nuclei such as $^{13}C$ and $^{15}N$ can be used to enhance certain signals when sensitivity is a challenge, as well as avoiding peak overlap
- Relaxation times can be used to predict both particle size and chemical degradation rates, especially in formulated products

## Detecting Polymorphs Using SSNMR Spectroscopy

SSNMR spectroscopy has several advantages for studying polymorphism in pharmaceuticals, including the fact that there are typically several NMR active nuclei that can be studied, where each can provide unique information about the sample. The most commonly studied nuclei for identifying multiple crystalline forms in both a drug substance and in a drug product are $^{13}C$ and $^{19}F$, with lesser studied nuclei including $^{15}N$, $^{31}P$, $^{23}Na$, $^{35}Cl$, and others. For $^{13}C$, there are typically between 10 to 30 peaks in the NMR spectrum for an average-size pharmaceutical compound that can be used to identify the particular crystalline form being studied. One advantage of $^{13}C$ SSNMR compared to other analytical techniques such as powder X-ray diffraction is that the peaks for the excipients tend to be between 60 to 110 ppm, whereas drugs will tend to have peaks between 0 to 50 ppm and 110 to 200 ppm. This means that there are many drug peaks that do not overlap with excipients and can be used to identify the particular crystalline form. In $^{19}F$ SSNMR, the only peaks in the spectrum are due to drug substance, because no excipients contain fluorine.

SSNMR spectroscopy can also provide information about the number of crystallographically equivalent sites in the crystal lattice. In particular for $^{13}C$ SSNMR, the presence of two peaks for one particular carbon usually indicates that there are two different conformations/arrangements of molecules in the asymmetric unit. Likewise, there could be three or four peaks for each carbon, corresponding to that number of molecules in the asymmetric unit. In $^{19}F$ SSNMR, multiple peaks may sometimes be observed for a single polymorphic crystal with one molecule in the asymmetric unit because both a $^{19}F$ and a $^{1}H$ atom have a similar atomic diameter, and so sometimes ring flips can create a different environment for $^{19}F$, which would not typically be considered a new polymorphic from. For this reason, it is not as easy to identify the number of crystallographically equivalent sites using $^{19}F$ NMR.

SSNMR spectroscopy has relatively low limits of detection and quantitation compared to other analytical techniques. It is inherently a quantitative technique, in that it is possible to quantify the relative amounts of forms without the need to prepare a calibration curve. It can also have substantially lower detection limits than other analytical techniques, especially for high abundance nuclei such as $^{1}H$, $^{31}P$, and $^{19}F$. Additional details about quantitation are discussed in the next section.

## Quantitation and Limit of Detection in SSNMR Spectroscopy of Polymorphs

One of the benefits of SSNMR spectroscopy is that, unlike most other analytical techniques, it is an inherently quantitative technique. In NMR spectroscopy, including SSNMR spectroscopy, the response function of each nucleus in the molecule in theory should be the same. This means that the integration of the peaks in an NMR spectrum reflect the amount of each form that is present in the sample. This assumes, of course, that the spectra were acquired using quantitative conditions. In most other analytical techniques, a calibration curve has to be prepared, because the response function of the instrument, whether it be the extinction coefficient in optical spectroscopy, such as UV-Vis spectroscopy, or the peak intensity in a powder X-ray diffraction pattern, which could depend upon things such as preferred orientation or particle size affects, is unknown. In most analytical methodologies, including NMR spectroscopy, the signal-to-noise ratio of 3 to 1 is often used to determine the limit of detection (LOD), and a signal-to-noise ratio of 10 to 1 is often used to determine the limit of quantification (LOQ).

