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Advanced Approaches in Solid-State Analysis of Pharmaceuticals

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Omprakash G. Bhusnure[1,*], Sachin B. Gholve[1], Kamble Nitin[2], Padmaja S. Giram[3], Varsha M. Gaikwad[2], Trupti M.Rajmane[1],

[1]Channabasweshwar Pharmacy College (Degree), Depart. of Quality Assurance, Latur(MS), India.

[2] Channabasweshwar Pharmacy College (Degree), Depart. of Pharmaceutics, Latur(MS),India.

[3] Channabasweshwar Pharmacy College (Degree), Depart. of Pharmacology, Latur(MS), India.

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ABSTRACT

Current analytical techniques for characterizing solid-state pharmaceuticals includes powder x-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, infrared spectroscopy, Raman spectroscopy, electron microscopy and nuclear magnetic resonance. Powder x-ray diffraction and differential scanning calorimetry are mainstream techniques but they lack spatial resolution. Scanning electron microscopy and micro-Raman spectroscopy provide good chemical and optical characterization but they are not capable of analysing very small nanoparticles. Transmission electron microscopy and nano-thermal analysis can provide explicit characterization of nanoparticles but they are invasive. Nuclear magnetic resonance offers good spatial resolution but its use is mainly limited by poor sensitivity and high costs. In view of the many challenges posed by existing methods, new and novel techniques are being continually researched and developed to cater to the growing number of solid formulations in the pipeline and in the market. Innovation in research and development is a key target for the pharmaceutical sector to address some of the challenges it currently faces. This review discusses these challenges in the context of pharmaceutically relevant surfaces and interfaces. The surface properties of materials determine many pharmaceutically important interactions and can be drastically different from the material's bulk properties. We first introduce current challenges in the surface and interface analysis of pharmaceutical materials in the context of material design, administration and fabrication. We review recent scientific and technological advances aimed to address these issues and discuss examples that illustrate the capabilities of these techniques.

INTRODUCTION

A major factor which determines the overall success of a pharmaceutical product is the solid-state form in which the active pharmaceutical compound exists. It is a well-known fact that most pharmaceuticals possess the ability to exist in different solid forms. The solid-state of a compound significantly influences the physico-chemical and mechanical properties exhibited by the drug. Subsequently, these properties not only affect the processability and stability of a drug, but also the dissolution and bioavailability thereof. Optimal drug performance depends to a large extent on the solid-state form used in the design and development of a pharmaceutical product. Considering the above mentioned facts it becomes quite evident why it is so essential to select the best solid-state form of a given drug to be incorporated into a dosage form. The appropriate solid-state form should be selected to ensure that the specific form will remain unchanged during processing, manufacturing as well as during distribution and storage of the final product. Knowledge of solid-state forms of drugs and the identification of possible transformations have developed from mere scientific interest to matters that must be addressed for every dosage form. It has been known that pharmaceutical solids can exist in more than one form, be it amorphous or crystalline poly-morphs. The different solid forms exhibit different physico-chemical properties including melting point, solubility, stability, powder flow, tabletting behaviour and dissolution. These properties, in turn, affect bioavailability of the drug and therapeutic outcome in the end user. It is therefore crucial to fully characterize a solid drug to ensure that the right form is incorporated into the final pharmaceutical product and maintained throughout its shelf life. Typically, the most thermodynamically stable form of the drug is chosen for development into the final product but more recently, metastable forms with higher solubilities have been used to enhance the dissolution or bioavailability of poorly soluble drugs.

The development and application of solid-state NMR spectroscopy (SSNMR) in pharmaceutical analysis continues to progress through research in both academic and industrial settings, particularly as laboratories continue to implement and extend recent accomplishments in the wider field of SSNMR.[1-2] In particular, high static field strengths, experiments based on homonuclear dipolar decoupling sequences, and experiments based on dynamic nuclear polarization (DNP) have seen increasing usage in applications to solid pharmaceutical materials and organic materials with similar properties.[3-5]

These methods and other recent developments have enabled access to previously underutilized nuclei, such as ^{1}H,^{14}N, ^{17}O, and ^{35}Cl, which are of common interest in pharmaceuticals, and has also allowed for improved sensitivity in studies of a variety of crystalline and amorphous phases.[6-8] Both magic-angle spinning (MAS) and static methods continue to be of interest in these applications.

This article highlights recently-developed approaches with broad potential applicability to pharmaceutical studies involving ^{1}H SSNMR at high fields and with homonuclear dipolar decoupling, DNP enhancement of pharmaceutical SSNMR spectra, for example using amorphous solid dispersions, a common solid form used in drug delivery.

It has been known that pharmaceutical solids can exist in more than one form, be it amorphous or crystalline polymorphs. The different solid forms exhibit different physicochemical properties including melting point, solubility, stability, powder flow, tabletting behaviour and dissolution. These properties, in turn, affect bioavailability of the drug and therapeutic outcome in the consumer. It is therefore crucial to fully characterize a solid drug to ensure that the right form is incorporated into the final pharmaceutical product and maintained throughout its shelf life. Typically, the most thermodynamically stable form of the drug is chosen for development into the final product but more recently, metastable forms with higher solubilities have been used to enhance the dissolution or bioavailability of poorly soluble drugs. Current analytical techniques for characterizing solid state pharmaceuticals include powder x-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, infrared spectroscopy, Raman spectroscopy, electron microscopy and nuclear magnetic resonance. Powder x-ray diffraction and differential scanning calorimetry are mainstream techniques but they lack spatial resolution. Scanning electron microscopy and micro-Raman spectroscopy provide good chemical and optical characterization but they are not capable of analysing very small nano particles. Transmission electron microscopy and nanothermal analysis can provide explicit characterization of nanoparticles but they are invasive. Nuclear magnetic resonance offers good spatial resolution. but its use is mainly limited by poor sensitivity and high costs. In view of the many challenges posed by existing methods, new and novel techniques are being continually researched and developed to cater to the growing number of solid formulations in the pipeline and in the market.

Some of the recent advances attained in the solid-state analysis of pharmaceutical are presented below.

CHALLENGES IN PHARMACEUTICAL CHARACTERISATION

According to the Food and Drug Administration's (FDA's) guideline on drug substances it is a regulatory requirement that the solid-state form of a drug be controlled throughout all processing and manufacturing steps. It is also required that the solid-state form of a drug must be known at any given time during the product manufacturing process, as well as during storage and distribution. Guidelines established by the International Conference for Harmonization (ICH) require a complete polymorphic study of a new drug prior to the product development stage.

The existence of different solid-state forms such as polymorphs, solvates, hydrates, and amorphous form in pharmaceutical drug substances and excipients, along with their downstream consequences in drug products and biological systems, is well documented. Out of these solid states, amorphous systems have attracted considerable attention of formulation scientists for their specific advantages, and their presence, either by accident or design is known to incorporate distinct properties in the drug product. Identification of different solid-state forms is crucial to anticipate changes in the performance of the material upon storage and/or handling. Quantitative analysis of physical state is imperative from the viewpoint of both the manufacturing and the regulatory control aimed at assuring safety and efficacy of drug products. Numerous analytical techniques have been reported for the quantification of amorphous/crystalline phase, and implicit in all quantitative options are issues of accuracy, precision, and suitability. These quantitative techniques mainly vary in the properties evaluated, thus yielding divergent values of crystallinity for a given sample.

Surface and interface analysis has become an integral part of pharmaceutical research and technology development. Advances in analytical capabilities have contributed significantly to understanding the performance of pharmaceutical products; yet, significant challenges remain. The nature of these challenges is diverse and ranges from science and technology to management and infrastructural aspects. Some of them are highlighted in Figure 1 and will be discussed below in the context of design, administration and manufacturing of pharmaceutical products.

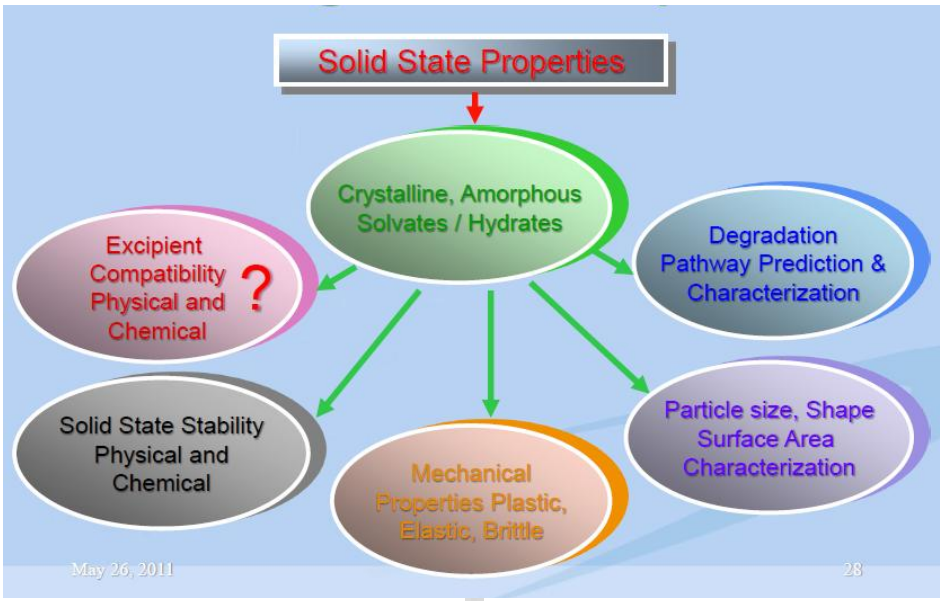

Figure 1: Nature of Challenges of Solid state

Characterizing pharmaceutical formulations physicochemical properties of pharmaceutical formulations, next to other aspects such as potency, scalability, cost etc., are among the main factors that drive pharmaceutical performance and efficacy. For example drug availability, stability and the rate of dissolution can vary greatly with properties such as morphology, roughness and distribution of a drug in a matrix. Equally, the chemical properties of a surface affect the interaction of materials at an interface within a system or indeed between that system and its environment. Identifying and characterizing these properties is therefore essential to understand why formulations are successful or fail. It is the understanding of these surfaces and interfacial properties and interactions that are particularly important. Bulk chemical and physical analytical methods such as NMR, infrared spectroscopy, traditional mass spectrometry, X-ray diffraction, dynamic light scattering etc. have been the mainstay of material characterization catalogues. While providing necessary and important information, these techniques do not offer insight into interfacial and surface phenomena. For example, active pharmaceutical ingredient (API) distribution and morphology can be markedly different on the surface of a tablet as compared to the bulk material. Standard bulk characterization techniques are unable to identify these subtle differences whereas surface sensitive analysis techniques are able to distinguish between the top molecular layer and the bulk of the material.

APPROACHES FOR SOLIDE STATE ANALYSIS

High Field ^{1}H SSNMR and Homonuclear Dipolar Decoupling

The use of high static fields for ^{1}H Solid State NMR enables detailed observation of effects such as hydrogen bonding in pharmaceutical materials of interest. This is particularly relevant in studies of Pharmaceutical co-crystals, which generally involve a molecular complex of two or more components that when separate are also solid phases. The great variety of cocrystals that can be formed with a particular drug provides flexibility and in some cases the ability to tailor the physical properties of the resulting cocrystalline phase, and provides new possibilities for drug molecules that are not amenable to the formation of salts.

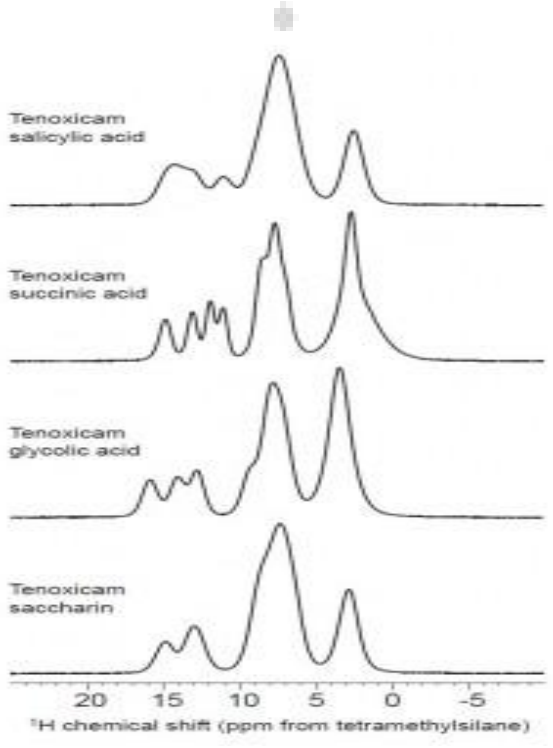

Figure 2. ^{1}H SSNMR spectra of four cocrystals of the drug tenoxicam at obtained at a MAS spinning rate of 35 kHz and a static magnetic field strength of 16.4 T. A measurement temperature of 283 K was used. For details about the interpretation of these spectra[9].

For example, Fig. 1 illustrates the ^{1}H spectra obtained at a static field of 16.4 T and at a MAS rate of 35 kHz for four cocrystals of the drug tenoxicam.[9] The spectra were obtained using a 2.5 mm double-resonance probe that also enables observation of heteronuclei with a lower

gyromagnetic ratio, such as ^{14}N, ^{17}O, and^{35}Cl,. The spectra offer relatively high resolution and allow for observation of ^{1}H signals for many proton positions of interest.[9] The spectral region from approximately 9 ppm to 15 ppm is commonly populated by resonances assigned to hydrogen bonding protons, which are usually of significance in cocrystal studies because of the strong influence of hydrogen bonding within the crystal structures of these phases. The use of high field ^{1}H SSNMR experiments can enable rapid assessment of the success of the formation of a cocrystal along while also providing information on the resulting hydrogen bonding trends, all without the need to obtain a crystal structure. In addition to higher field strengths, the use of homonuclear dipolar decoupling in the observation of ^{1}H spectra also offers many benefits for studies of pharmaceutical materials.

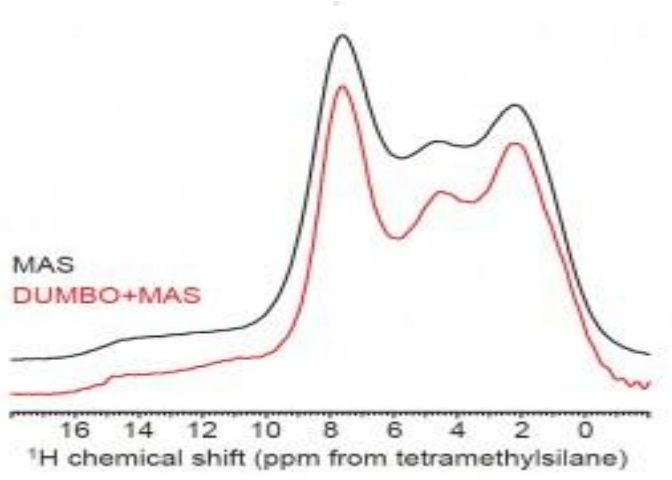

Figure 3. ^{1}H SSNMR spectra of amorphous quinapril hydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA) obtained using conventional MAS and with DUMBO dipolar decoupling at a static field of 16.4 T. The MAS spectrum and DUMBO spectrum were obtained using MAS rates of 35 and 30 kHz, respectively. A measurement temperature of 283 K was used.