LOD and LOQ for polymorphs for SSNMR spectroscopy can vary significantly depending upon line widths, relaxation times, and compound molecular weight. In general, LOD of 0.1-1% are easily achievable for drug substances that have molecular weights around 400 g/mol, line widths of 0.5 ppm or less, and $^{1}H$ T1 relaxation times of 10 s or less, which are typical of many drug substances. Low LOD and LOQ are often limited in drug substances by the ability to resolve the different form peaks in

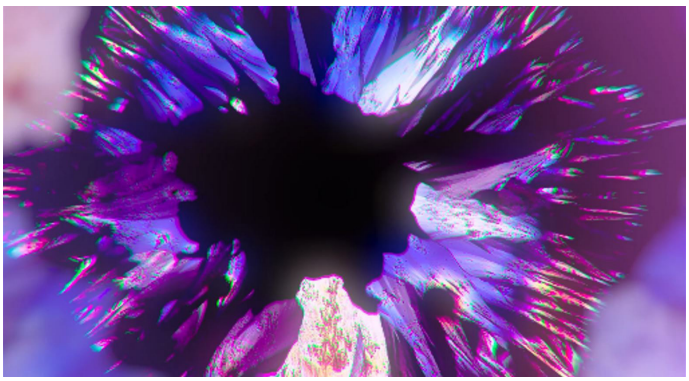
the spectrum. If the forms have different relaxation properties, then experiments that can reduce or remove one component selectively can be used to observe the desired component. The detection limits are absolute in that low dose drug products, e.g. 1-5% of drug substance by weight in a drug product, still have a drug substance detection limit of 0.1-1%, but being able to resolve the different forms is less of a problem.

## Drug Product Analysis Using SSNMR Spectroscopy

SSNMR is one of the best analytical techniques to analyze both active pharmaceutical ingredients (API) and inactive ingredients, i.e. excipients, in drug products. One of the reasons is that the peaks from the API are typically in a different part of the $^{13}C$ SSNMR spectrum than the excipients. Typically, most of the API peaks are between 0-50 ppm and 110-200 ppm in the spectrum, whereas the majority of the excipient peaks tend to be between 60-110 ppm. Unlike other analytical techniques, $^{13}C$ SSNMR usually avoids interference between the active pharmaceutical ingredients and the inactive ingredients, or excipient. For other nuclei, such as $^{19}F$, there are no fluorine nuclei present in the excipient, ensuring that the only signals that are observed come from the API. Besides $^{13}C$ and $^{19}F$, other nuclei such as $^{1}H$,$^{31}P$, $^{15}N$, $^{23}Na$, and $^{35}Cl$ can also be studied in the solid state. These nuclei are not studied as routinely in the solid state because of poor resolution ($^{1}H$), lack of presence in API molecule ($^{31}P$), poor sensitivity ($^{15}N$), or the specialized techniques needed to study them in the solid state ($^{23}Na$, $^{35}Cl$). The LOD for SSNMR spectroscopy are the same for drug substance and drug product. The primary limitation is that detecting a 1% polymorph content in an API-only sample may be difficult because of peak overlap, i.e. lack of resolution, between the two forms. However, if the API is present at 10% in the drug product, then 1% total API content in the drug product corresponds to 10% of the total API present in the sample, making resolution less of an issue.

## Amorphous Drugs and Amorphous Solid Dispersions

Amorphous solid dispersions (ASD) are being more frequently used in drug products to increase the amount of drug that is available to the body, i.e. its bioavailability. An ASD can increase this amount several fold, which reduces the pill burden on the patient, and may even make a drug candidate viable. One of the challenges of an ASD is that the active pharmaceutical ingredient is in a metastable state, i.e. it would prefer to be in the crystalline state. Since even a small amount of crystalline material could result in decreased bioavailability, regulatory agencies often require monitoring of the drug product for crystallinity. SSNMR spectroscopy is an excellent technique for determining crystallinity in a drug product. For $^{13}C$ SSNMR, the amorphous peaks are often about an order of magnitude broader than the crystalline peaks, and the crystalline peaks may be offset from the amorphous peaks (typically 0-4 ppm for $^{13}C$ SSNMR), making them easy to identify and quantify. In $^{19}F$, the spectrum of an amorphous drug is also similar to the crystalline drug, except that the line widths are often about an order of magnitude broader than the crystalline drug, depending upon the functional group, and the peak locations are slightly different. As noted in the section on polymorphs, one advantage of $^{19}F$ SSNMR compared to other nuclei is that fluorine is only present in the API, and not in the excipients.