Many pharmaceutical materials of interest are amorphous and lack long range order, which limits the applicability of studies using diffraction methods. These systems can still be beneficially studied using ^{1}H SSNMR. In Fig. 2, the ^{1}H SSNMR spectrum of amorphous quinapril hydrochloride obtained using MAS alone at 16.4 T is compared to the ^{1}H spectrum obtained using the windowed eDUMBO-1_{22} pulse sequence for homonuclear ^{1}H decoupling at the same field.

In the case of amorphous quinapril hydrochloride, the use of DUMBO decoupling reveals the presence of at least two proton resonances in the hydrogen bonding region of the spectrum, which are likely related to the carboxylic acid and protonated amine groups in the amorphous solid.

Even without detailed interpretation, these observations highlight the ability of SSNMR to probe structure in amorphous pharmaceutical materials. The superior resolution available from the use of high fields and homonuclear dipolar decoupling enables detailed assignments of ^{1}H spectra obtained directly and through ^{1}H-^{1}H, ^{1}H-^{13}C, and other 2D correlation methods with or without the availability of a crystal structure and an accompanying DFT chemical shielding calculation.[1-2]

Dynamic Nuclear Polarization (DNP) Enhanced NMR

Conventional solid-state NMR techniques utilise crosspolarization magic angle spinning (CP/MAS) pulse sequences to obtain high-resolution spectra from dilute, spin- 1/2 nuclei. With CP/MAS, the sample is rotated around an axis inclined at a "magic angle" (= 54°44') to the magnetic field so that line broadening is significantly suppressed and resolution is greatly enhanced. This to a large extent, overcomes the overlapping problem of active ingredient by excipients in a pharmaceutical formulation. Although crosspolarization considerably enhances the signals in the NMR spectra, active ingredients still produce relatively low signals compared to excipients due to their small fraction in pharmaceutical formulations. This issue of sensitivity is one of the key reasons behind the recent interest in dynamic nuclear polarization (DNP) techniques.

In DNP NMR, the sample is cooled to < 4K in a strong magnetic field in the presence of a polarizing agent such as trityl-type radicals and tyrosyl radicals. Under these conditions, unpaired electrons become strongly polarized, and the polarization can be transferred to nearby atomic nuclei using microwave irradiation. For solid-state analysis, DNP at high magnetic fields requires relatively powerful and stable microwave sources, yet low enough so that the relative positions of electronic and nuclear spin are frozen. Commercial instruments to accommodate this requirement have recently emerged, such as those combining low-temperature MAS with in situ microwave irradiation. Dynamic nuclear polarization (DNP)-enhanced solid-state NMR

spectroscopy has been shown to hold great potential for functional studies of membrane proteins at low temperatures due to its great sensitivity improvement. With the availability of such modern instrumentation, DNP is proving to be quite useful in the signal enhancement of spin-1/2 nuclei such as 13C and 15N. The effect of signal enhancement can be appreciated from a study analysing an amorphous solid dispersion containing 30% diflunisal in polyvinyl pyrrolidone (PVP) by 13C CP/MAS with DNP (microwaves on) and without DNP (microwaves off). The dispersion was impregnated with bis-TEMPO-bisketal radical as polarizing agent and using 1,1,2,2- tetrachloroethane as solvent. A relaxation delay of 7 s was used, during which DNP buildup occurs when microwave irradiation is applied. Spectra were obtained using a MAS rate of 8 kHz with a static field of 9.4 T, at a measurement temperature of 100K. In another more extensive study, the crystalline and amorphous forms of cetirizine, povidone (excipient), as well as four brands of cetirizine tablet formulations (Life, CVS, Reactine and Wal-Zyr) were analysed by both 13C and 15N CP/MAS NMR under DNP. The tablets containing 4.8 to 8.7 % w/w of cetirizine dihydrochloride were gently ground by hand with a mortar and pestle and then impregnated with a small volume of 1,1,2,2- tetrachloroethane as solvent and nitroxide biradical TEKPol as polarizing agent. Spectra were obtained using a MAS rate of 8 kHz with a static field of 9.4 T, at a measurement temperature of 105K. It is obvious from the 13C and 15N NMR spectra that all four formulations were of the amorphous form. Sensitivity enhancements of 2 orders of magnitude were obtained with the use of DNP. The use of DNP methods has been shown to allow for significant signal enhancements in studies of microcrystalline solids with longer ^{1}H T_1values[4] and in an amorphous organic molecule.[5] Recent developments in DNP instrumentation and in stable radical molecules have further extended the utility of DNP methods.[10]

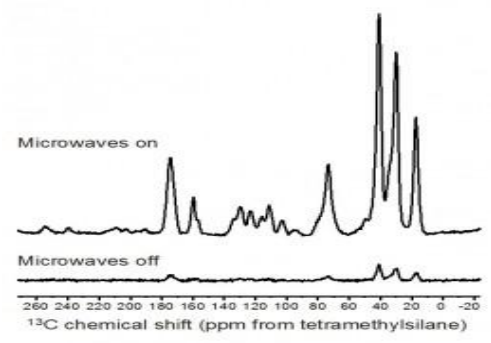

Figure 4. ^{13}C CP-MAS spectra obtained with DNP (microwaves on) and without DNP (microwaves off) of an amorphous solid dispersion containing 30% diflunisal in PVP.[7] The dispersion was impregnated with 16 mM of bCTbK radical (bis-TEMPO-bis-ketal, where TEMPO is (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl)) using 1,1,2,2-tetrachloroethane solvent (leading to signals in the 70-80 ppm region). Centerbands appear in the 180 to 10 ppm region. Each spectrum is the result of 16 scans. A relaxation delay of 7 s was used, during which DNP buildup occurs when microwave irradiation is applied. Spectra were obtained using a MAS rate of 8 kHz with a static field of 9.4 T. A measurement temperature of 100 K was used.

An example of the potential of DNP is shown in Fig. 3 for an amorphous solid dispersion (also known as a glass solution) containing 30% w/w of the drug diflunisal in the polymer polyvinylpyrrolidone (PVP)[7] . A significant enhancement is observed by comparison of the cross-polarization (CP) MAS spectra in Fig. 3, which were obtained at 100 K using microwave irradiation with a 263 GHz continuous-wave gyrotron source, microwave transmission line, 3.2 mm low temperature MAS probe, and 400 MHz Bruker Avance III wide-bore SSNMR spectrometer.[10]

Strong signals from the amorphous drug are seen e.g. in the 140 to 100 ppm region after 16 scans with microwave irradiation, highlighting the potential for both fast 1D analysis and improved sensitivity for 2D experiments. Amorphous solid dispersions represent an area of intense interest in pharmaceutical development, and the ability of DNP to enhance the signal of ^{13}C nuclei and other low sensitivity nuclei should enable greater structural information to be obtained from these systems.

DNP leverages polarization to enhance signal intensities for NMR

DNP-NMR makes it possible to transfer this large Boltzman polarization of the electron spin reservoir to the nuclear spin reservoir to provide a boost in NMR signal intensities by several orders of magnitude; thus increasing the signal intensity and data acquisition rate in a NMR experiment dramatically.

This is no new scientific area. First DNP-NMR experiments were performed in the early 1950s at low magnetic fields (1) but until recently the technique was of limited applicability because of the lack of high-frequency, high-power terahertz sources. Briefly, in DNP-NMR spectroscopy,

the large electron polarization of a polarizing agent is transferred to surrounding nuclei (typically protons, 1H) by terahertz (microwave) irradiation near or at the electron paramagnetic resonance (EPR) transition. The electron spin system (polarizing agent) required for DNP spectroscopy can either be an endogenous or exogenous paramagnetic system.

For liquid-state NMR the only DNP mechanism currently known is the Overhauser Effect (2) while for solid-state different DNP mechanisms can employed such as the solid-effect, thermal-mixing or the cross-effect (3,4).

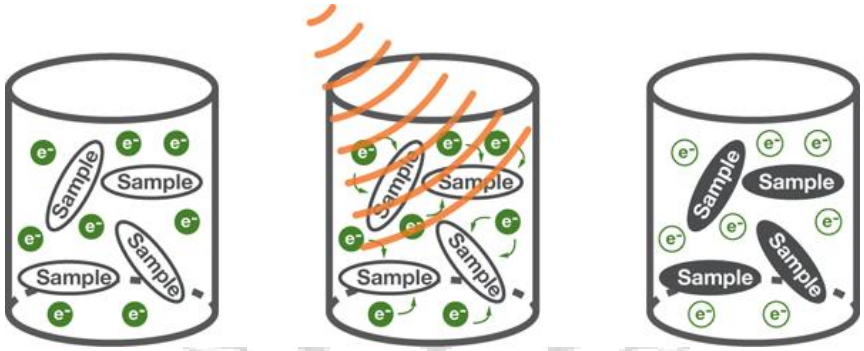

Figure 5: Schematic representation of the DNP process. Without DNP the sample is in its thermal equilibrium state. The DNP process is initiated and driven by microwave irradiation of the sample. During this process the large thermal polarization of the electrons spin reservoir is transferred to the nuclear spin reservoir.

At high magnetic fields, the cross-effect is the mechanism that yields the largest signal enhancements in dynamic nuclear polarization solid-state NMR experiments. The cross-effect can be exploited, if the homogenous linewidth (δ) and the inhomogeneous breadth (Δ) of the EPR spectrum of the paramagnetic polarizing agent, is larger compared to nuclear Larmor frequency (ω0I). The underlying mechanism is a two-step process involving two electrons with Larmor frequencies ω0S1 and ω0S2 separated by the ω0I (matching condition)[11-15]. The DNP-enhanced nuclear polarization then disperses throughout the bulk via spin diffusion[16]. To date most polarizing agents for high-field DNP experiments are based on TEMPO moieties, which employ the cross-effect (CE) as the DNP mechanism. No sample-shuttling is necessary, which makes in-situ DNP a straightforward approach to combine with solid-state NMR spectroscopy. For high-field, DNP-enhanced solid-state NMR spectroscopy biradicals such as TOTAPOL or

bTbk are very efficient[17-18] For liquid-state DNP experiments TEMPO can be simple added to the solution.

Magic Angle Spinning (MAS) NMR

Due to the properties of 19F which has a relatively high resonance frequency, spin quantum number of and 100% natural abundance, 19F NMR is a selective and sensitive multinuclear method for the analysis of complex mixtures of drugs or impurities in pharmaceutical formulations: Not surprisingly, it is the most common nucleus studied by this technique after 1 H and 13C NMR. The instrumentation setup and technique employed in 19F NMR is essentially the same as that of 1 H NMR. However, it is better to use a probe specifically designed for fluorine as fluorocarbon polymers often used in the construction of 1 H NMR probes can give rise to large, broad, baseline-distorting signals in a 19F NMR spectrum.

The advantage of 19F NMR over 1 H NMR is that there is no need to suppress the H2O solvent signal hence the use of expensive deuterated solvent is averted. A fluorine nucleus in molecules is on average surrounded by 9 electrons, rather than a single electron as is the case with hydrogen, the range of fluorine chemical shifts and the response of fluorine chemical shifts to the details of the local environment are much higher for fluorine than hydrogen. This means that 19F NMR can be applied not only in the identification and quantification of drug molecules but also in the study of drug-protein binding. Nowadays, 20-25% of drug in the pharmaceutical pipeline contain at least one fluorine atom12, making 19F NMR an increasingly popular analytical method for pharmaceutical products. A study examining amorphous forms of atorvastatin by 19F MAS NMR showed that acceptable spectra of tablets containing 1-5% active ingredient can be recorded within 10-30 minutes. The study also showed that the results obtained with 19F NMR were closely correlated with that obtained by 13C NMR, FTIR and X-ray diffraction techniques. In another study of amorphous ezetimibe adsorbed onto a mesoporous silica drug delivery system, 19F CP/MAS NMR was found to be a sensitive method for direct detection of the drugat concentrations of 2 - 16%. Full characterization to determine structural and dynamical properties of the drug as well as interactions between the drug and the silica substrate were successfully carried out. Active ingredients aside, 19F NMR has also been used to quantify polytetrafluoroethylene (PTFE) contamination in drugs that could potentially arise from pharmaceutical manufacturing or testing equipments. The study demonstrated the superb

sensitivity of the technique for the detection of fluoropolymer contaminants in drugs at levels as low as 0.02%.

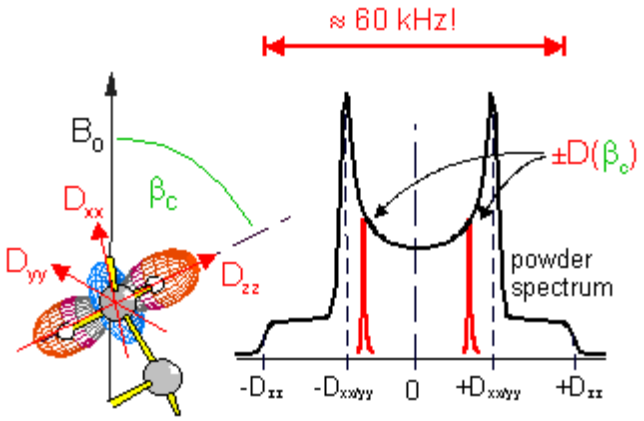

Figure 5a: Magic Angle Spinning (MAS) NMR

The most important aspect in solid-state NMR is the dominance of *orientation-dependent* interactions, which are usually averaged out in NMR spectra of solutions. They lead to substantial broadening of the spectra and a loss of spectral resolution.

High resolution can be re-gained by rapid sample rotation about the magic angle (54.7°), which, much like in the case of solutions, effectively averages all orientation-dependent interactions.

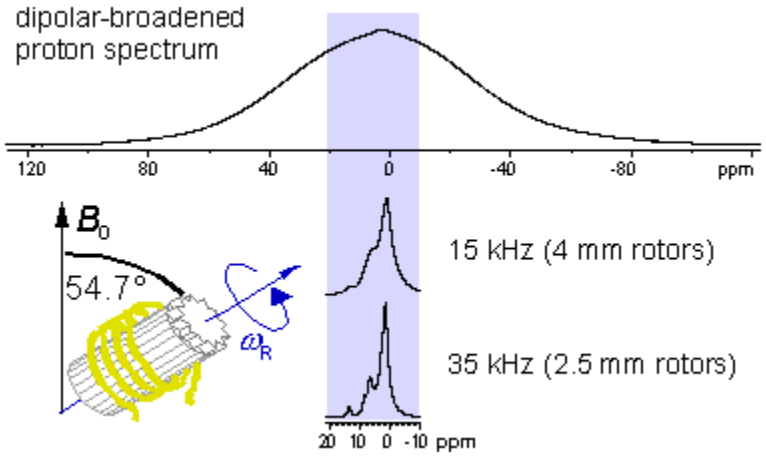

Figure 5b: Magic Angle Spinning (MAS) NMR

While almost liquid-like resolution is achievable for ^{13}C already at moderate spinning frequencies, very-fast MAS in excess of 25 kHz is needed in order to narrow dipolar-broadenend proton spectra. This is due to the strong multi-spin dipolar-coupling network among the abundant protons, which is also responsible for the smearing of the typical features of powder (Pake) spectra in the static limit.[19]