The LOD and LOQ for the crystalline component in an ASD are similar to that of polymorphs, except that the amorphous signal is usually substantially broader than the crystalline signal, making overlap less of an issue compared to a mixture of two crystalline forms. The LOD for an amorphous API is usually substantially higher, often an order of magnitude compared to the crystalline component, due to line widths that are significantly broader than the crystalline component.

## Emerging Technologies for SSNMR Analysis of Pharmaceuticals

SSNMR techniques continue to evolve, expanding both the current detection limits, with the development of cryogenically cooled probes for SSNMR [2], as well as new applications, such as dynamic nuclear polarization (DNP) [3] and time-domain (TD) SSNMR [4]. In the case of DNP SSNMR, there is the potential to dramatically decrease the detection limits for crystalline forms in drug products, while with TD SSNMR, there are potential applications where SSNMR can be used on-line or at-line in a pharmaceutical manufacturing facility. In these cases, the critical step is to translate the significant capabilities of SSNMR to the pharmaceutical industry.

## Conclusion

SSNMR spectroscopy is an outstanding technique for the analysis of both crystalline and amorphous pharmaceuticals, both in the drug substance and in the drug product. It is an inherently quantitative technique that can selectively detect the active pharmaceutical ingredient separately from the excipients. Multiple nuclei can be studied, where each nucleus can provide unique information about the sample. For example, selective labeling of $^{13}C$ nuclei can be used to both increase sensitivity and isolate signals from a particular functional group, while the $^{19}F$ SSNMR spectrum only contains signals from the API and not the excipients. Moreover, relaxation filters and multidimensional experiments can be used to isolate components and monitor dynamics, respectively. The detection limits for polymorphs are often superior to other analytical techniques and are usually unaffected by the presence of excipients in drug products.

## About Kansas Analytical

KAS has become a leading supplier of solid state NMR analytical services to the pharmaceutical industry. We provide an essential link between the pharmaceutical development process and the finished drug. Solid state NMR offers answers to important questions such as the type and quantity of a particular crystalline form present, the presence of amorphous material in a formulation, and predicting the stability of pharmaceutical compounds. These variables impact important parameters such as bioavailability and manufacturing processes and often cannot be measured by any other technique.

No data from KAS is provided in this article.

## References


1. John Bauer et al., Ritonavir: An Extraordinary Example of Conformational Polymorphism, Pharm Res 2001, Vol 18, No 6
2. Alia Hassan et al., Sensitivity Boosts by the CPMAS CryoProbe for Challenging Biological Assemblies, J Magn Reson 2020, 311: 106680
3. Qing Zhe Ni et al., In Situ Characterization of Pharmaceutical Formulations by Dynamic Nuclear Polarization Enhanced MAS NMR, J Phys Chem B, 2017, 121: 8132
4. Dirk Stueber and Stefan Jehle, Quantitative Component Analysis of Solid Mixtures by Analyzing Time Domain $^{1}H$ and $^{19}F$ T1 Saturation Recovery Curves (qSRC), J Pharm Sci 2017, 106: 1828


## Additional review articles


1. Thomas Offerdahl and Eric Munson, Solid-State NMR Spectroscopy of Pharmaceutical Materials, Am Pharm Rev 2004, 7: 109
2. Robert Berendt et al., Solid-State NMR Spectroscopy in Pharmaceutical Research and Analysis, Trends Analyt Chem 2006, 25: 977



**Bruker BioSpin**
info@bruker.com
bruker.com