Dipolar Couplings and Internuclear Distances

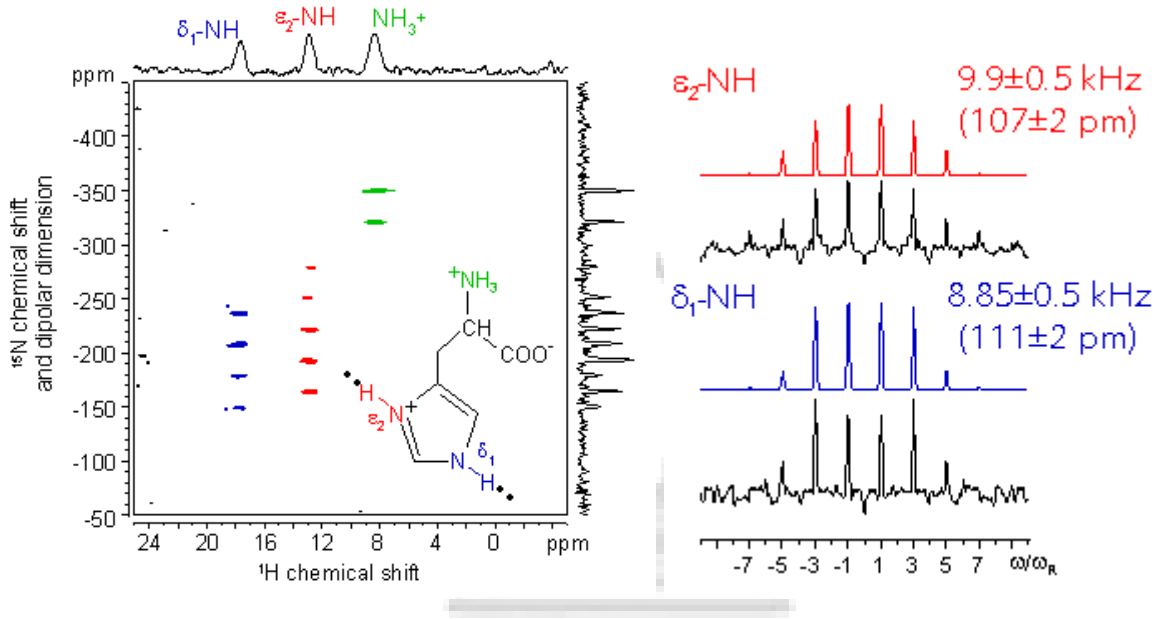

Figure 6: Dipolar Couplings and Internuclear Distances

Dipolar coupling constants are dependent on the distance between the coupled spins. Yet, their measurement is precluded when MAS is applied, because this valuable information is simply averaged away. The solution to this paradox is the application of *recoupling pulse sequences* of a specific duration as part of a two- or higher dimensional experiment. The variation of the recoupling time while monitoring the signal intensity provides access to the dipolar coupling. The experiment might embody the temporary excitation of higher-quantum coherences. A special variant of this approach comprises a second Fourier transformation over an indirect time domain, which leads to the appearance of coupling-specific *sideband patterns*.

The spectra shown here are special in that they were obtained on samples naturally abundant in ^{15}N (!) by using *inverse detection*. I.e., the detection of the signal takes place on protons instead of the weakly sensitive heteronucleus.

The distances which are extracted from the spinning sideband patterns are in good agreement with results from other methods. In fact, only neutron scattering or X-ray scattering involving substantial refinement is able to provide distance information with comparable accuracy.

Proton Multiple-Quantum Spectroscopy

The measurement of inter-proton dipolar couplings is particular attractive in that it provides information on dynamics in polymers, and has the advantage of being applicable to as-synthesized samples. Polymers, and other soft matter, are, unlike normal liquids, characterized by *anisotropic molecular motions*. This leads to the appearance of weak, *residual dipolar couplings*, which is proportional to the *order parameter* of the polymer chain. This order parameter provides a link to macroscopic (e.g. mechanical) properties, and can be used to put theories of polymer dynamics to a test.[20]

While line shape analysis might not be very reliable, due to the problem of multiply coupled spins and featureless spectra, *multiple-quantum* spectroscopy is able to provide faithful information. The technique resembles the recoupling experiments mentioned above, but can also be applied in static samples. Information on dipolar couplings is extracted by observing the signal intensity as a function of the duration of a double-quantum pulse sequence.

We have developed an experimental strategy, with which it is possible to not only determine the amount of liquid-like components and the average order parameter in a given network sample, but also to characterize the *order parameter distribution*. The method was tested on a series of bimodal network samples, which were obtained by end-linking mixtures of short and long precursor polymers.

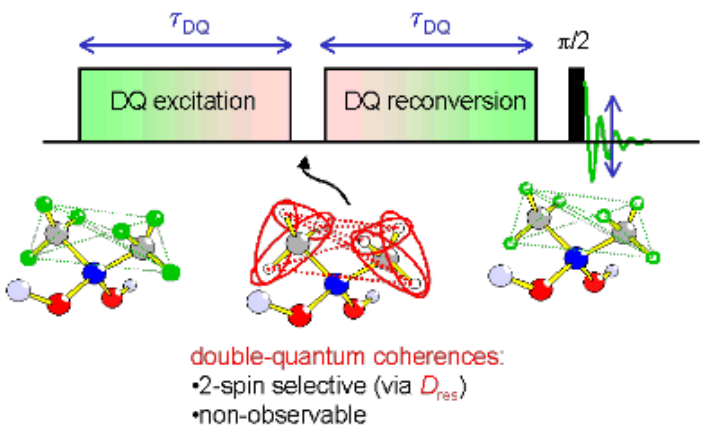

Figure 7: **Proton Multiple-Quantum Spectroscopy**

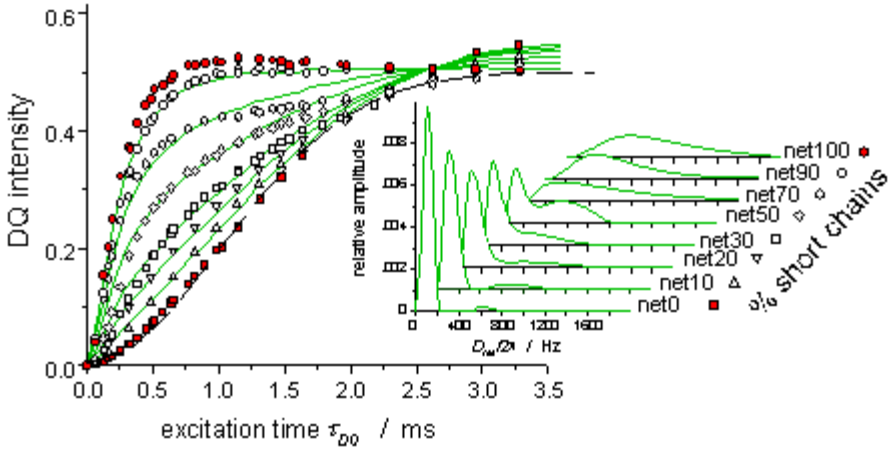

Figure 8: ***Proton Multiple-Quantum Spectroscopy***

NMR MICROIMAGING

In NMR microimaging, the drug is micronized into micro- or nanoparticles then it is embedded in a polymer carrier such as hydroxypropylmellose (HPMC) by means of melting, hot melt extrusion or solvent evaporation before being pressed into a tablet. To observe the hydration and dissolution of the tablet, a small quantity (~ 2 ml) of deuterated water is layered on top of the tablet in the magnet. Then 1 H and 2 H NMR imaging is performed by fast low angle shot imaging pulse sequence (FLASH) under constant time imaging (CTI). 1 H NMR signals are primarily from the solid polymer matrix while 2 H NMR signals are due to water. Vertical slices, parallel to the tablet axis, and horizontal slices at different heights within the tablets can be

acquired, all with a slice thickness of about 0.5 mm, at different times during the water penetration process. This allows complete profiling of the dissolution or disintegration kinetics of the tablet.

In a study conducted on a 15% antipyrine solid dispersion system using HPMC as the carrier, 1 H NMR microimaging was carried out every 4th minute to observe the water distribution in the tablet.

In another similar study, 15% flutamide in HPMC solid dispersion tablets were analysed by 1 H and 2 H NMR for water distribution as well as 19F NMR for selective drug characterization since flutamide possesses 3 fluorine atoms. The study succeeded not only in observing changes in dissolution of the tablet, but also the process of recrystallization and with a very high spatial resolution.[21]

SPATIALLY OFFSET RAMAN SPECTROSCOPY (SORS)

Verification of incoming raw materials is a basic regulatory requirement in pharmaceutical manufacturing. To do so, these materials are usually taken through an inspection area and sampled before being analysed by conventional methods such as attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy. This creates a major bottleneck in pharmaceutical manufacturing with associated high costs, the need for highly trained employees and a special chemical handling environment, as well as potential safety hazards to employees. Furthermore, raw materials that are exposed to the air can undergo rapid degradation from moisture, oxygen and light, or could be cross-contaminated by previously inspected materials or sampling tools. In view of these problems, Spatially Offset Raman Spectroscopy (SORS) has recently been proposed as a rapid and non-invasive method to identify raw materials, including API without the need to open the packaging.

In contrast to conventional Raman spectroscopy, spectra of SORS are typically collected from two or more regions that are spatially separated from the laser illumination point. Two Raman spectra are obtained at different spatial offsets, and then processed using a scaled subtraction of one from the other to yield pure Raman spectra of the individual layers. Since light transmits in straight paths through transparent objects but is diffusely scattered through coloured/ opaque/ translucent objects, a special SORS optical arrangement utilizing an obliquely angled laser beam

is necessary to cater both the illumination situations. As with conventional Raman spectroscopy and other types of optical spectroscopy, use of SORS is limited to non-metallic containers and containers which are not black or darkly coloured such as cardboard drums. Compared to conventional Raman spectroscopy, however, SORS is capable of overcoming interfering signals and even fluorescence from containers be it transparent or not, thus allowing identification of materials through packaging.

Using SORS, a study was carried out to identify raw materials in a wide variety of packaging materials such as amber glass bottle, thick white plastic high density polyethylene (HDPE) tub, blue plastic sack and multiple layers of coloured paper sacks. Laser beam was delivered to the sample at an angle of 40° then Raman scattered light was collected using a lens, filtered and imaged onto a detector. Zero offset spectra (for container surface) were acquired in < 0.5 s, while offset spectra (for container and content) were obtained between 2 and 10 s. The SORS spectra produced were processed by subtraction of the two spectra to cancel out the container contribution and yield pure spectra of the content. They were then compared with conventional Raman spectra and spectra of the pure material. In all the packaging materials analysed, the processed SORS spectra showed good correlation with the pure material, indicating successful identification and verification of raw materials. This study proves the viability of SORS in the non-invasive and nondestructive identification of pharmaceutical raw materials.

SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS) WITH MICROSCOPY:

The study on distribution and homogenity of drugs and excipients in solid formulations is an important consideration for a number of reasons. Because distribution of active ingredient in solid dosage forms influences the rate of dissolution, it will affect bioavailability and hence therapeutic outcome. Moreover, distribution of active ingredient in powders determines how homogenous the powder blend will be and eventually influences the uniformity of content of the final product. Detection of counterfeit drugs may also be possible by studying drug distribution as different manufacturing processes give rise to different distributions. Therefore, a combination of techniques comprising surface-enhanced Raman spectroscopy (SERS), microscopic imaging and mapping stage is necessary to obtain spatial images of drug distribution in solid pharmaceutical dosage forms.

SERS is a surface-sensitive technique that enhances Raman scattering by drug molecules adsorbed on rough metal surfaces such as silver. In a study investigating drug distribution in aspirin tablets by SERS, tablets containing aspirin and lactose were prepared by two different processes: “dry technology” and “wet technology”. In the dry technology, both materials were blended in a mortar and pestle while in the wet technology, both materials were blended and dissolved before evaporating the solvent. Tablets were compressed in a KBr disk press and then coated on the top with a silver colloid SERS solution. This will cause aspirin molecules to be adsorbed into the silver particles and enhance the Raman signal. Raman mapping spectra were then collected from 1750-550 cm-1 across a selected area of the tablet and maps of different technologies were compared.[22]

The spatial maps show that the active ingredient (white areas) were homogenously distributed throughout the whole tablet due to wet technology while it was scattered less homogenously in tablets from dry technology. Due to surface enhancement by the silver nanoparticles, trace amount of the drug (0.25%) could be detected, which would not be possible with conventional Raman chemical imaging. SERS also offered the added advantage of a decreased mapping acquisition time as compared with conventional Raman techniques. In the study, mapping for conventional Raman chemical imaging took 14 hours while the SERS chemical imaging only took 20 minutes. Therefore, the study proved the capability of Raman spectroscopy, in particular SERS as a rapid and sensitive tool in drug distribution studie.

TERAHERTZ PULSED SPECTROSCOPY COMBINED WITH CHEMOMETRICS:-

The terahertz region of the electromagnetic spectrum lies between the mid-IR and microwave regions, from 3 - 100 cm-1. Unlike vibrational spectroscopy, molecules excited by terahertz photons undergo torsional low-energy motions. This enables studies on drug polymorphism since the weak intermolecular forces found in crystal lattices are absent in amorphous forms. This means that crystalline forms of a drug will have a well-defined spectrum while the amorphous forms will be essentially invisible in the terahertz region. Apart from that, water shows very strong terahertz signals allowing this technique to be used to determine moisture content in pharmaceuticals.

In terahertz pulsed spectroscopy (TPS) or timedomain terahertz spectroscopy, ultra short laser pulses are generated on the order of femtoseconds. TPS offers the advantages of rapid analysis (full acquisition of spectra in < 1 minute), high sensitivity, low maintenance, suitability for imaging as well as the ability to determine absorption coeffficients and refraction indexes of materials. The relatively low powered laser compared to Raman spectrometers also reduces the risk of thermal-induced changes or photodecomposition of the sample.

Due to the large amount of complex data generated by spectroscopic techniques, chemometrics, a type of multivariate analysis is being increasingly used to analyse them. Qualitative data analysis by principle component analysis (PCA), hierarchical cluser analysis (HCA), linear discriminant analysis (LDA) and soft independent model class analogy (SIMCA) have proven to be effective techniques for identifying and differentiating between groups of drugs. On the other hand, regression techniques such as multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS) regression are vital tools in quantifying and predicting drug content in pharmaceutical formulations. The usefulness of chemometrics lie in its ability to filter out unnecessary information or noise and extract only the essential and relevant portions of the data for analysis.

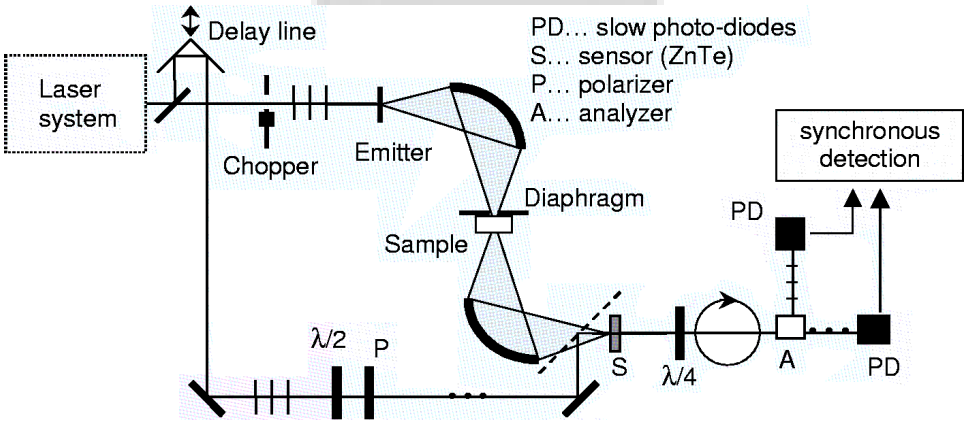

Figure 8: Terahertz spectrometer

In a recent study on alkaloids of natural products in solid crystals, terahertz spectroscopy in combination with multiple linear regression were employed for qualitative and quantitative analysis. Different mixtures of the alkaloids were investigated to determine the best combination that would give the lowest signal to noise ratio.[23]

MATRIX ASSISTED LASER DESORPTION/ IONIZATION (MALDI) MASS SPECTROMETRY IMAGING (MSI):

In the last decade or so, mass spectrometry imaging (MSI) has garnered considerable interest in the pharmaceutical community. It detects the actual molecules in the image based on their characteristic mass-to-charge ratios (m/z) and does not require the use of labeled compounds such as those used in chemical imaging. In MSI, spectra are collected from the sample, creating a grid of points on the surface of the sample. Then each point is converted into a two-dimensional spatial coordinate before an image is finally constructed by displaying the intensity of a specific m/z at each coordinate. The main advantage of MSI has been mass accuracy, mass resolution and spatial resolution. Mass accuracy describes the agreement of an ion's detected mass to its theoretical mass while mass resolution refers to the minimum difference between two m/z that can be identified as unique ions. Spatial resolution is the minimum distance between two objects in an image at which they can be distinctly discerned.

One of the ionization source technique often employed in pharmaceutical analysis is matrix assisted laser desorption/ ionization (MALDI). In MALDI MSI. The drug is coated with a thin layer of matrix and irradiated with a laser beam. The matrix absorbs much of the energy from the incident laser and provides a very “soft” ionization for the analyte compounds. To produce a good spectra, the matrix must absorb light at the laser wavelength and must not react with the analyte. MALDI MSI is capable of imaging molecules small and large, making it a very versatile technique for pharmaceutical drugs.

A study to examine the distribution of active ingredient and excipients in perindopril tablets was carried out using MALDI MSI with multivariate chemometric analysis. The tablet was first eroded to obtain a flat surface for higher quality and reproducibility of spectra acquisition. Then 2,5- dihydroxybenzoic acid and -cyano-4-hydroxycinnamic acid were deposited by sublimation method onto the tablet before acquiring mass spectra images. This study demonstrated that the use of multivariate algorithms complemented MALDI MSI techniques for explicit identification and examination of drug distribution in tablets.

TIME OF FLIGHT SECONDARY ION MASS SPECTROMETRY (TOF-SIMS) IMAGING:-

Time of flight secondary ion mass spectrometry (ToFSIMS) imaging is a highly sensitive surface analytical technique which enables examination of complex surfaces of solid pharmaceutical dosage forms. In ToF-SIMS, primary ion pulses are accelerated and focused onto the surface of a sample held under ultra-high vacuum. The bombarding particle impacts the surface and cause a sputter plume containing atoms, whole molecules and fragments of molecules. A certain portion of these will possess a charge that allows acceleration into a time-of-flight region. Since each ion has equal kinetic energy, their drift velocity will be governed by the relation of kinetic energy to mass. Thus by measuring the time it takes for the secondary ions to reach the detector, the mass-to-charge ratio can be deduced and used to separate them. A significant advantage of ToF-SIMS imaging is the acquisition of full mass spectra. This enables the study of any fragment within the mass spectrum, or to extract a spectrum from any region of interest from an image. In addition to that, depth profiling can offer very sensitive compositional and molecular. information as a function of depth as well as provide full 3-dimensional distributions within the analysed volum.. Time-of-flight Moreover, using tandem mass spectrometry (MS/MS) will increase confidence in drug identification and improve the dynamic range of the analysis.instruments have resolving powers ranging from 10,000 to 100,000 and can routinely achieve less than 5 ppm mass accuracy. A diagram depicting the process of ionization is given below.[24]

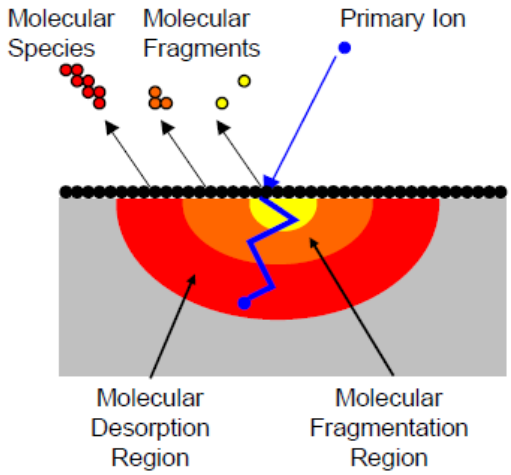

Figure 9. MSI ionization sources

Several studies have used ToF-SIMS to map the distribution of drugs within solid state drug delivery systems by direct analysis of sample cross-sections. Cross-sections of three different controlled release pellet formulations (paracetamol, theophylline and prednisone) were studied with ToF-SIMS imaging and the distribution of drug, excipients and coating characterised. This methodology has also been successfully used to determine the distribution of different drug forms (hydrated and non-hydrated) within a controlled release pellet.[25-29]

Typical Applications • Polymer/Organic Coatings: on plastics, glass, metals, and paper • Surface Contamination: additives, mold release agents, surfactants, defects • Delamination • Surface Modification Chemistry • Trace Impurities • Catalyst Surface Characterization • Bio-materials Characterization • Thin Film Depth Profiling

NANOTHERMAL ANALYSIS (NTA) WITH ATOMIC FORCE MICROSCOPY (AFM):-

The necessity for development of new drug delivery systems for poorly soluble drugs and therapeutics which targets directly the pathogenic area requires the design of complex systems in nanoscale. Atomic Force Microscopy (AFM) since its discovery offers a unique tool to develop novel drug delivery systems. Its capability to investigate, characterise surfaces and measure forces with spatial resolution at nano-scale respectively contributes to develop and analyse pharmaceutical systems and biomedical devices with complex structures and chemistries. Specifically, polymeric nanoparticles and liposomal drug formulation have been studied extensively by using AFM where their size and morphology where revealed. However, apart from topographical information, AFM can provide details on the local compositions of the sample. There are many modes that can be used to achieve this but the most common is to monitor the phase shift of the oscillating cantilever in tapping mode Such 'Phase imaging' can be used to detect nanoscale variation in composition, adhesion, friction, viscoelasticity, and other properties of the materials. Changes in the phase lag often indicate changes in the properties of the sample surface. Phase imaging has proved an extremely useful tool for pharmaceutical characterization. Phase imaging has been used to reveal polymeric forms from single crystal measurements to confirm phase separation of two copolymers for drug delivery, to establish the stability of the formulation on different environments and to identify formation of amorphous domains during milling of crystalline salbutamol[12-16]

One of the strategies to improving dissolution and bioavailability of poorly soluble drugs is by preparing them in nanoparticles from dried nanosuspensions. However, experimental techniques that can obtain information about the state of nanometer-sized drug particles are scarce. Scanning electron microscopy and micro-Raman spectroscopy provide good chemical and optical characterization but they are not capable of analysing very small nanoparticles. Characterization by conventional powder X-ray diffraction and differential scanning calorimetry (DSC) is limited due to their lack of sensitivity and spatial resolution. Thus nanothermal analysis (nTA) is an emerging technique which combines the high resolution imaging capabilities of atomic force microscopy (AFM) with the ability to characterize the thermal properties of drugs. Compared to its predecessor, scanning thermal microscopy, it offers significantly enhanced spatial resolution.

In nTA the conventional silicon tip used in AFM is replaced by a microfabricated silicon-based probe with a miniature heater that has an imaging spatial resolution of around 5 nm and a thermal property measurement spatial resolution of up to 20 nm. This imaging probe enables utilization of the most widely applied AFM imaging mode, tapping mode, to allow the analysis of softer samples, such as polymers, without damage from the imaging probe. NTA can be used to map thermal properties during imaging, or to carry out local thermal analysis at defined points on a surface. In such analysis, the probe is heated in a temperature cycle similar to DSC whilst in contact with the sample, providing quantitative information on thermal phase transitions. The use of a microfabricated probes is advantageous because of their ability to measure samples down to femtograms and nanoliters. rapid heating and cooling rates exceeding 100o C/s, and high sensitivity to small temperature changes and heat flow.

The application of nTA with AFM can be seen in a study on a nano-dispersed pharmaceutical system containing carbamazepine and hydroxypropylmethyl cellulose (HPMC) [24]. The surface of a 50% carbamazepine-HPMC formulation could be clearly visualised with the nanothermal probe.

In another study on a solid nano-dispersion of felodipinepolyvinylpyrrolidone (PVP), surface morphology could be visibly appreciated by nTA supported by AFM. The formulation was subjected to 95% relative humidity and examined at 0, 1 and 3 days. These studies showed that onset of crystallization can be detected, which is a critical consideration in the stability of poorly soluble drugs formulated in amorphous forms.

SYNCHROTRON RADIATION X-RAY COMPUTED MICROTOMOGRAPHY (SR-CT):-

X-ray microtomography, like tomography and x-ray computed tomography, uses x-rays to create cross-sections of a physical object that can be used to recreate a virtual model (3D model) without destroying the original object. The prefix micro- (symbol: μ) is used to indicate that the pixel sizes of the cross-sections are in the micrometre range. These pixel sizes have also resulted in the terms high-resolution x-ray tomography, micro–computed tomography (micro-CT or μCT), and similar terms. Sometimes the terms high-resolution CT (HRCT) and micro-CT are differentiated, but in other cases the term high-resolution micro-CT is used. Virtually all tomography today is computed tomography.

Oral controlled release dosage forms make up a large fraction of the pharmaceutical market due to the ease of administration and patient compliance. The conventional in vitro dissolution tests for controlled release formulations with HPLC or LC/MS analysis can quantify the extent and rate of the drug release. However, they do not provide any insight into the internal structure of the tablet cores. Eventhough tight dissolution specifications are introduced to monitor the quality of the final products, a number of dosage forms fail after approval, and consequently have to be recalled from the market each year. Therefore, modern in vitro microtomography techniques are being utilised as new and efficient tools that can directly reveal the internal structure and dynamic characteristics of the controlled release tablet core at different stages of the drug release process.

In synchrotron radiation computed microtomography (SR-CT), the sample is illuminated by an extended parallel x-ray beam and projection images of the sample are recorded with a 2D detector, commonly a camera coupled with x-ray scintillator. The sample is carefully rotated relative to the Xray beam and the process is repeated to produce additional two-dimensional images from various viewpoints. Using a sophisticated Fourier transform algorithm, the 2D images are then combined to generate a complete 3D map of the sample. In very simple terms, SR-CT can be thought of as creating a three-dimensional map of the relative atomic density of the sample under evaluation. Accurate sample structure can be reconstructed, given suitable angular sampling. Grayscale images also can be manipulated using standard image analysis techniques to produce binary or multiple-coloured images and to obtain dimensional information

about the sample. Furthermore, SR-CT is a non-destructive technique that has a high penetration ability and provides a reasonable level of resolution (~5–20 mm). felodipine sustained release tablets observed at various time points during dissolution. In another study, tablet swelling behaviour of various in-house polymeric preparations was investigated over time using SR-CT. The reconstructed vertical image of a 10% hydroxypropylmethylcellulose (HPMC) + 90% pregelatinised starch tablet showed crack formation after 60 minutes .

Besides observing dynamic changes in tablets, SR-CT has also been applied to examine the structures of fine pharmaceutical granules. In the study, two granules were prepared: a bromhexine HCl granule coated with Kollicoat and a wax-matrix acetaminophen granule. It was shown that SR-CT was capable of visualizing the internal structure as well as coating layer of the granules. Moreover, it successfully visualized the high talc regions of the Kollicoat layer that could not be detected by scanning electron microscopy. All these studies prove the high potency of SR-CT in elucidating internal and external shapes and structures of solid pharmaceutical dosage forms.

CONCLUSION

Surface and interface analysis has been an important aspect of pharmaceutical research for many years and provided significant insight into pharmaceutical problems. Several recent examples have been highlighted here to illustrate how state of the art analysis techniques can elucidate important aspects of pharmaceutical applications such as the surface properties of pharmaceutical formulations and their interfacial interactions during administration. The ongoing development of instrumental capabilities will continue to contribute to the advancement of our understanding of interfacial phenomena in drug formulation, administration and manufacturing. The recent progress and prospects for a number of powerful surface analysis techniques discussed here illustrate that technological advancement is critically reliant on close collaborations between industry and academia. Translation of these novel or advanced technologies to industry is not straightforward but can be facilitated by collaborative centres that provide the necessary access to facilities and expertise. The combined development of technological and infrastructural capabilities in surface and interface analysis presents the pharmaceutical sector with the necessary prerequisites to tackle important challenges in drug discovery and development. Surface and interface analysis can be expected to continue to play a

significant role in addressing the scientific pharmaceutical challenges that lie ahead and improve the R&D pipeline of pharmaceutical products.

It is evident that recent advances in the analysis of solid-state pharmaceuticals are made possible with the improvement and adaptation of conventional techniques of NMR, Raman, mass spectrometry, thermal analysis and x-ray computed tomography. The progress of such techniques provides critical knowledge and understanding of complex phenomena such as polymorphism, disorder in the crystalline state, molecular-level interactions in crystalline and amorphous materials, and detection of phase impurities. It also enables accurate and vibrant mapping of solid dosage forms at high resolution and expands the capabilities for quantitative analysis of solid formulations even at low levels. The various strengths and weaknesses of each technique can be exploited to achieve full and reliable characterization of solid drugs. It is hoped that the continual progress and advancement in analytical techniques will contribute to better understanding and ultimately effective quality control of solid pharmaceutical dosage forms.

FUTURE DIRECTION

The examples described here represent just a small portion of the recent developments in SSNMR spectroscopy, which also feature increased access to quadrupolar nuclei, greater use of paramagnetic probes and isotope labeling, improved computational methods for spectral prediction and analysis, and increased application of multidimensional methods.

Translation of these methods for use with pharmaceutical materials has provided more insight into the structure and ultimately the performance of these materials. As advances in SSNMR methods continue, the adaptation of these developments for use with pharmaceutical systems is also expected to continue.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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